Microorganisms for Sustainability 43
Series Editor: Naveen Kumar Arora

Dinesh Kumar Maheshwari
Shrivardhan Dheeman *Editors*

Sustainable Agrobiology

Design and Development of Microbial Consortia

Springer

Microorganisms for Sustainability

Volume 43

Series Editor

Naveen Kumar Arora, Environmental Microbiology, School for Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

Dinesh Kumar Maheshwari •
Shrivardhan Dheeman
Editors

Sustainable Agrobiology

Design and Development of Microbial Consortia

Springer

Editors
Dinesh Kumar Maheshwari
Department of Botany and Microbiology
Gurukula Kangri (Deemed to be University)
Haridwar, Uttarakhand, India

Shrivardhan Dheeman
Department of Biotechnology and Microbiology, School of Allied Health Sciences
MVN University
Palwal, Haryana, India

ISSN 2512-1901 ISSN 2512-1898 (electronic)
Microorganisms for Sustainability
ISBN 978-981-19-9569-9 ISBN 978-981-19-9570-5 (eBook)
https://doi.org/10.1007/978-981-19-9570-5

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2023
This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

To attain the unprecedented food demand for burgeoning population, tremendous pressure on the crop yield enhancement is at present a challenging task. The limited cultivated land disturbs ecosystem and climate change congested and stressed to meet out the requirement for feeding the hungry billions of the world. Due to the impact of climate change, plant pests that ravage major crops are becoming more destructive and posing an increasing threat to food severity and the environment. In addition, pests destroy up to 40% of global crops and cost $220 billion in losses.

Sustainable plant health management is fundamental to achieve a sustainable agri-food system. The United Nations declared 2020 as the International Year of Plant Health (IYPH), and it was extended until July 1, 2021, due to the COVID-19 pandemic. Since then, the conditions built on this legacy to raise awareness on how protecting plant health can reap hunger, preserve eco-environment, and boost overall sustainable developments.

More research till date, on majority of plant–microbe interactions considered so far a concern by using single microorganisms as antagonist to a pathogen, given that each pathogen is likely to have a specific ecological niche and optimum physiological conditions of temperature and pH to grow. Hence, how a single microbial strain covers all these situations is a competitive environment. But alteration is now not to expect a single organism to perform effectively against a wide range of biotic and abiotic stresses. It is, therefore, microbial consortium where two or more interacting organisms have synergistic, additive, or mutual complementary in nature contains the desired beneficial effects on plants and soil. The huge impact of microbial consortium on the rhizosphere microbial community is an added advantage to the concept of microbe engineering and sustainable growth and development of crops.

The contents of the book have 17 chapters that cover facets in three sections. Section I includes basic and fundamentals of microbial consortium, section II comprises contributions to agriculture and sustainability while section III is based on ecosystem and productivity. A due account is provided on microbial consortium with special emphasis on bacterial combinations and their mixture based on diverse genera for exploitation of beneficial bacteria in improving agricultural system with economic benefits is sound production of human food.

The book will be useful not only for researchers but also for students interested in strengthening their knowledge in Agricultural Microbiology, Phytopathology, Ecology, Environmental Science, and Agronomy. The book is also a benefit to those who are interested in Organic farming and Crop science.

We would like to express our sincere thanks to all contributors for their much-needed scholarly contribution and mutual co-operation. The updated and authoritative information organized in a befitted manner is definitely of great scientific value. We acknowledge the assistance rendered by our research scholars.

We wish to record our special thanks to Prof. Naveen Arora, series editor, for his professional suggestions. Our heartful gratitude to Ms. Akanksha Tyagi and her team for extending valuable support in multivarious ways to facilitate completion of this task. Thanks are due to UGC, New Delhi for awarding BSR fellowship to D.K.M. that served as a prologue to arrange the base for compilation of this book.

Haridwar, India Dinesh Kumar Maheshwari
Palwal, India Shrivardhan Dheeman

Contents

Part I Basic and Fundamentals: Microbial Consortia

Part II Contribution to Agriculture and Sustainability

Part III Contributions to Ecosystem and Crop Production

Part IV Biofertilizer, Biocontrol Agents, and Crop Growth

Part V Conclusion: A Future Perspective

Editors and Contributors

About the Series Editor

Naveen Kumar Arora Fellow of International Society of Environmental Botanists (FISEB), PhD in Microbiology, is Professor and Head of the Department of Environmental Science at Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India. He is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is plant–microbe interactions, particularly plant growth-promoting rhizobacteria. He has more than 75 research articles published in premium international journals and several articles published in magazines and dailies. He is an editor of 25 books, published by Springer. He is a member of several national and international societies, Secretary General of Society for Environmental Sustainability, serves on the editorial board of four journals, and a reviewer of several international journals. He is also the editor in chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures at conferences and seminars around the globe. He has a long-standing interest in teaching at the PG level and is involved in taking courses in bacteriology, microbial physiology, environmental microbiology, agriculture microbiology, and industrial microbiology. He has been advisor to 134 postgraduate and 11 doctoral students. He has been awarded for excellence in research by several societies and national and international bodies/organizations. Although an academician and researcher by profession, he has a huge obsession for wildlife and its conservation and has authored a book, *Splendid Wilds*. He is the President of Society for Conservation of Wildlife and has a dedicated website www.naveenarora.co.in for the cause of wildlife and environment conservation.

About the Editors

Dinesh Kumar Maheshwari former Vice-Chancellor (Gurukula Kangri Deemed to be University, Haridwar), a renowned Botanist and Microbiologist, is holding MHRD-UGC-BSR Eminent Faculty Fellowship after his superannuation. Prof. Maheshwari worked in Biology Research Centre (Szeged), Hungary in the year 1983–1984. He was Visiting Professor, Science University of Tokyo, Noda (Japan), University of Ulm (Germany), University of Gent (Belgium), University of Barcelona (Spain), University of Tokyo (Japan), and Daegu University (Korea). He has received several recognitions and has been awarded "Prof. Y.S. Murty Medal" and most prestigious "Birbal Sahni Award" of the Indian Botanical Society in the year 1992 and 2018, respectively, for his outstanding contribution.

Shrivardhan Dheeman is working as an Associate Professor and Head in the Department of Biotechnology and Microbiology, School of Allied Health Sciences, MVN University, Palwal, Haryana, India. Dr. Dheeman has research interests in Plant–Microbe Interaction and Microbial Ecology. He is involved in teaching Microbiology and Biotechnology to different disciplines in undergraduate and post-graduate classes. Dr. Dheeman was nominated for "Young Scientist" of Indian Science Congress in the year 2016 for his outstanding contribution to Microbial Diversity and Plant-Microbe Interactions.

Contributors

Hossein Ali Alikhani Department of Soil Science, College of Agriculture and Natural Resource, University of Tehran, Tehran, Iran

Naveen Kumar Arora Department of Environmental Sciences, School of Earth and Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, India

Olubukola Oluranti Babalola Food Security and Safety Niche, Faculty of Natural & Agricultural Sciences, North-West University, Mmabatho, South Africa

Federico J. Berli Grupo de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo, Chacras de Coria, Argentina

M. Hossein Borhan Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada

Rubén Bottini Instituto Argentino de Veterinaria Ambiente y Salud, Universidad Juan A. Maza, Guaymallén, Argentina

Rene Carlson Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of Pretoria, Pretoria, South Africa

José A. Carrasco Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain
Earlham Institute, Norwich, Norfolk, UK

K. Chandrashekar Department of Biotechnology, R V College of Engineering, Bengaluru, India

Ankita Das Soil and Environmental Microbiology Lab, Department of Microbiology, Assam University, Silchar, India

Shrivardhan Dheeman Department of Biotechnology and Microbiology, School of Allied Health Sciences, MVN University, Palwal, Haryana, India

Rihab Djebaili Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Flemming Ekelund Department of Biology, University of Copenhagen, Copenhagen, Denmark

Somayeh Emami Department of Soil Science, College of Agriculture and Natural Resource, University of Tehran, Tehran, Iran

Laura Ercoli Sant'Anna School of Advanced Studies, Research Centre of Crop Sciences, Pisa, Italy

Tahmish Fatima Department of Environmental Sciences, School of Earth and Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, India

Lamenew Fenta Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia
Department of Biology, Assosa University, Assosa, Ethiopia

Noris J. Flores-Duarte Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

Maddalena Del Gallo Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Kindu Geta Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia
Debre Tabor University, Debre Tabor, Ethiopia

Dweipayan Goswami Department of Microbiology and Biotechnology, University of School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

Ahmed Idris Hassen Agricultural Research Council, Plant Health and Protection, Pretoria, South Africa

Tofazzal Islam Institute of Biotechnology and Genetic Engineering (IBGE), Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

Ritika Jain Department of Microbiology and Biotechnology, University of School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

Vida Kardgar Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Tehran, Iran

Mulugeta Kibret Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia

S. C. Kipngetich Department of Crops, Horticulture and Soils, Egerton University, Njoro, Kenya

H. Korir Department of Crops, Horticulture and Soils, Egerton University, Njoro, Kenya

Mukesh Kumar Department of Botany and Microbiology, Faculty of Life Sciences, Gurukul Kangri (Deemed to be University), Haridwar, Uttarakhand, India

Prashant Kumar Department of Botany and Microbiology, Faculty of Life Sciences, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India

Sachin Kumar Department of Botany and Microbiology, Faculty of Life Sciences, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India

Sandeep Kumar Department of Botany and Microbiology, Faculty of Life Sciences, Gurukul Kangri (Deemed to be University), Haridwar, Uttarakhand, India

Dinesh Kumar Maheshwari Department of Botany and Microbiology, Faculty of Life Sciences, Gurukul Kangri (Deemed to be University), Haridwar, Uttarakhand, India

Enrique Mateos-Naranjo Department of Plant Biology and Ecology, Faculty of Biology, University of Seville, Seville, Spain

Habtamu Mekonnen Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia

Jennifer Mesa-Marín Earlham Institute, Norwich, Norfolk, UK

M. A. Baset Mia Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

Afrina Momotaj Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

N. W. Mungai Department of Crops, Horticulture and Soils, Egerton University, Njoro, Kenya

Salvadora Navarro-Torre Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

Vidya Niranjan Department of Biotechnology, R V College of Engineering, Bengaluru, India

Marco Nuti Sant'Anna School of Advanced Studies, Research Centre of Crop Sciences, Pisa, Italy
Department of Agriculture, Food and Environment (DAFE), University of Pisa, Pisa, Italy

Giancarlo Pagnani Faculty of Bioscience and Technologies for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

Eloísa Pajuelo Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

Piyush Pandey Soil and Environmental Microbiology Lab, Department of Microbiology, Assam University, Silchar, India

Marika Pellegrini Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Elisa Pellegrino Sant'Anna School of Advanced Studies, Research Centre of Crop Sciences, Pisa, Italy

Patricia N. Piccoli Grupo de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo, Chacras de Coria, Argentina

Zalak R. Rathod Department of Microbiology and Biotechnology, University of School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

Susana Redondo-Gómez Department of Plant Biology and Ecology, Faculty of Biology, University of Seville, Seville, Spain

Ignacio D. Rodríguez-Llorente Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

Nader Rokni Department of Science, Jundi-Shapur University of Technology, Dezful, Iran

M. Victoria Salomon Grupo de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo, Chacras de Coria, Argentina

Meenu Saraf Department of Microbiology and Biotechnology, University of School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

Anagha S. Setlur Department of Biotechnology, R V College of Engineering, Bengaluru, India

Sarita Sharma Department of Microbiology and Biotechnology, University of School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

Sinosh Skariyachan Department of Microbiology, St. Pius X College, Kasaragod, Kerala, India

Daniela M. Spera Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Clement Kiing Fook Wong Department of Agriculture and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Kampar, Perak, Malaysia

Part I

Basic and Fundamentals: Microbial Consortia

Chapter 1
An Overall Insight Into the Attributes, Interactions, and Future Applications of "Microbial Consortium" for Plant Growth Promotion with Contemporary Approaches

Dinesh Kumar Maheshwari, Ankita Das, Shrivardhan Dheeman, and Piyush Pandey

Abstract Plant-associated microorganisms in the form of microbial consortia play an important role in agricultural production. The use of single strain or individual microorganism-based bioformulation has limitations. Thus, having a microbial consortium, where two or more interacting microorganisms have additive, synergistic, or mutual complementarity in nature, results in the desired effects on plants and soil. In this review, we have discussed the insights of interactions and mechanisms through which an effective microbial consortium promotes plant growth, improves nutrient utilization efficiency, enhances yield, induces tolerance to abiotic stresses, may contribute toward pest and phytopathogen management., etc. within the rhizosphere under their efficient root colonization and biofilm formation. In addition, the activity of microbial consortia has also been highlighted, mainly as a species of plant growth- and health-promoting bacteria. Furthermore, there is a huge impact of microbial consortia on the rhizosphere, which is enhanced by the concept of microbiome engineering and strain improvement. Augmentation of soil with synthetic microbial communities (SynComs), which are extended versions of traditional consortia, is recently being realized as a tool to modulate the complete rhizosphere microbiome for beneficial effects. This article is aimed to explain the wide horizon of the use of microbial consortia that facilitates the sustainable development of agriculture and its applications for human welfare.

D. K. Maheshwari
Department of Botany and Microbiology, Faculty of Life Sciences, Gurukul Kangri (Deemed to be University), Haridwar, India

A. Das · P. Pandey (✉)
Soil and Environmental Microbiology Lab, Department of Microbiology, Assam University, Silchar, India

S. Dheeman
Department of Biotechnology and Microbiology, School of Allied Health Sciences, MVN University, Palwal, Haryana, India

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_1

Keywords Microbial consortium · Formulations · PGPR · Metagenome · Microbiome engineering · SynComs

1.1 Introduction

The soil-plant ecosystem's complexity often presents challenges for the single strain bioinoculants, when applied for plant growth promotion and disease control. By combining several microorganisms in the multi-species consortium, multiple beneficial activities are also added, and thus it is assumed that at least this group of microbes, i.e., the microbial consortium, will have more functional traits; hence, they perform better than respective individual microbial isolates (Woo and Pepe 2018; Nuti and Giovannetti 2015).

A microbial consortium is a group of different species of beneficial microorganisms, mainly plant growth-promoting bacteria, that act together as a microbial community. The co-culture of two or more microbial populations interacting synergistically forms a microbial consortium. It can perform diverse functions more efficiently which is difficult (not so efficient) or even impossible to carry out by a single organism. Since the division of labor is quite common in nature, it can be easily characterized in microorganisms established due to microbe-microbe interactions (Tshikantwa et al. 2018). Microbial consortia are "microbial cell factories" representing new synthetic biology approaches (Roell et al. 2019).

The microbial consortium proved effective in plant-microbe interactions, improvement of the soil profile, and soil nutrient status, which is supportive to induce plant growth, plant development in general, and enhancement of crop productivity. The microbial consortium helps in biofertilization, bioremediation, phytostimulation, and biological control of pests and pathogens (Sharma et al. 2018). They act in the solubilization of minerals in the soil, secreting phytohormones, producing enzymes (i.e., ACC deaminase) and chemical metabolites, and contributing to the bio-removal of soil pollutants and heavy metals (Arora et al. 2010; Pandey et al. 2012; Zhang et al. 2019a, b; Santoyo et al. 2021).

The synergism of microorganisms comprises the microbial consortium offering a new scope in agro-practices toward sustainable development. This may also avoid the agricultural requirements of microbial inoculants' trade-off in individual microbial populations.

1.2 Microbe-Microbe Interactions

The interactions between/among the species or strains play a major role in the beneficial effects of bacterial consortia (Singh et al. 2019). The consortium (bearing bacteria) can be classified into three types. It is based upon the effect on each other, for example, (a) positive or stimulatory, (b) negative or inhibitory, and (c) neutral. The positive interactions comprise generating a network supporting individual members through cross-feeding wherein one bacterium utilizes the metabolic end products as nutrients for another member. Mutualism, protocooperation, and commensalism are some of the features. In mutualism, each or one of the members is benefitted in an obligatory association due to the exchange of required substances or mutual removal of toxins (Roell et al. 2019), while in the case of protocooperation, the interaction that occurs between species is beneficial to the growth rate of both populations but is not required either to persist. Similarly, commensalism is a positive one-way interaction in which one member benefits while the other remains unaffected (Dubey and Maheshwari 2022).

The suppression or inhibitory action of one another leads to the negative interactions that occur due to their growth inhibition of the structure and function. Such processes are (i) amensalism, (ii) predation, (iii) parasitism, and (iv) competition. When the growth of one of the members is affected due to the secretion of inhibitory substances (unidirectional), it is called amensalism, while predation and parasitism involved the growth of one species that depends upon the other species. Competition is mainly due to nutrition or space; therefore, the fast-growing organism dominated. In neutral interactions, members of the consortium do not influence each other. It occurs when two or more species consume different nutrients and neither produces any inhibitory compound to another consortium (Chaneton and Bonsall 2000).

1.3 Microbial Consortia

1.3.1 Definition and Design

A microbial consortium constitutes two or more compatible microorganisms of diverse/similar genera of different species in synergistic or additive interactions (Stockwell et al. 2011; Sarma et al. 2015). Long back, Higa and Parr (1994) advocated the use of effective microorganisms (EM) in the growth promotion of crops. The EM may also contain non-microbial biostimulants and stress-mediated/stress-protective nutrients. Even less-defined microbial populations originating from the fermentation of various natural substrates, farmyard manure, or composting processes are recommended as inoculants. The microbial consortia contain a network of microorganisms and represent an elegant way to identify specific microbes that have a more central position in the network, often defined as “keystone” species or “hub.” Such microorganisms generally co-occur with other taxa and likely exert a strong influence on the structure of microbial communities. The identified “hub” species may act on microbial communities and/or indirectly through (a) cascade modifications in the interconnected microbial network, (b) competition for space and nutrients, (c) alteration of the plant immunity, and (d) modification of the host physiology as identified (Kang et al. 2020). Microbial consortia consists either (i) a synthetic assembly by combining several isolated strains (Puentes-Téllez and Salles 2018) or (ii) complex microbial communities from nature (Skariyachan et al.

2017). In this scenario, the enrichment process is often used to get the desired microbial consortia.

1.3.2 Types, Process, and Development

Bashan and Prabhu (2020) highlighted the formation of advanced consortia with microbe-based products. Two types of consortia, i.e., simple and complex, are based on their differences in fermentation strategy (production of a large population of bacteria to be later formulated into an inoculant). For this, strains are grown individually or in combination including staggering into other species/strains in a suitable medium for all the plant growth-promoting (PGP) organisms. The consistency in results under field conditions is a benchmark of the success of bacterial consortia application which not only depends upon the type and function of strains but also includes their adaptation to adverse climate conditions, survival, and persistence in the soil after application (Verbruggen et al. 2013; Gosal and Kaur 2017).

1.4 Formulations: Difficulties and Success

Consortia formulation can be carried out by using selected PGP bacteria by combining a uniform bacterial cell concentration of all the participating strains. Later, after mixing, inoculant suspensions are prepared to achieve a final bacterial concentration of approximately 10^8 CFU/ml (~OD 600) as described by Gomez et al. (2021). To ensure different genera of PGP bacteria, for consortia formulations, the strain must be evaluated for some traits such as N fixation, P solubilization, siderophore production, IAA production, biofilm formation, ACC deaminase activity, etc. Thus, PGPR selected are recommended to design and construct microbial-based bioformulation for their application in a wide range of agro-ecosystem (Pandey et al. 2005, 2010). Santoyo et al. (2021) described plant growth stimulation by microbial consortia. Although many publications are appearing on plant-microbe interaction, it is significant to note that comparatively few appeared on the use of microbial consortium to perform plant growth and development enhancement to perform a variety of tasks in an ecosystem.

Consortium communication is governed by molecular signals. In this, quorum sensing plays a major role in the compatibility of bacterial communities comprising consortium formulations. Quorum sensing (QS) allows bacteria to switch between two gene expression programs: (i) at low density for individual and social behavior and (ii) at high cell density for social and group behaviors which are preferential for consortia (Ng and Bassler 2009). The QS enables bacterial cells in a formulation to function in unison, and they carry out as a collective, not allowing the desired effect of compatible consortia (Schikora et al. 2016).

A proper description of the consortium, the taxonomic affiliation of the strains and identification protocols, the process of formulation, the effect of edaphic and other related parameters, and the population of consortia formulations can be carried out by using selected PGP bacteria. The PGP strain is to be evaluated for their nature not to inhibit the growth of each other by the "cross-streaking" method of Pierson and Weller (1994). This was further confirmed by the filter paper disk method as given by Sindhu et al. (1999). The strains are further listed for their consortium-forming abilities following the spectrophotometric method of Shanmugam et al. (2002). In one of the reports from our research group, we have designed different combinations of bacteria, viz., (i) *Pseudomonas aeruginosa* KRP1 + *B. licheniformis* KRB1, (ii) *B. licheniformis* KRB1 + *Sinorhizobium meliloti* RMP1, (iii) *S. meliloti* RMP1 + *P. aeruginosa* KRP1, and (iv) KRP1 + RMP1, a multi-species bacterial consortium of all the above strains (Maheshwari et al. 2010). The healthy seeds of *Brassica campestris* (Indian mustard) were bacterized with KRP1, KRB1, or RMP1 and by consortia as given above, and the maximum enhancement of vegetative growth parameters was observed in the consortium, in comparison to those that emerged due to individual treatment with KRP1, KRB1, or RMP1. This application of bacterial consortium proved to be most desirable for plant growth and development of *B. campestris* (Maheshwari et al. 2010).

According to Nuti and Giovannetti (2015), microbial consortia are based on multiple PGP microbial strains with complementary properties. Sometimes non-microbial biostimulants and stress-protective nutrients are added to reduce the product cost. Molina-Romero et al. (2021) observed the potential of a second-generation consortium formulated with *Azospirillum brasilense* SP7, *Pseudomonas putida* KT2440, *Acinetobacte*r sp. EMM02, and *Sphingomonas* sp. OF-178A. The bacterial strains present in the consortium proved compatible and efficient for field applications and resistant to desiccation.

1.5 Root Colonization and Biofilm Formation

PGPR-plant interaction is an intricate and interdependent relationship that involved not only the microorganisms but also other abiotic and biotic factors of the rhizosphere region that also play a role in their successful partnership (Kshetri et al. 2015). Root colonization and biofilm formation by the microbial community and the underlying principles are also behind the success of these organisms to tide over unfavorable conditions as suggested (Dutta and Podile 2010). The nature of bacterial genera and their relationship with host plants are exhibited by aggressive root colonization due to adequate adhesiveness to its surface. The adhesion improved when the strains of *Azotobacter brasilence*, *Acinetobacter* spp., and *Sphingomonas* spp. were applied to *Zea mays* together in a consortium. The inoculation of the bacterial consortium also improves the root colonization capacity in comparison to that of individual treatments. De Oliveira et al. (2006) observed the root colonization of a consortium formulated with *Gluconacetobacter diazotrophicus*, *Herbaspirillum* *seropedicae*, *H. rubrisubalbicans*, *Azospirillum amazonense*, and *Burkholderia tropica*. Even the different isolates of *Burkholderia* sp. RHT8 and RHT12 led to synergism and root colonization in fenugreek's rhizosphere (Kumar et al. 2017). The combined effects of rhizo-competitive rhizosphere and non-rhizosphere *Bacillus* species enhanced the growth and yield in *Eleusine coracana* (Dheeman et al. 2020).

Root zone or "rhizosphere effect" is pronounced due to the successful establishment of bacterial consortia. This phenomenon is a crucial step to obtaining the beneficial effect of consortia on the host plant, which is further improved due to adequate adhesion and colonization (Shahzad et al. 2013). A significant difference was seen in maize when inoculated with *A. brasilense*, *P. putida*, *Acinetobacter*, and *Sphingomonas* spp. together. The plant taxa, variety, and other morphological features are also supportive of bacterial colonization formulated with *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Azotobacter amazonense*, and *Burkholderia tropica* in sugarcane (De Oliveira et al. 2006). The colonization capability of consortia of *P. striata* and *Piriformospora indica* is also dependent on corn varieties as observed by Singh et al. (2009). Earlier, Gusain and Bhandari (2019) studied the root colonization of *Sinorhizobium meliloti*, *A. chroococcum*, *Serratia marcescens*, and *P. aeruginosa* in different combinations of consortia which showed quite effective colonization in comparison to their counterparts. Santoyo et al. (2021) described plant growth stimulation and root colonization by microbial consortia.

The bacterial biofilm formation occurs quite commonly on the root surface and represents a hotspot for microbial interactions assisting them to form a consortium. It plays a significant role in the ecological network for shaping microbial communities for playing their role in sustainable agrobiological practices. The desired role of microbe-microbe interaction or mixed consortium involved in stimulating ecosystem functioning as well as in the enhancement of plant productivity (Pandit et al. 2020).

Currently, bioinformatics tools have been devised and used to investigate inter-microbial co-occurrence networks from community profiling or metagenomic data (Faust and Raes 2012); Layeghifard et al. 2017) study of the microbial networks. Plant interaction tends to indicate that positive correlation dominates among microbes from the same kingdom, whereas negative interaction primarily occurs through inter-kingdom microbe-microbe interaction (Agler et al. 2016). Thus, the role of microbial consortia is complex, and a more holistic understanding of microbial networks for holobiont fitness, is required (Hassani et al. 2018).

Aggressive bacterial genera in the root rhizosphere must have adequate adhesion and root colonization. Molina-Romero et al. (2021) highlighted that the adhesion improved when strains of *A. brasilense*, *Acinetobacter* sp., and *Sphingomonas* sp. were applied to maize together in a consortium. The inoculation of the bacterial consortium improved the bacterial colonization capacity in comparison to that of individual treatments. In another study, De Oliveira et al. (2006) observed the colonization of a consortium formulated with *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Azospirillum amazonense*, and *Burkholderia tropica*.

In addition, the bacterial consortium offered an alternative allowing the efficient use of half of the recommended dose of nitrogen fertilizer. The use of the consortium allowed the lowering of a 50% mineral N application and generated beneficial agronomic practices along with the lower cost to the cultivars (Molina-Romero et al. 2021). Recently, a new approach is devised wherein the effect of microbial consortia is applied as fertilizer coating. For this, the use of illumine high-throughput sequencing (HTS) is involved to influence the bulk soil and rhizosphere microbial community applied to potato fields (Overbeek et al. 2021). On the other hand, bacterial consortium acts as a substitute to chemical fertilizers such as urea, DAP, etc. because decreased application of chemical fertilization along with bacterial consortium exhibited a similar effect on plant growth and yield as revealed while applying the recommended doses of chemical fertilizers (Kumar et al. 2010; Da Costa et al. 2013).

Other characteristics such as strain evaluation to salinity stress under drought resistance cannot be ruled out, to stimulate crop growth and improve tolerance to abiotic stresses, and prove more effective in extreme climate change conditions. Microbial inoculants may improve salt tolerance by altering hormonal root-shoot signaling that manages IAA production in plants by bacterial action, thus having the potential in enhancing salt tolerance (Etesami and Maheshwari 2018). Such an approach is beneficial for a realistic assessment of the potential of microbial consortia in a climate change world.

1.6 Abiotic Stress: Action and Mechanism

The application of microbial consortia can reduce the negative effects that arise due to abiotic stress conditions on crops. But for their effective application in the crops, novel approaches are required to explore bacteria-bacteria and plant-bacteria interactions or bacteria-fungi interactions. Isolating and identifying the stress-tolerant or stress-resistant microbes to recalcitrant agrochemicals and heavy metals is important (Xia et al. 2020; Katiyar et al. 2021).

Abiotic stresses inhibit plant growth and development due to oxidative damage attacking DNA and cellular membranes. The antioxidant enzymes neutralize the reactive molecules; thus, cells are protected. PGPB having catalase and peroxidase properties are proven more protective. The beneficial bacteria also produce trehalose which also benefits the plants to abiotic stress (Glick 2015; Kumar and Verma 2018). Microbial production of phytohormones also protects plants by the involvement of various physiological actions. PGPB induces the level of proline in plants. Proline scavenges reactive oxygen molecules and acts to stabilize proteins through molecular chaperons in stress conditions (Meena et al. 2019). The effect of the consortium of *Bacillus cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21 was reported to develop healthy cucumber plants, with much darker green leaves containing increased proline and chlorophyll contents, and induce superoxide dismutase activity (Wang et al. 2012). An increase in ethylene level is injurious to plants causing senescence and other deleterious effects which occur due to the accumulation of a consortium of ACC deaminase-producing bacteria (*Ochrobactrum pseudogrignonense*, *Pseudomonas* sp., and *B. subtilis*) that significantly increased early vegetative growth plant parameters in *Vigna mungo* and *P. sativum*.

As human populations continue to increase, the disturbance of the soil ecosystem to enhance productivity may place greater demand on supplying soil essential nutrients. Therefore, it is essential to increase the understanding of the biological, physical, and chemical properties of soil along with the soil-microbe-plant relationship to enhance productivity with available nutrient pools (Millard and Singh 2010). The soil native ability to supply sufficient nutrients continuously decreases and emerges as a greater challenge for enhancing the productivity of crops and the quality of water, air, and fragile soil ecosystems. The relationship of soil-plant-microbes especially soil interaction influences plant compounds accurately, identifies the yield-limiting potential factors and growth and development, and minimizes the influence of those to manage the enhancement productivity (Metcalfe et al. 2011). Most of the research during the last decades was focused on the use of fertilizers and manures. Thus, information on the integrated approach of plant nutrition on the sustainability of soil fertility and crop productivity is necessary.

Soil fertility is the status or the inherent capacity of the soil to supply nutrients to plants in adequate amounts in suitable proportions. On the other hand, soil productivity is the capacity of the soil to produce crops with a specific system of management and is expressed in terms of yields (Van Ittersum et al. 2013). All productive soils are fertile, but all fertile soils are not necessarily productive. To produce crops of economic value and to maintain the health of the soil without deterioration is most important. Modern farming, driven by economic constraints, is forced to use artificial fertilizers, often to the detriment of the soil's natural fertility (Rana and Rana 2011).

1.7 Metagenomics and Biotechnological Approach to Increase Efficiency of Microbial Consortium for Plant Growth Promotion

When compared to single microorganisms, consortia are superior throughout many situations. The selection of consortium members in a way that maximizes performance is a significant obstacle. Microbial consortia have the advantage of being more adaptive to environmental changes due to their high stability, resilience, and multifunction. Human health, bioremediation and biodegradation, chemical and bioenergy generation, and food manufacturing are just a few of the areas where microbial consortia are playing crucial roles in the developing sector (Lee et al. 2013). Recent breakthroughs in synthetic biology have significantly enhanced both the synthesis of microbial consortia and the comprehension of microbial communication mechanisms (Song et al. 2014). Cell-cell interactions in relatively small synthetic microbial consortia have recently been studied. Synthetic microbial consortia are typically less complex and easier to genetically modify than real microbial consortia, making the interaction and control processes easier to explore (Sanchez-Gorostiaga et al. 2019).

1.7.1 Microbiome Engineering

Microorganisms found on or within a plant have been shown to have beneficial effects, such as promoting growth or inhibiting pathogens (Ab Rahman et al. 2018). Altering the microbiome with plant growth-promoting rhizobacteria (PGPR) can improve plant development and reduce infections and abiotic stress (Kumar et al. 2018). Microbiome engineering can enhance agricultural yields and resilience by manipulating the plant holobiome. The plant's genotype is also very important for the formation and function of rhizospheric microbiomes and for getting the most out of PGPR (Arif et al. 2020). Beneficial interactions between plants and microbes have been studied to learn how to change plant genomes to attract and keep beneficial microbiomes. Different plant genotypes attract helpful and disease-suppressing microorganisms to varying degrees, reorganizing the microbiome assembly (Gao et al. 2021). The endophytic microbiome of plants also influences functional genes related to plant growth promotion (Singha et al. 2021).

The study of plant functional genomics during mutually beneficial plant-microbe interactions has allowed the manipulation of plant genomes to entice and sustain such microbiomes (Rosier et al. 2018; Vandana et al. 2021). This led to the idea of "designer plants." These genetically modified plants can release hormones or other substances that attract and keep good microbiomes (Stringlis et al. 2018). The targeted crop's yield can be dramatically increased through the application of a consortium that is compatible with the chosen plant and able to repair the rhizospheric microbiome (Tabacchioni et al. 2021). Several studies have also pointed out that wild-type relatives of domesticated crops can help us learn more about the role of genes in wild plants that are linked to microbiome assembly (Pérez-Jaramillo et al. 2018). The microbiome is often commonly referred to as the brain of a given environment because of the significant impact it has on the general health and well-being of that environment (Lavazza and Sironi 2019). Inoculated groups of microorganisms can rebuild the structure and function of the microbiome in plants and soil. Microbes create functional consortia in the rhizosphere; soil conditioning and important microbial strains can modify the rhizosphere microbiome's structure (Voges 2019). It is feasible to create artificial consortia with several functions for promoting plant development. This could fix some of the problems with traditional microbial biofertilizers, like not getting along with the host, not being able to compete well with native microbes, and not being able to adapt to the local environment (Hart et al. 2018). The development of the optimum artificial microbial consortium involves studying the microbes' origin, getting and cultivating the microorganisms, optimizing microbial interactions as per compatibility, and finally investigating the consortia's performance. Microbiome breeding is another technique by which the microbiome can be altered for betterment. It requires allowing the host to filter which populations of bacteria are permitted to interact with it and will be passed straight to their progeny, thus indirectly affecting the microbiome (Mueller and Linksvayer 2022). This strategy involves spreading a microbiome-influenced phenotype of the host. For example, to study the microbial influence on the flowering pattern of *Arabidopsis thaliana*, the early and late flowering microcosms are studied over generations, and it was found that more phenotypic inflorescence was observed in the plants inoculated with microbiome from late flowering plants. The repeatability of flowering phenotypes shows that microbiomes can be regulated to influence plant characteristics and coordinate soil resource pools (Panke-Buisse et al. 2015, 2017). In the same study, an increase in total biomass and increased enzyme activity for the mineralization of nitrogen were observed in *Brassica rapa* when inoculated with the same (Panke-Buisse et al. 2015). Likewise, microbiome transformation is another technique where the beneficial microbiota from one species was inoculated in other species to promote plant growth (Arif et al. 2020). For example, *Leptospermum scoparium* is reported to release antibacterial agents to counter the growth of *Pseudomonas* pathogens. A similar biocontrol activity was observed in the kiwi plant when PGP bacterial microbiome from this species was inoculated in it (Wicaksono et al. 2018).

1.7.2 Molecular Tools to Increase Efficiency of Microbiome Engineering

Understanding the physiological and biochemical functionality of the consortium can be greatly aided by genetic engineering or the use of molecular tools in the microorganism involved with plant growth promotion. The extensive collection of genes that are engaged in the processes will be taken into consideration as potential targets to achieve an accurate comprehension of the function that each gene is carrying out (Kumar et al. 2020). The discovery of RNA interference (RNAi) and CRISPR is the most recent and commonly used biotechnological development in genetic tools in this regard (Boettcher and McManus 2015; Schultenkämper et al. 2020). RNAi relies on an endogenous process that regulates gene expression with short RNAs. Synthetic tiny RNAs (siRNAs or short hairpin RNAs) can be used to seize the indigenous RNAi mechanism. Either way, the inserted RNA is put into the RNA-induced silencing complex (RISC), which promotes target mRNA destruction (Carthew and Sontheimer 2009; Mohr et al. 2014). Reduced amounts of the target protein are the result achieved post-translationally by targeting the expression of the corresponding mRNA (Boettcher and McManus 2015).

CRISPR is a revolutionary way to change the genes of plants to improve specific traits, and thus it has become one of the most useful tools in the field of functional genomics (Pérez-Jaramillo et al. 2018). One important use of CRISPR-based genetic

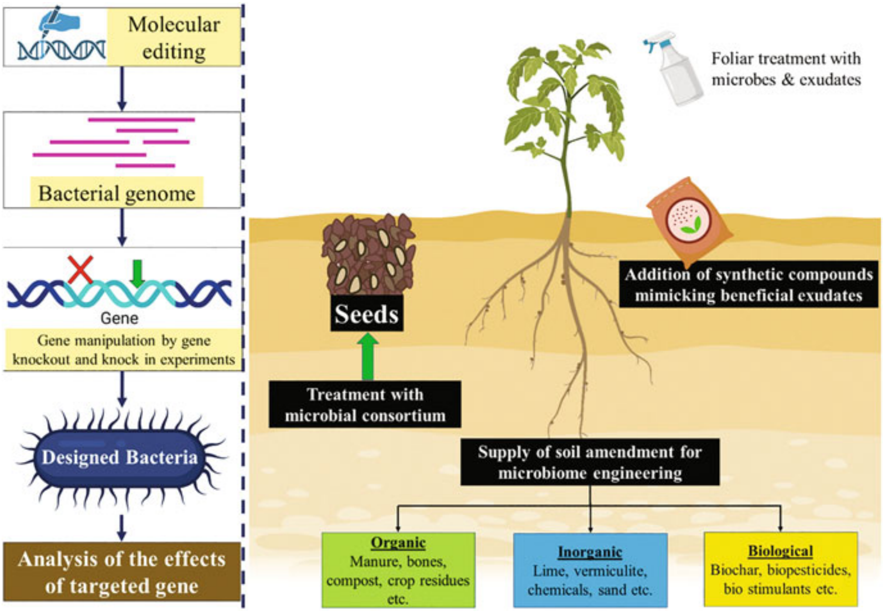


Fig. 1.1 Different approaches for employing microbiome engineering through the application of consortium

engineering tools is to alter the genes of plants or microbes to study how the genes work. One of the best things about the CRISPR tool is that it can completely shut down the target gene. To do this, designer plants could be genetically engineered using the CRISPR tool to make and release mass hormones or exudates that attract and keep beneficial microbial populations in the rhizosphere microbiome ecosystem (Bisht et al. 2019) (Fig. 1.1).

1.7.3 Next-Generation Microbial Synthetic Communities (SynComs) for Plant Yield Promotion

From basic natural or synthetic consortia to a more complex applied consortium of microbes, omics-based techniques can paint a comprehensive picture of a consortium's operation. Based on single omics approaches, metabolomics, metagenomics, transcriptomics, and metaproteomics have been developed to make it easier to study groups of microbes (Chandran et al. 2020). Quantifying the meta-proteome in a group of microorganisms is important for understanding how different protein functions work together and how they change over time which makes it the best of the different meta-omics approaches at showing how a microbial consortium's system works (Franzosa et al. 2015). Rapid developments in mass spectrometry have led to the creation of many quantification strategies. Among these techniques, the isobaric leveling method and isobaric tags for relative and absolute quantitation (iTRAQ) are widely utilized for comparative proteomic research because of their high sensitivity and accuracy. The metabolomic analysis of the consortium dedicated to any process gives a prediction of how the combined effects the production of intermediate metabolites so that the best can be grouped to obtain the best results (Ma et al. 2019).

SynComs are microbial consortia designed to imitate the natural microbiome. The goal is to minimize the sophistication of the microbiome while keeping some of the natural interactions between bacteria and hosts, offering a spectrum of capabilities unattainable by a single bacterium. Additionally, synergistic interactions between members of SynComs may improve community stability (Kaminsky et al. 2019; McCarty and Ledesma-Amaro 2019). To unlock the potential of soil microbes and boost agricultural yields, microbial synthetic communities (SynComs) have been proposed as a useful technique that incorporates both microbial ecology and genetics in the construction of inoculants. The goal of this strategy is to identify and then recruit a group of microbes that can stimulate plant development in a variety of climates and the face of harsh events (York 2018). In recent times, the focus has been given to the development of microbially based goods due to the worldwide potential of these SynComs to boost agricultural production and sustainability (Singh et al. 2020).

Computational approaches, such as machine learning algorithms, will improve the screening and identification of beneficial bacteria, as well as the process of establishing the optimal microbe combination for a particular plant phenotype (Harfouche et al. 2019). The growing number of reference genomes and metagenomes in public databases helps to find bacteria with desirable features, and by using these genomic information and gene expression patterns, one can choose microorganisms with plant-beneficial functional features or metabolic capabilities (Vorholt et al. 2017). Genome surveys for several gene markers will be critical to finding relevant microorganisms because important properties like colonization efficiency and frequency of other attributes are likely to relate to multiple genes, and to solve this problem, genomics-based datasets filter microbiological candidates on a genomic markers' basis (Finkel et al. 2017). Thus, genome and metagenome sequencing, together with microbial characterization, could assist in building SynComs that bestow stable plant phenotypes and increase plant colonization and permanence (De Souza et al. 2020). A systematic flow to develop a successful SynCom is presented in Fig. 1.2.

In another way, SynComs help in understanding the physiology and function of microorganisms and the parameters regulating community assembly by manipulating a SynCom formulation by adding, removing, or replacing microorganisms (Vorholt et al. 2017). For example, removing a single strain of *Enterobacter cloacae* reduced the activity of microbial consortium which was related to reducing the severity of maize blight disease (Niu et al. 2017). Similarly, a SynCom with more microorganisms from the *indica* strain had a bigger effect on rice growth than a SynCom with more microorganisms from the *japonica* strain (Zhang et al. 2019a, b).

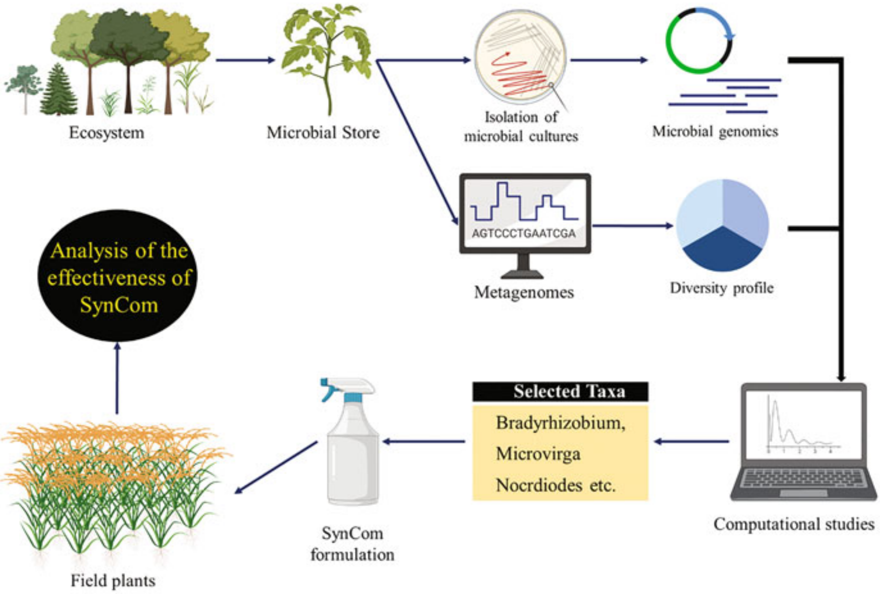


Fig. 1.2 A blueprint for the customization of stable and efficient synthetic microbial communities (SynComs), with the goal of increasing the resistance of crops to environmental challenges and yield production

Few obstacles or problems exist with the employment of SynComs. Most microbial species are likely to be uncultivable, making it difficult to assemble in a microbial consortium. Further, the cost of sequencing hundreds of thousands of samples is extremely expensive (Lewis et al. 2021). In addition, soil microbiomes are complicated, and the relationships between soil single taxa and environmental factors are inadequately documented, limiting our understanding of microbial candidates that might be employed to increase plant growth and productivity in the wild (Jayaraj et al. 2016). Therefore, most of the recently developed SynComs are comprised of bacteria only and a group of culturable microbes equipped with good plant growth-promoting dexterities.

1.8 Application: Microbial Inoculation and Soil Community

Microbial inoculation directly impacts the soil microbial community to increase the relative abundance of inoculated microbial genera. The rhizospheric microbial community composition differed substantially from the bulk soil microbial community composition (Overbeek et al. 2021). For example, in the case of potato roots, enrichment of the rhizosphere community over bulk soil was observed for

Proteobacteria and Eurotiomycetes. A similar difference in the microbial community was also observed by several workers (Berendsen et al. 2012; Xue et al. 2018). The external input of microorganisms closely associated with the rhizosphere contributed as core microorganisms and the alteration in the rhizospheric microbiome help in designing microbial inoculants beneficial to the plants growing under a variety of soil conditions (Sathya et al. 2017).

The biological management for the growth and development of plants is still at an early stage of development, while the approach appears to have tremendous potential, and many of the basic concepts necessary for the implementation are in place, and apparent obstacles such as information on biomass, formation of a product, site of application, and registration difficulties exist (Kumar et al. 2017). For increasing crop productivity and the maintenance and improvement of soil fertility for sustainable crop production, the multifunctional formulation may be promoted that involves microbial consortium utilizing the PGPR, which has been proven better and eco-friendly in comparison to that of formulation alone (Kshetri et al. 2017).

The microbial consortia are also used to control and optimize various industrial processes. Puentes-Téllez and Salles (2018) described the construction of effective minimal active microbial consortia for lignocellulose degradation. The simplification of the microbial community makes it easier to help and understand the individual roles of the strains in the consortia.

Skariyachan et al. (2018) worked on polymer degradation by novel thermophilic consortia of *Brevibacillus* spp. and *Aneurinibacillus* sp. associated with waste management landfills and sewage treatment plants. Earlier, the authors formulated bacterial consortia from plastic-contaminated cow dung. It is interesting to note that Subhashchandrabose et al. (2011) studied the biotechnology potential of consortia of cyanobacteria/microalgae and bacteria.

The PGP strain was evaluated for their nature to inhibit the growth of each other by the “cross-streaking” method of Pierson and Weller (1994). This was further confirmed by the filter paper disk method as given by Sindhu et al. (1999). The strains are further listed for their consortium-forming abilities following the spectrophotometric method of Shanmugam et al. (2002). Recently, Baliyan et al. (2022) reviewed the bacteriophage cocktails and antibacterial agents in crop protection.

1.9 Conclusions

The development of artificial consortiums developed with multifarious characteristics is a growing interest in using similar or diverse genera of beneficial bacteria in agriculture applications. The microbial consortia offer consistency and higher reproducibility of data under various environmental conditions and provide a broader array of the mechanism of action in comparison to that of individual beneficial bacteria applied alone for friendly crop production and protection system in agriculture. However, some critical challenges are yet to be resolved. Certain issues with the registration and marketing of formulations comprising mixed cultures limit their potential use in modern agriculture. The difficulties in understanding the specific role of each component of microbial consortium and their desirable effects may limit the predicted effect on the growth and development of crops. The molecular tools and biotechnological approach involving plant-microbe interaction, soil-microbe engineering, metagenomic soil profile, and next-generation synthetic microbial consortia are some of the most useful tools to make it easier to study microbe involved in the design and construction of microbial consortium systems. Further, bioinformatics and computational tools may improve the understanding of function of microbial consortia and their products for sustainable agriculture.

Acknowledgments PP acknowledges DBT, Govt of India; and AD acknowledges DST-INSPIRE, Govt of India for financial assistances.

References

Ab Rahman SFS, Singh E, Pieterse CM, Schenk PM (2018) Emerging microbial biocontrol strategies for plant pathogens. Plant Sci 267:102–111

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Wiegel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol 14(1):1–9

Arif I, Batool M, Schenk PM (2020) Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trend Biotechnol 38(12):1385–1396

Arora NK, Khare E, Maheshwari DK (2010) Plant growth promoting rhizobacteria: constraints in bioformulation, commercialization, and future strategies. In Plant growth and health promoting bacteria, Springer, Berlin, Heidelberg, pp 97–116

Baliyan N, Dhiman S, Dheeman S, Vishnoi VK, Kumar S, Maheshwari DK (2022) Bacteriophage cocktails as antibacterial agents in crop protection. Environ Sustain:1–7

Bashan Y, Prabhu SR, de-Bashan LE, Kloepper JW (2020) Disclosure of exact protocols of fermentation, identity of microorganisms within consortia, formation of advanced consortia with microbe-based products. Biol Fertil Soils 56:443-445

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trend Plant Sci 17:478–486

Bisht DS, Bhatia V, Bhattacharya R (2019) Improving plant-resistance to insect-pests and pathogens: the new opportunities through targeted genome editing. Semin Cell Dev Biol 99:65–78

Boettcher M, McManus MT (2015) Choosing the right tool for the job: RNAi, TALEN, or CRISPR. Mol Cell 58(4):575–585

Carthew RW, Sontheimer EJ (2009) Origins and mechanisms of miRNAs and siRNAs. Cell 136(4): 642–655

Chandran H, Meena M, Sharma K (2020) Microbial biodiversity and bioremediation assessment through omics approaches. Front Environ Chem 1(570326):1–22

Chaneton EJ, Bonsall MB (2000) Enemy-mediated apparent competition: empirical patterns and the evidence. Oikos 88(2):380–394

Da Costa PB, Beneduzi A, De Souza R, Schoenfeld R, Vargas LK, Passaglia LMP (2013) The effects of different fertilization conditions on bacterial plant growth promoting traits: guidelines for directed bacterial prospection and testing. Plant Soil 368:267–280

De Oliveira ALM, De Canuto EL, Urquiaga S, Reis VM, Baldani JI (2006) Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. Plant Soil 284:23–32

De Souza RSC, Armanhi JSL, Arruda P (2020) From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. Front Plant Sci 11(1179):1–7

Dheeman S, Baliyan N, Dubey RC, Maheshwari DK, Kumar S, Chen L (2020) Combined effects of rhizo-competitive rhizosphere and non-rhizosphere *Bacillus* in plant growth promotion and yield improvement of *Eleusine coracana* (ragi). Can J Microbiol 66(2):111–124

Dubey RC, Maheshwari DK (2022) Textbook of microbiology. S. Chand and Company Limited, New Delhi

Dutta S, Podile AR (2010) Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. Critic Rev Microbial 36(3):232–244

Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Safe 156:225–246

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10: 538–550

Finkel OM, Castrillo G, Paredes SH, González IS, Dangl JL (2017) Understanding and exploiting plant beneficial microbes. Curr Opin Plant Biol 38:155–163

Franzosa EA, Hsu T, Sirota-Madi A, Shafquat A, Abu-Ali G, Morgan XC, Huttenhower C (2015) Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nat Rev Microbiol 13(6):360–372

Gao M, Xiong C, Gao C, Tsui CK, Wang MM, Zhou X, Cai L (2021) Disease-induced changes in plant microbiome assembly and functional adaptation. Microbiome 9(1):1–18

Glick BR (2015) Stress control and ACC deaminase. In: Principles of plant-microbe interactions. Springer, Cham, pp 257–264

Gomez JA, Höffner K, Barton PI (2021) Production of biofuels from sunlight and lignocellulosic sugars using microbial consortia. Chem Eng Sci 239(116615):1–14

Gosal SK, Kaur J (2017) Microbial inoculants: a novel approach for better plant microbiome interactions. In: Probiotics in agroecosystem. Springer, Singapore, pp 269–289

Gusain P, Bhandari BS (2019) Rhizosphere associated PGPR functioning. J Pharmacog Phytochem 8(5):1181–1191

Harfouche AL, Jacobson DA, Kainer D, Romero JC, Harfouche AH, Mugnozza GS, Altman A (2019) Accelerating climate resilient plant breeding by applying next-generation artificial intelligence. Trend Biotechnol 37(11):1217–1235

Hart MM, Antunes PM, Chaudhary VB, Abbott LK (2018) Fungal inoculants in the field: is the reward greater than the risk? Funct Ecol 32(1):126–135

Hassani MA, Durán P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6(1):1–17

Higa T, Parr JF (1994) Beneficial and effective microorganisms for a sustainable agriculture and environment, vol 1. International Nature Farming Research Center, Atami, pp 1–16

Jayaraj R, Megha P, Sreedev PJIT (2016) Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdiscip Toxicol 9(3-4):90

Kaminsky LM, Trexler RV, Malik RJ, Hockett KL, Bell TH (2019) The inherent conflicts in developing soil microbial inoculants. Trends Biotechnol 37(2):140–151

Kang D, Samuel J, Herschend J, Wei S, Nesme J, Sorensen SJ (2020) Construction of simplified microbial consortia to degrade recalcitrant materials based on enrichment and dilution-to-extinction cultures. Front Microbiol 10(3010):1–10

Katiyar P, Dubey RC, Maheshwari DK (2021) ACC deaminase-producing Ensifer adhaerens KS23 enhances proximate nutrient of *Pisum sativum* L. cultivated in high altitude. Arch Microbiol 203(5):2689–2698

Kshetri L, Nevita T, Pandey P (2015) Plant growth promoting rhizobacteria (PGPR) and their application for sustainable agriculture in north eastern region of India. ENVIS Bull Himal Ecol 23:41–47

Kshetri L, Pandey P, Sharma GD (2017) Solubilization of inorganic rock phosphate by rhizobacteria of *Allium hookeri* Thwaites and influence of carbon and nitrogen sources amendments. J Pure Appl Microbiol 11:1899–1908

Kumar A, Singh S, Gaurav AK, Srivastava S, Verma JP (2020) Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front Microbiol 11(1216):1–41

Kumar A, Singh VK, Tripathi V, Singh PP, Singh AK (2018) Plant growth-promoting rhizobacteria (PGPR): perspective in agriculture under biotic and abiotic stress. In Crop improvement through microbial biotechnology, pp. 333–342

Kumar A, Verma JP (2018) Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52

Kumar H, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L) var. Manak by bacterial combinations amended with chemical fertilizer. Crop Prot 29(6):591–598

Kumar H, Dubey RC, Maheshwari DK (2017) Seed-coating fenugreek with *Burkholderia* rhizobacteria enhances yield in field trials and can combat Fusarium wilt. Rhizosphere 3:92–99

Lavazza A, Sironi VA (2019) Are we ready for a "microbiome-guided behaviour" approach? Camb Q Healthc Ethics 28(4):708–724

Layeghifard M, Hwang DM, Guttman DS (2017) Disentangling interactions in the microbiome: a network perspective. Trends Microbiol 25:217–228

Lee DJ, Show KY, Wang A (2013) Unconventional approaches to isolation and enrichment of functional microbial consortium–a review. Bioresour Technol 136:697–706

Lewis WH, Tahon G, Geesink P, Sousa DZ, Ettema TJ (2021) Innovations to culturing the uncultured microbial majority. Nat Rev Microbiol 19(4):225–240

Ma Q, Bi YH, Wang EX, Zhai BB, Dong XT, Qiao B, Yuan YJ (2019) Integrated proteomic and metabolomic analysis of a reconstructed three-species microbial consortium for one-step fermentation of 2-keto-L-gulonic acid, the precursor of vitamin C. J Ind Microbiol Biotechnol 46(1):21–31

Maheshwari DK, Kumar S, Kumar B, Pandey P (2010) Co-inoculation of urea and DAP tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as integrated approach for growth enhancement of *Brassica juncea*. Indian J Microbiol 50(4):425–431

McCarty NS, Ledesma-Amaro R (2019) Synthetic biology tools to engineer microbial communities for biotechnology. Trends Biotechnol 37(2):181–197

Meena M, Divyanshu K, Kumar S, Swapnil P, Zehra A, Shukla V, Upadhyay RS (2019) Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. Heliyon 5(12):1–20

Metcalfe DB, Fisher RA, Wardle DA (2011) Plant communities as drivers of soil respiration: pathways, mechanisms, and significance for global change. Biogeosciences 8(8):2047–2061

Millard P, Singh BK (2010) Does grassland vegetation drive soil microbial diversity? Nutr Cycl Agroecosyst 88(2):147–158

Mohr SE, Smith JA, Shamu CE, Neumüller RA, Perrimon N (2014) RNAi screening comes of age: improved techniques and complementary approaches. Nat Rev Mol Cell Biol 15(9):591–600

Molina-Romero D, Juárez-Sánchez S, Venegas B, Ortíz-González CS, Baez A, Morales-García YE, Muñoz-Rojas J (2021) A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. Front Sustain Food Syst 4(616757):1–14

Mueller UG, Linksvayer TA (2022) Microbiome breeding: conceptual and practical issues. Trends Microbiol 30(10):997–1011

Ng WL, Bassler BL (2009) Bacterial quorum-sensing network architectures. Annu Rev Genet 43: 197–222

Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. Proc Natl Acad Sci 114(12):2450–2459

Nuti M, Giovannetti G (2015) Borderline products between bio-fertilizers/bio-effectors and plant protectants: the role of microbial consortia. J Agric Sci Technol 5:305–315

Overbeek W, Jeanne T, Hogue R, Smith DL (2021) Effects of microbial consortia, applied as fertilizer coating, on soil and rhizosphere microbial communities and potato yield. Front Agron 3(717400):1–13

Pandey P, Aeron A, Maheshwari DK (2010) Sustainable approaches for biological control of *fusarium* wilt in pigeon pea (*Cajanus cajan* L. Millspaugh). Plant Growth Health Promoting Bacteria 18:231–249

Pandey P, Bisht S, Sood A, Aeron A, Sharma GD, Maheshwari DK (2012) Consortium of plant-growth-promoting bacteria: future perspective in agriculture. Bacteria Agrobiol 9:185–200

Pandey P, Kang SC, Gupta CP, Maheshwari DK (2005) Rhizosphere competent *Pseudomonas aeruginosa* GRC1 produces characteristic siderophore and enhances growth of Indian mustard (*Brassica campestris*). Curr Microbiol 51(5):303–309

Pandit A, Adholeya A, Cahill D, Brau L, Kochar M (2020) Microbial biofilms in nature: unlocking their potential for agricultural applications. J Appl Microbiol 129(2):199–211

Panke-Buisse K, Lee S, Kao-Kniffin J (2017) Cultivated sub-populations of soil microbiomes retain early flowering plant trait. Microb Ecol 73(2):394–403

Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. ISME J 9(4):980–989

Pérez-Jaramillo JE, Carrión VJ, de Hollander M, Raaijmakers JM (2018) The wild side of plant microbiomes. Microbiome 6(1):1–6

Pierson EA, Weller DM (1994) To suppress take-all and improve the growth of wheat. Phytopathology 84(1):940–947

Puentes-Téllez PE, Salles JE (2018) Construction of effective minimal active microbial consortia for lignocellulose degradation. Microb Ecol 76:419–429

Rana SS, Rana MC (2011) Cropping system. Department of Agronomy, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, p 80

Roell GW, Zha J, Carr RR, Koffas MA, Fong SS, Tang YJ (2019) Engineering microbial consortia by division of labor. Microb Cell Factories 18:1–11

Rosier A, Medeiros FH, Bais HP (2018) Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. Plant Soil 428(1):35–55

Sanchez-Gorostiaga A, Bajić D, Osborne ML, Poyatos JF, Sanchez A (2019) High-order interactions distort the functional landscape of microbial consortia. PLoS Biol 17(12):1–34

Santoyo G, Guzmán-Guzmán P, Parra-Cota F, Santos-Villalobos SDL, Orozco-Mosqueda MDC, Glick BR (2021) Plant growth stimulation by microbial consortia. Agronomy 11(219):1–24

Sarma BK, Yadav SK, Singh S, Singh HB (2015) Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. Soil Biol Biochem 87:25–33

Sathya A, Vijayabharathi R, Gopalakrishnan S (2017) Plant growth promoting *Actinobacteria*: a new strategy for enhancing sustainable production and protection of grain legumes. 3Biotech 7: 102–102

Schikora A, Schenk ST, Hartmann A (2016) Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the N-acyl homoserine lactone group. Plant Mol Biol 90: 605–612

Schultenkämper K, Brito LF, Wendisch VF (2020) Impact of CRISPR interference on strain development in biotechnology. Biotechnol Appl Biochem 67(1):7–21

Shahzad SM, Arif MS, Riaz M, Iqbal Z, Ashraf M (2013) PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (*Zea mays* L.) under fertilized conditions. Eur J Soil Biol 57:27–34

Shanmugam V, Senthil N, Raguchander T, Ramanathan A, Samiyappan R (2002) Interaction of Pseudomonas fluorescens with Rhizobium for their effect on the management of peanut root rot. Phytoparasitica 30:169–176

Sharma CK, Bishnoi VK, Dubey RC, Maheshwari DK (2018) A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen *Macrophomina phaseolina* in mung bean. Rhizosphere 5:71–75

Sindhu SS, Gupta SK, Dadarwal KR (1999) Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of growth of green gram (*Vigna radiata*). Biol Fertil Soils 29(1): 62–68

Singh BK, Trivedi P, Egidi E, Macdonald CA, Delgado-Baquerizo M (2020) Crop microbiome and sustainable agriculture. Nat Rev Microbiol 18(11):601–602

Singh G, Singh N, Marwaha TS (2009) Crop genotype and a novel symbiotic fungus influences the root endophytic colonization potential of plant growth promoting rhizobacteria. Physiol Mol Biol Plants 15(1):87–92

Singh R, Ryu J, Kim SW (2019) Microbial consortia including methanotrophs: some benefits of living together. J Microbiol 57:939–952

Singha KM, Singh B, Pandey P (2021) Host specific endophytic microbiome diversity and associated functions in three varieties of scented black rice are dependent on growth stage. Sci Rep 11(1):1–17

Skariyachan S, Patil AA, Shankar A, Manjunath M, Bachappanavar N, Kiran S (2018) Enhanced polymer degradation of polyethylene and polypropylene by novel thermophilic consortia of *Brevibacillus* sps. and *Aneurinibacillus* sp. screened from waste management landfills and sewage treatment plants. Polym Degrad Stab 149:52–68

Skariyachan S, Setlur AS, Naik SY, Naik AA, Usharani M, Vasist KS (2017) Enhanced biodegradation of low and high-density polyethylene by novel bacterial consortia formulated from plastic-contaminated cow dung under thermophilic conditions. Environ Sci Pollut Res 24:8443–8457

Song H, Ding MZ, Jia XQ, Ma Q, Yuan YJ (2014) Synthetic microbial consortia: from systematic analysis to construction and applications. Chem Soc Rev 43(20):6954–6981

Stockwell VO, Johnson KB, Sugar D, Loper JE (2011) Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. Phytopathology 101:113–112

Stringlis IA, Zhang H, Pieterse CM, Bolton MD, de Jonge R (2018) Microbial small molecules–weapons of plant subversion. Nat Prod Rep 35(5):410–433

Subhashchandrabose SR, Ramakrishan B, Megharaj M, Venkatesawrlu K, Naidu R (2011) Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. Biotechnol Adv 29(6):896–907

Tabacchioni S, Passato S, Ambrosino P, Huang L, Caldara M, Cantale C, Schlüter A (2021) Identification of beneficial microbial consortia and bioactive compounds with potential as plant biostimulants for a sustainable agriculture. Microorganisms 9(2):426

Tshikantwa TS, Ullah MW, He F, Yang G (2018) Current trends and potential applications of microbial interactions for human welfare. Front Microbiol 9:1156

Van Ittersum MK, Cassman KG, Grassini P, Wolf J, Tittonell P, Hochman Z (2013) Yield gap analysis with local to global relevance—a review. Field Crops Res 1(143):4–17

Vandana UK, Rajkumari J, Singha LP, Satish L, Alavilli H, Sudheer PD, Pandey P (2021) The endophytic microbiome as a hotspot of synergistic interactions, with prospects of plant growth promotion. Biology 10(2):101

Verbruggen E, van der Heijden MGA, Rillig MC, Kiers ET (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197:1104–1109

Voges M (2019) Molecular principles to engineer plant microbiomes. Stanford University, Stanford, CA, pp 1–24

Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing causality: opportunities of synthetic communities for plant microbiome research. Cell Host Microbe 22(2):142–155

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7(12):1–10

Wicaksono WA, Jones EE, Casonato S, Monk J, Ridgway HJ (2018) Biological control of *Pseudomonas* syringae pv. actinidiae (Psa), the causal agent of bacterial canker of kiwifruit, using endophytic bacteria recovered from a medicinal plant. Biol Control 116:103–112

Woo SL, Pepe O (2018) Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. Front Plant Sci 9(1801):1–13

Xia Y, Farooq MA, Javed MT, Kamran MA, Mukhtar T, Ali J, Chaudhary HJ (2020) Multi-stress tolerant PGPR *Bacillus xiamenensis* PM14 activating sugarcane (*Saccharum officinarum* L.) red rot disease resistance. Plant Physiol Biochem 151:640–649

Xue D, Christenson R, Genger R, Gevens A, Lankau RA (2018) Soil microbial communities reflect both inherent soil properties and management practices in Wisconsin potato fields. Am J Potato Res 95:696–708

York A (2018) Pick of the crop microbiome. Nat Rev Microbiol 16(10):583–583

Zhang J, Liu YX, Zhang N, Hu B, Jin T, Xu H, Guo X (2019a) NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nat Biotechnol 37(6):676–684

Zhang LN, Wang DC, Hu Q, Dai XQ, Xie YS, Li Q, Liu HM, Guo JH (2019b) Consortium of plant growth-promoting rhizobacteria strains suppresses sweet pepper disease by altering the rhizosphere microbiota. Front Microbiol 10(1668):1–10

Chapter 2
Beneficial Microbial Mixtures for Efficient Biocontrol of Plant Diseases: Impediments and Success

Shrivardhan Dheeman, Mukesh Kumar, and Dinesh Kumar Maheshwari

Abstract Microbial consortium is a naturally occurring beneficial microbiome in the vicinity of plant root system. Artificially, bacterial mixture can be prepared after selecting them for a shared purpose of benefits such as nutrients, space, and other multifunctional benefit to the plants. The mixture of strains formulated in the form of bio-inoculants of microbial consortium can be seen in various ways including its use for biological control of plant diseases. Extensively, the applications of the microbial consortium have been proven more beneficial to the plants in comparison to the use of individual microorganisms, precisely contributing to sustainable development of overall ecosystem. The exploitation of microbial consortia is challenging to study synergism at eco-microbiological level thus offering a new scope of research towards rhizospheric bioengineering. Microbe–microbe interaction, etc., at theoretical and practical level may pay attention towards rediscovering. Their role as potential contender of boosting agricultural productivity is for the future benefits of the ever-increasing population. This review highlights the present state of knowledge and purpose of this scientific commentary to develop novel methods of using microbial mixtures that have relevance to developing microbial consortia for agriculture use. The success, impediments, and failure have also been presented to bring new scientific insight and technologies for wide dissemination of knowledge in scientific community. The research-based evidence clearly shows the advantages of microbial consortia whose function to plants or soil rhizosphere stimulate the natural process to overall benefits to plants in agro-ecosystems.

Keywords Microbe–microbe interaction · Phytopathogens · Disease control · Microbial consortium · Biocontrol agents

---

S. Dheeman
Department of Biotechnology and Microbiology, School of Allied Health Sciences, MVN University, Palwal, Haryana, India

M. Kumar · D. K. Maheshwari (✉)
Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India
e-mail: mukesh.kumar@gkv.ac.in

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_2

2.1 Introduction

The concept of biological control of plant diseases in fact arose in the 1920s and 1930s when some plant pathogens were suppressed and were not able to cause diseases that occurred by introducing antibiotic producing microbes to the natural habitats. Garret (1965) defined biological control in plant disease as "Any condition under which, or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself), with the result that there is a reduction in incidence of disease caused by the pathogens." On the other hand, Beirner (1967) has proposed that "Biological control is effect by another organism or group of organisms." But the most comprehensive definition of biological control is "the reduction of inoculum density or disease producing activities of pathogen or parasite in its active or dormant state, by one or more organisms, host, or antagonists" (Bakker and Cook 1974).

More than one organism can control the wide spectrum of pathogens by applied antagonists largely remains an unfulfilled goal for biological control. There are three main approaches to achieve this goal: (i) modify the genetics of the biocontrol agent to add mechanisms of disease suppression that are operable against more than one pathogen, (ii) alter the environment to favor the biological control agent and to disfavor competitive microflora, and (iii) develop strain mixtures or consortia with superior biocontrol activity (Janisiewicz 1988). Several strategies for formulating consortia of biocontrol agents could be envisioned including consortia of organisms with differential plant colonization patterns; consortia of antagonists that control different pathogens; consortia of antagonists with different mechanisms of disease suppression; consortia of taxonomically different organisms; and consortia of antagonists with different optimum temperature, pH, and moisture conditions. Microorganisms as biocontrol agents typically have a relatively narrow spectrum of activity compared with synthetic pesticides as described by Bakker (1991) and Janisiewicz (1996) that often exhibited inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for suppression of plant pathogens (Backman et al. 1997).

In more research till date, in biological control, majority of plant–microbe interactions considered so far concern single biocontrol agents as antagonist to a single pathogen. Considerably, why do we expect a single biocontrol strain to perform effectively against a wide range of plant pathogens given that each pathogen is likely to have a specific ecological niche and a specific set of conditions (nutrients, temperature, pH, etc.) under which it is active? How can a single biocontrol strain cover all these situations in a competitive environment where its own activities are being constrained? Shanmugam et al. (2002) stated that it is not possible to isolate a single microorganism for effective biocontrol activity in a particular soil type that may act in similar manner across a range of quite different soil types. It is therefore a promising trend in the field of inoculation technology, the use of mixed inoculants or consortia (combination of microorganisms) that interact synergistically. Long back, Pierson and Weller (1994) have shown that a combination of biocontrol strains gives

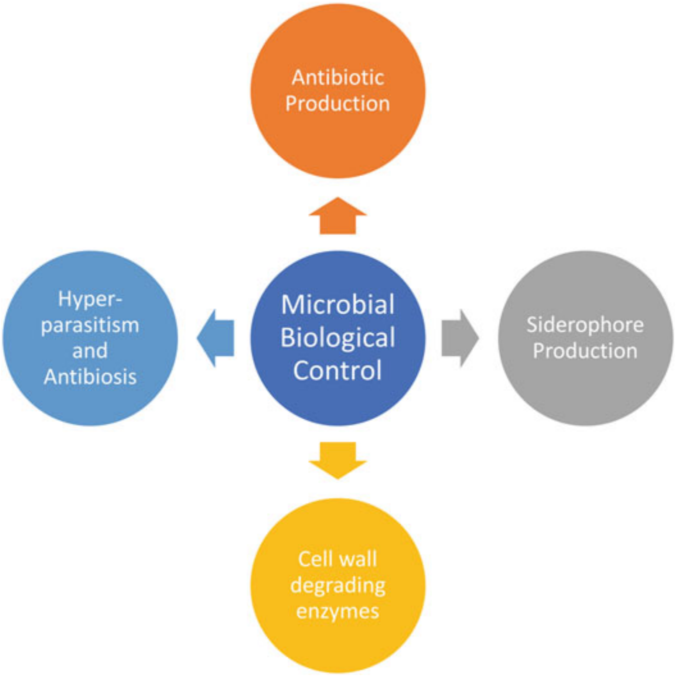


Fig. 2.1 Microbial agents and their mode of action in biocontrol

high level of plants' protection, suppresses multiple plant diseases, and reduces variability of biological control (Guetsky et al. 2002). Several others (Nandakumar et al. 2001; Jetiyanon and Kloepper 2002, Jetiyanon et al. 2003; de Boer et al. 2003; Romano and Kolter 2005) have shown that combining microorganisms not only improved plant growth but also enhanced control over several bacterial and fungal plant diseases (Fig. 2.1).

Since a single biocontrol agent is not likely to be active in all kinds of soil environment and agriculture ecosystem, the use of consortia or mixtures of two or more microbial strains is one of the better approaches to enhance the level and consistency in disease control (Raupach and Kloepper 1998; Fukui et al. 1999; de Boer et al. 2003). Mondal et al. (2020) addressed some important issues of microbial consortium as an integrated past of below ground plant-soil ecosystem for their invaluable role in sustainable crop production due to the wide array of remunerative roles to their host plants.

The artificially constructing microbial strain combinations, i.e., consortia formation strictly empirical and based on the premise of combining several effective strains which may lead to additive or synergistic effect. Currently, there is no in vitro test that predicts which strain will have biocontrol activity and which ones will be compatible in consortium. Thus, combinations must be screened in situ as individual strains are. We initially assumed that PGPR strains comprising effective combinations would be mutually non-inhibitory because of overlapping niche in the rhizosphere and the proven ability of PGPR strains to produce inhibitory secondary metabolites (Keel et al. 1992; Kempf et al. 1993).

2.2 Protocol Strategy: Artificial Microbial Consortia, Construction, and Mode of Applications

Interest in synthetic or artificial microbial consortia has immense importance in diverse fields of applied microbiology and biotechnology. In plant disease control, applications with higher reproducibility provide a breakthrough in field research. Still there are challenges to commercialize the microbial consortia in the market. Registration process of individual microorganisms as biopesticide is quite clear all over the globe but in case of microbial consortia, the issue is still in its infancy, and hence discourage the scientists. Czajkowski et al. (2020) described the problems of using microbial consortia in biological control are mainly due to lack of proper guidelines of registration and commercialization. Other difficulties such as specific refer of each microorganism of a consortium as well as their mechanism of action yet to be understood both at physiological and molecular level. These genuine issues are required to be emphasized on a more advanced level and subject of research for their application to suppress multiple plant diseases. Although, modern scientific tools such as omic approach is available now to resolve various issues, e.g., identification of genome of bacterial strains sequences for construction of consortia, their action and mechanism, feasibility in agro-biological application for environment-friendly crop protection. Maciag et al. (2022) have addressed some of the above issues by sequencing the complete genome of bacterial strains comprising the great five (GF) synthetic microbial consortium effective against potato soft rot caused by *Pectobacterium* and *Dickeya* spp. (Krzyzanowska et al. 2019; Maciag et al. 2020).

The in vitro spectrophotometric interaction studies for growth parameters indicated establishment of protocooperation among *S. meliloti* MPR4, *P. aeruginosa* LRP7, *A. chroococcum* TRA2, and *S. marcescens* MTCC97. This is due to the non-reactive nature of secondary metabolites produced by *S. marcescens* action among each other. Interaction of four rhizobacteria showed that all were able to grow even in the presence of culture filtrates of one another. Earlier, Protocooperation action of different groups of bacteria was observed by number of workers (Pierson and Weller 1994; Duffy et al. 1996; Guetsky et al. 2002; Shanmugam et al. 2002; de Boer et al. 2003). Gera and Sharma (2004) prepared a consortium of *Azotobacter*, *Azospirillum*, *Pseudomonas*,, and *Rhizobium* growing all the bacteria in a single medium and found that the bacteria did not affect the growth of other bacteria in the consortium. Hence, protocooperation behavior among *S. meliloti* MPR4, *P. aeruginosa* LRP7, *A. chroococcum* TRA2, and *S. marcescens* MTCC97 proved their significance.

Gera and Sharma (2004) developed a consortium (Biomix) containing the species *Azotobacter, Azospirillum, Pseudomonas*, and *Rhizobium.* Inoculation of Biomix on pearl millet increased the plant growth parameters over un-inoculated control as well as over plants inoculated with individual cultures. Vishwakarma et al. (2020) highlighted the importance of plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture besides giving suggestions to understand microbial formulation compositions, their concrete identification for better utilization in various plant species. Niu et al. (2020) emphasized the interaction-related factors to be considered when constructing multiple-strain biological control consortia and proposed a workflow for assembling them by applying a reductionist synthetic community approach. A diverse group of PGP consortia having antagonistic nature against disease-causing pathogens in *Camellia sinensis* have shown their worth in curbing soil-borne as well as foliar/stem diseases; however, very limited commercial bioformulations have found field applications, despite so many cutting-edge research (Bora and Bora 2021). Eze et al. (2021) studied the diversity and metagenome analysis of a bacterial consortium involved in pollution control of terrestrial and aquatic environment by petroleum contaminants. The dominant genus *Acidocella* could serve as an effective inoculum for the bioremediation of sites polluted especially with diesel fuel. A similar approach may also resolve certain basic issues in sustaining agro-biological practices. Rokni et al. (2021) observed the significance of the tripartite consortium for the biocontrol activity against *Phytophthora capsici* on *Capsicum annum;* such combination provides a promising feature as a complement for crop nutrition and enhances the plant tolerance to *P. capsici.*

The beneficial effects of microbial consortia generally occur due to their mode of application in controlling diseases in plants. The effect mainly occurred because of different nature of microorganisms inhabiting this complex system and contribute to the application of consortia success with increased consistency in effective control of the pathogens present in the plant/and or in soil. Hence, designing of the synthetic or artificial consortia for biological protection depends upon the recruitment of beneficial microorganisms. It is now generally accepted that applications of microbial consortia and their mode of action contribute to understanding plant health and fitness success.

2.2.1 Cocktail and Combined Effect

By combining microorganisms, multiple antifungals, as well as PGP traits, can be combined, and one may assume that at least one biocontrol agent will be functional under the conditions faced by the released biocontrol agent. Certain combinations of PGPR performed better than individual strains and suggest the importance of additive and interactive effects among introduced bacteria in biological control (Pierson and Weller 1994). A seed application of a combination of three PGPR, *Bacillus pumilus*, *Bacillus subtilis,* and *Curtobacterium flaccumfaciens* provided greater control of several pathogens on *Cucumis sativus* L. than their individual application (Raupach and Kloepper 1998). The combinations of *Paenibacillus* spp. and *Streptomyces* spp. suppressed fusarium wilt of cucumber better than when either used alone (Singh et al. 1999). The combinations of *Escherichia coli* S17R1 and *Burkholderia cepacia* BC-B provided greater suppression of cucumber seedlings pathogenesis in field soil naturally infested with *Pythium* spp. and *Fusarium* spp. than seed treated with the strain BC-B or S1781 or *Enterobacter cloacae* 501R3 (Roberts et al. 1997). A combination of two *Pseudomonas* strains, antagonizing by competition for iron and inducing resistance, respectively reduced fusarium wilt in radish more than each strain by itself (de Boer et al. 1999). Palmieri et al. (2017) concluded that a microbial consortium comprised of *Serratia marcescens*, *P. fluorescens*, *Rahnella aquatilis,* and *B. amyloliquefaciens* efficiently controlled *F. oxysporum* and *F. solani* with a consistently higher efficacy. The strains were screened based on mutual compatibility when grown in a mixture and a process of antagonism against both Fusarium pathogens. Moreover, combinations of biocontrol strains are expected to result in a higher level of protection (Dunne et al. 1998) by reduced variability of biological control (Guetsky et al. 2002), and hence have the potential to suppress multiple plant diseases (Jetiyanon and Kloepper 2002). Nautiyal et al. (2005, 2006) developed a microbial consortium comprising *B. subtilis* and *B. lentimorbus* and a method of production involving synergistic composition. The microbial consortium comprising only bacterial strains showed effectiveness in checking damping-off caused by *Pythium aphanidermatum* in chili whereas combined effect of two different strains of *P. putida* having different disease-suppressive mechanisms enhanced suppression of fusarium wilt in radish (de Boer et al. 2003). Dutta et al. (2008) reported combined use of *B. cereus* and *P. aeruginosa* with rhizobia for induction of systemic resistance against fusarium wilt in *Cajanus cajan*. Two or more than two microbial components associated with consortia formation were found more effective than that of an individual organism (Maheshwari et al. 2013).

A microbial consortium is a group of different species of microorganisms that act together as a community. For developing a consortium one can choose microorganisms that are resistant to environmental shock, fast acting, synergistically active, producing natural enzymatic activity, easy to handle, have long shelf life, good sustainability, non-pathogenic, non-corrosive of consistent quality, and economical in nature. For the success of consortia, compatibility of microbes used in the mixture, their cooperation in mutual interaction (beneficial), and establishment in the rhizosphere besides lack of competition among them are prerequisite and necessary requirements for a microbial consortium to quality the eligibility criteria for sustainable strategy and application.

2.2.2 Co-inoculation

Recent studies showed a promising trend in the field of inoculation technology, where the use of mixed inoculants or consortia (combinations of microorganisms) that interact synergistically are currently being devised. Microbial studies performed without plants indicate that some mixture allow the bacteria to interact positively with each other through physical or biochemical metabolic activities that may enhance some beneficial aspects of their physiology such as nitrogen fixation. An example of this is *Azospirillum*, one of the most studied bacterium that is associated with plants (Bashan and Holguin 1997). It may associate with sugar of polysaccharide degrading bacteria (PDB), establishing a metabolic association where the sugar degrading bacteria produce degradation and fermentation products used by *Azospirillum* as a carbon source that in turn provides PDB with nitrogen. Shanmugam et al. (2002) determined the effectiveness of root rot control with co-inoculation to *Pseudomonas fluorescens* and *Rhizobium* sp. Plant studies have shown that the beneficial effects of *Azospirillum* on plants can be enhanced by co-inoculation with other microorganisms (Alagawadi and Gaur 1992; Belimov et al. 1995). Co-inoculation, frequently, increased growth and yield compared to single inoculation provided the plants with more balanced nutrition and improved absorption of nitrogen, phosphorus, and mineral nutrients.

Apparently, co-inoculation (inoculation of more than one microorganism in the same culture. medium) is a delicate approach in which differences in root colonization ability of bacterial growth rate easily affect successful growth promotion. Co-inoculation (Xavier and Germida 2002) and co-culture (Hogan and Kolter 2002) of microbes have been observed to perform tasks better than their individual microbes. To summarize a few, a number of different mixtures of bacteria (Pierson and Weller 1994; Raaijmakers et al. 1995; Kloepper 1996; de Boer et al. 1997, 2003; Schisler et al. 1997; Sung and Chung 1997a, b; Nandakumar et al. 2001; Shanmugam et al. 2002), fungi (Paulitz and Baker 1990; Budge et al. 1995; de Boer et al. 1997) and mixtures of fungi and bacteria (Park et al. 1988; Lemanceau and Alabouvette 1991; Duffy and Weller 1995; Janisiewicz 1996; Leeman et al. 1996; Leibinger et al. 1997; Romano and Kolter 2005) have shown significance in growth and development of crop plants (Fig. 2.2).

Co-inoculation frequently increases growth and yield, compared to single inoculation providing the plants with more balanced nutrition and improves absorption of nitrogen, phosphorus, and mineral nutrients (Xavier and Germida 2002). Co-inoculation of *Bacillus* spp. with *Bradyrhizobium japonicum* enhanced soybean plant growth (Bai et al. 2003). Jensenm et al. (2002) have determined the effectiveness of root rot control with co-inoculation of *B. subtilis* and *Rhizobium* spp. Hence, synergistic behavior between *P. aeruginosa* KRP1, *S. meliloti* RMP1, and *B. licheniformis* KRBI proved their significance for their use as co-inoculants consortium. In one of our work plans, we observed seven different PGPR strains, viz., *P. aeruginosa* KRP1, KRP7, GRCI, *B. licheniformis* KRB1, and *B. licheniformis* MTCC424. *Sinorhizobium meliloti* RMP1 and *Serratia marcescens*

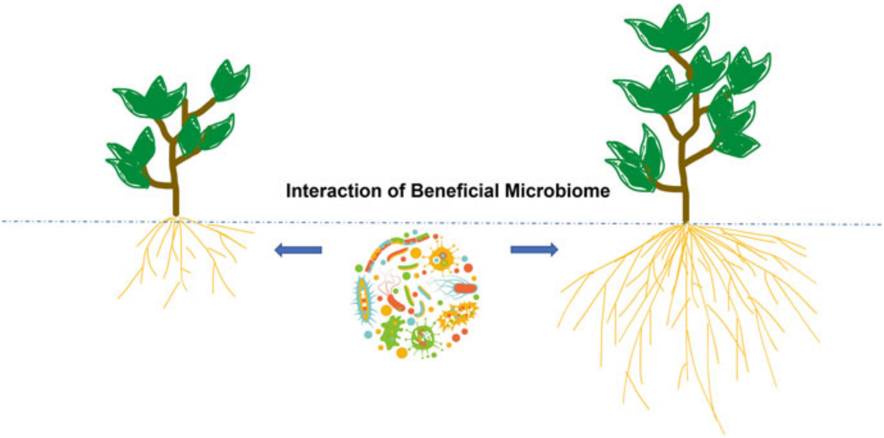


Fig. 2.2 Effect of beneficial microbial mixture on growth of plant

Table 2.1 In vitro interaction among selected PGPR for the preparation of microbial consortium

Strains	KRP1	KRP7	GRC1	KRB1	MTCC 429	RMP1	MTCC 97
KRP1	+	–	–	+	–	+	–
KRP7	–	+	+	–	+	–	+
GRC1	–	+	+	+	+	–	+
KRB1	+	–	+	+	–	+	–
MTCC 429	–	+	+	–	+	–	–
RMP1	+	–	–	+	–	+	+
MTCC 97	–	+	+	–	–	+	+

Abbreviation: KRP1, KRP7, GRCI—*P. aeruginosa*; MTCC429 *B. licheniformis*; KRB1—*B. licheniformis*; RMP1 *Sinorhizobium meliloti*; MTCC 97—*Serratia marcescens* (+), growth present; (–), growth was inhibited

97 form consortium (Table 2.1). The screening of the strains was based on spectrum inhibition (antagonism) during bacterial–bacterial interactions. Since, in vitro fungal growth inhibition assay by PGPR in consortia (*P. aeruginosa* KRP1 + *B. licheniformis* KRB1+ *S. meliloti* RMP1) showed 89% and 85% inhibition of radial growth of *S. sclerotiorum* and *F. oxysporum*, which is comparatively higher with respect to their co-inoculated and application of individual strains. Thus, antagonistic action was more pronounced in mixed culture of *P. aeruginosa* KRP1, *B. licheniformis* KRBI, and *S. meliloti* RMP1. It is therefore confirmed that compatible nature of bacterial metabolites is significant in disease suppression and plant growth promotion.

To develop a mutualistic relationship among each other, antagonistic actions of the selected bacterial strains were first tested by cross streaking, which was later confirmed by filter paper disc method in vitro. *P. aeruginosa* KRP1, *B. licheniformis* KRB1, and *S. meliloti* RMP1 did not inhibit the growth of each other under co-culture conditions. Thus, no antagonistic behavior of organisms towards each another was noticed. While other strains, viz., *P. aeruginosa* KRP7 and GRC1, *B. licheniformis* MTCC429, and *S. marcescens* 97 inhibited the growth of KRP1, KRB1, and RMP1 as evident by their growth-suppressing activities on solid plates which ruled out the possibility of using the above strains in consortium preparation (Table 2.1).

2.3 Biofilm and Quorum Sensing

In general, bacteria experience a certain degree of shelter and homeostasis when residing in a biofilm. Competition for substrate is one of the major evolutionary driving forces among different microorganisms which may effectively out-compete others for better utilization of a given energy source. Both *P. aeruginosa* KRP1 and *S. meliloti* RMP1 could grow together, and this cooperation maybe because both belong to the same group with similar nutritional requirements. The strains showed a mutual relationship with each other, which accounts that their growth corresponds to their metabolic products also proved beneficial to plants (Saxena and Tilak 1994). The results of in vitro interaction studies showed synergistic cooperation among *P. aeruginosa* KRP1, *B. licheniformis* KRB1, and *S. meliloti* RMP1 due to the non-inhibitory nature of secondary metabolites produced by these strains against each other. A similar type of mutualistic, synergistic actions of different groups of bacteria such as pseudomonads, rhizobia, bacilli, and *Azotobacter* were observed earlier by many researchers (Pierson and Weller 1994; Lorito et al. 1994; Duffy et al. 1996; Dunne et al. 1998; Shanmugam et al. 2002; Guetsky et al. 2002; de Boer et al. 2003).

Berggen et al. (2001) observed neutral action of *P. putida* towards *R. leguminosarum* during microbial interaction studies. On the other hand, Shanmugam et al. (2002) reported positive interaction between *P. fluorescens* and *Rhizobium* sp. under in vitro as well as in vivo conditions. Earlier most of the workers have demonstrated the role of microbial consortia (containing different PGPR) directly applied in the farmer's field (Nandakumar et al. 2001; Guetsky et al. 2002; Shanmugam et al. 2002; Jetiyanon and Kloepper 2002; de Boer et al. 2003). Recently, Wang et al. (2022) highlighted the improvement of biocontrol efficacy of antagonists using a combination of microbial antagonists and additions. Several recently postulated mechanisms of action such as biofilm formation and an oxidative burst of reactive oxygen species are also described.

2.4 Factors Affecting the Efficacy of Consortia

The complexity of the soil ecosystem is constraint that make biological control of these root pathogens by introducing antagonists as a challenge. The use of high efficacy of biocontrol agents is generally observed under controlled environments.

Occasionally, introduction of antagonists that have been highly effective under controlled environments is only moderately effective sometimes totally ineffective when applied in the phyllosphere of commercially grown plants. For example, a survey of 64 greenhouse experiments conducted all over the world revealed that in approximately 70% of them, *Trichoderma harzianum* T59 suppressed *Botrytis cinerea* infections in tomato and cucumber similarly to that chemical fungicide in efficacy. In case of biocontrol, in 20% of the experiments conducted so far, the efficacy of the biocontrol agents was found significantly increased in comparison to that of the fungicides (Shtienberg and Elad 1997).

A commercial product of mixture of three *Bacillus subtilis* strains with several biocontrol mechanisms proved effective in controlling fungal soil pathogens after disinfection by seed treating fungicides. Sung and Chung (1997a, b) showed that a mixture of bacteria that produce chitinases and antibiotics could effectively suppress rice sheath blight caused by *Rhizoctonia solani*. Dunne et al. (1998) demonstrated that combining phloroglucinol-producing *P. fluorescens* and proteolytic bacteria *Stenotrophomonas maltophilia* improved biocontrol of *Pythium* mediated damping-off of sugar beet. Sung and Chung (1997a, b) demonstrated that chitinase-producing *Bacillus cereus* when used in conjunction with antibiotic producing *P. fluorescens* exhibited synergistic effect on the suppression of rice sheath blight.

Combinations of fungi and bacteria have also been shown to provide enhanced biocontrol. For instance, *Trichoderma koningii* combined with either *Pseudomonas chlororaphis* 30-84 or *P. fluorescens* Q2-87 provided greater suppression of take-all of wheat than *T. koningii* alone (Duffy et al. 1996). The non-pathogenic *Fusarium oxysporum* Fo 47 combined with *Pseudomonas putida* WCS358 provided better suppression of fusarium wilt of flax caused by *F. oxysporum* f. sp. *lilii* than either alone (Duijff et al. 1999). To evolve a consortium, by combining effective strains may lead to additive or synergistic effects. Hence, PGPR comprising effective combinations as evidenced by their mutually non-inhibitory nature because of overlapping niche in the rhizosphere proved ability of strains to produce inhibitory secondary metabolites (Keel et al. 1992; Kempf et al. 1993). Such enhanced disease suppression may involve not only different disease-suppressive mechanisms but can also result from interaction between the introduced strains that positively influence growth, root colonization, and/or activity of the strains.

Competition for substrate nutrition is one of the major evolutionary driving forces in the bacterial world, and numerous experimental data obtained under well-controlled conditions revealed how different organisms may effectively out-compete others because of better utilization of a given energy source. *S. meliloti* MPR4 and *P. aeruginosa* LRP7; *A. chroococcum* TRA2 and *S. marcescens* MTCC297 were successfully grown as mixed cultures (unpublished result). The cooperation of *S. meliloti* MPR4 and *P. aeruginosa* LRP7 was because both belong to the same group being Gram-negative, aerobic non-spore-forming rods, fast growing, and having quite similar nutritional requirements. The strains having synergistic action on each other's growth would be ideal due to the reason that the microbial growth correspondents to their metabolic products which are beneficial to plants (Saxena and Tilak 1994). Both *S. meliloti* MPR4 and *P. aeruginosa* LRP7 produced similar types of siderophore (hydroxamate) in addition to IAA production and phosphate solubilization. During microbial interaction with *P. putida* towards *R. leguminosarum*, nutritional action was observed (Berggen et al. 2001). Shanmugam et al. (2002) obtained positive interaction between *P. fluorescens* and *Rhizobium* sp. under in vitro as well as in vivo conditions.

Many of the strains that might be members of microbial consortium were either strongly inhibitory to or strongly inhibited other members of the consortium in the in vitro assay. For example, strain *P. aeruginosa* GRC1 inhibited *P. aeruginosa* LRP7, *Bacillus licheniformis* MTCC429, *Bradyrhizobium* (Arachis) ARH2, *Gluconacetobacter diazotrophicus; Sinorhizobium meliloti* MPR4 was inhibitory for *S. meliloti* RMP1, *Bradyrhizobium* (Arachis) AHR2, and *Gluconacetobacter diazotrophicus* and itself inhibited by *P. aeruginosa;* GRC1 inhibited *P. aeruginosa* LRP7, and *Bacillus licheniformis* MTCC429. They found some strains of *Pseudomonas* inhibited 8 of 10 PGPR and inhibited by 7 of 10 strains. In our study, volatile substance HCN producing strains inhibited HCN deficient strains of the same group of bacteria. It may be since HCN-producing bacteria themselves are resistant to it but others may show susceptibility (Glick 1995). Thus, for the microbial consortium preparations *S. meliloti* MPR4, *P. aeruginosa* LRP7, *A. chroococcum* TRA2, and *S. marcescens* MTCC97, out of 10 were selected because of their non-inhibitory nature to each other.

Disease management of root rot and wilt in *Sesamum indicum*. Combinations of MPR4 + LRP7, LRP7 + *Serratia,* and microbial consortium involving *S. meliloti* MPR4, *P. aeruginosa* LRP7, *A. chroococcum* TRA2, and *S. marcescens* MTCC97 reduced root rot incidence by 67%, 65%, and 74%, respectively and the wilt disease incidence by 61%, 60%, and 69%, respectively. To suppress take-all disease in wheat PGPR consortium gave better results in comparison to their single inoculation (Pierson and Weller 1994). Consortium reduced the sheath blight disease in rice more effectively than their single inoculants (Nandakumar et al. 2001). de Boer et al. (2003) found 20% more reduction of wilt disease incidence by the application of the combination of two fluorescent pseudomonads. Mineral uptake of phosphate due to acid production, nitrogen assimilation, and nitrogen fixation besides indirect role of siderophore in disease suppression results in enhanced plant growth and yield in various crops (Noel et al. 1996; Antoun et al. 1998; Katiyar and Goel 2003; El-Komy 2005). Jain et al. (2015) reported an increase in phenolics in plants because of microbial consortium in control of *Sclerotinia sclerotiorum* in *Pisum sativum*.

On the other hand, interactions between two or more introduced biocontrol agents can also negatively influence disease control. de Boer et al. (1999) demonstrated that *Pseudomonas putida* strain RE8 inhibits the growth of *P. fluorescens* strain RSIII in vitro and both the strains RE8 and RSIII did not suppress *Fusarium* wilt of radish, whereas the combination of RE8 with RSIII-9, i.e., a spontaneous mutant of RSIII, was no longer inhibited growth by RE8, enhanced disease suppression. Such results may be due to genetic modification of the strains RSIII that could yield desirable results. Pierson and Weller (1994) established threshold densities of an antagonist as a key factor in biological control, the rationale of using a mixture of antagonists' isolates is a logical approach because mixtures more closely mimic microbial communities and have multiple mechanisms of disease suppression (Schisler et al. 1997). It is likely that most cases of naturally occurring biological control result from mixtures of antagonists, rather than from a high population of a single antagonist. For example, mixture of antagonists is considered due to their action for protection in disease-suppressive soils (Lemanceau and Alabouvette 1991; Schippers 1992). Consequently, application of mixture of introduced biocontrol agents would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity, enhance the efficacy and reliability of various mechanisms without the need for genetic engineering (Hubbard et al. 1983). More recently, Minchev et al. (2021) hypothesized that microbial consortia are more versatile, across wide application strategies in biocontrol of both root and foliar diseases in tomatoes. The consortia exhibited an extended functionality against *Fusarium oxysporum* and *Botrytis cinerea.*

2.5 Reason for Failure

In most of the investigations, the efficacy of individual biocontrol strain or combination of different strains was assessed by screening these agents against a single isolate of the target pathogen. This could be one of the reasons for failure of commercial bioinoculant as for evaluation no consideration of the genetic diversity of the target pathogen population may exist among geographic region, within a given field, or even among lesions of the same plant (Mc Donald et al. 1994; Budge et al. 1995).

Previous studies, of combinations of biological agents for plant diseases have included mixtures of fungi (Budge et al. 1995; Datnoff et al. 1995; de Boer et al. 1997), mixtures of fungi and bacteria (Duffy et al. 1996; Janisiewicz 1996; Leeman et al. 1996; Hassan et al. 1997; Leibinger et al. 1997), and mixtures of bacteria (Pierson and Weller 1994; Janisiewicz and Bors 1995; Mazzola et al. 1995; Wei et al. 1991; de Boer et al. 1997, 2003; Roberts et al. 1997; Sung and Chung 1997a, b; Nandakumar et al. 2001; Shanmugam et al. 2002). Most of these reported on biological control agents do not result in improved suppression of disease compared with the individual antagonists (Hubbard et al. 1983; Sneh et al. 1984; Miller and May 1991; Dandurand and Kundsen 1993). This incompatibility of the co-inoculants is due to the action of biocontrol agents on each other as well as on the target pathogen(s) (Leeman et al. 1996). This is an important prerequisite attribute of the co-inoculated microorganisms (Baker 1990; de Boer et al. 1997). Enhancing biological control by using mixtures of antagonists may have advantages as it may broaden the spectrum of activity, may enhance the efficacy and reliability of the biological control, and allows the combination of various traits without employment of genetic engineering (Mc Donald et al. 1994).

2.6 Success Stones and Bottlenecks

For evaluating the activity and efficacy of microbial consortia to disease management, it is a prerequisite to study the planting material, i.e., seeds seedling tubers cutting even the root in soil (Czajkowski et al. 2020). The Synthetic or artificial consortia if introduced into the native soil is more advantageous to the plant in comparison to that of consortia synthesized by using non-native strains and applied to the native soil. Their establishment near the root system is smoother and easier in niche or even from effective all physiological reactions in an equilibrium supporting the overall performance of the consortium as also observed by Sarma et al. (2015). Combining beneficial microorganisms or co-inoculating the microorganisms with prescreened and multifarious inherent traits in the associated microbes in consortia seems to be the most strength forward approach to obtain them for use in plant fitness.

The evaluation of the mechanistic compatibility of microbial consortia to check the phytopathogens showed to be prioritized for their biocontrol activity. In microbial consortia, strains exhibited diverse activity, e.g., *Bacillus pumilus* INR 7, *B. subtilis* GB03, and *Curtobacterium flaccumfaciens* ME1 showed biocontrol activities wherein strain INR7 and ME1 impart ISR against *R. solani* and *Fusarium* up, but when combined, they could control several pathogens causing soil and foliar disease in plant. The origin for designing the microbial consortia preferably is to obtain from the same habitat and positively influence compatibility of the consortium (de Vrieze et al. 2018). The consortia in the field should not import any mammalian toxicity and be ecologically and environment-friendly in the application to the plant health and fitness under variable pathogen pressure.

Another desirable property of artificial microbial consortia is to recruit those organisms in design which could develop disease-suppressed soil when subjected to biotic stress. Berendsen et al. (2018) suggested designing synthetic microbial consortia for biological control/plant protection. Using this approach, a consortium of three bacteria namely, *Xanthomonas* sp. WCS 2014-23, *Stenotrophomonas* sp. WCS 2014-113, and *Microbacter* sp. WCS 2014-259 active against *Hyaloperonospora arabidopsidis*. The pathogen-free *Arabidopsis thaliana* is obtained if microbial consortia are applied prior to infection. It is also pertinent that consortia remain active after application as supported by their effect in the natural environment which further depends on nutrient availability between artificial media and national resources (Kamilova et al. 2006). It is now generally accepted that designing and constructing microbial consortia for application requires a better understanding of the plant–microbe interactions, with special reference to the knowledge on how plant recruits and colonize with the beneficial microbiome. The dominating bacterial genera *Bacillus* and *Pseudomonas* species are more frequently proved beneficial for biocontrol activity due to their antagonistic nature to plant pathogens hence used both in proof-of-concept in vitro and in vivo (Hass and Defago 2005). Both beneficial and harmful microorganisms live in the vicinity of plant roots, but plants exploit a few of them for their mutual benefits and support. Both can reach an equilibrium state where invasion of the new microbe from external sources is quite different (Scheuring and Yu 2012). If any, deviation occurs either due to a sudden change in the microbiome or by the acquisition of the growth substances (root exudates) the state of un-equilibrium leads to infection or damage. To avail the benefits of microbial consortia application, impact of other stress such as effect of climate change cannot be ruled out.

Acknowledgment DKM acknowledges UGC-BSR Fellowship.

Conflict of Interest Author(s) declares no conflict of interest.

References

Alagawadi AR, Gaur AC (1992) Inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria on yield of sorghum (*Sorghum bicolor* L. moench) in dry land. Trop Agric 69:347–350

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalinde R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67

Backman PA, Wilson M, Murphy JF (1997) Bacteria for biological control of plant diseases. In: Environmentally safe approaches to crop diseases control. CRC Lewis Publishers, Boca Raton, FL, pp 95–109

Bai Y, Zhou X, Smith DL (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci 43:1774–1781

Baker R (1990) An overview of current and future strategies and models for biological control. In: Hormby D (ed) Biological control of soil borne plant pathogens. CAB International, Wellinford, pp 375–388

Bakker R (1991) Diversity in biological control. Crop Prot 10:85–94

Bakker KF, Cook RJ (1974) In: Biological control of plant pathogens, W. H. Freeman and Co, San Francisco, CA. Amer Phytopathol Soc, St. Paul, Minnesota, 433

Bashan Y, Holguin G (1997) *Azopsirillum*- plant relationships: environmental and physiological advances (1990-1996). Can J Microbiol 43:102–121

Beirner BP (1967) Biological control and its potential. World Rev Pest Cont 6:7–20

Belimov AA, Kojemiakov AP, Chuvarliyea CV (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate solubilizing bacteria. Plant Soil 173:29–37

Berendsen RL, Vismans G, Yu K, Song Y, De Jonge R, Burgman WP, Burmelle M, Herschend J, Bakker P, Pieterse CMJ (2018) Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J 12:1496

Berggen I, Vuurde JWL, Martensson AM (2001) Factors influencing the effect of deleterious *Pseudomonas putida* rhizobacteria on initial infection of pea roots by *Rhizobium leguminosarum* bv. viceae. Appl Soil Ecol 17:97–105

Bora P, Bora LC (2021) Microbial antagonists and botanicals mediated disease management in tea, *Camellia sinensis* (L.) O. Kuntze: an overview. Crop Prot 148:105711

Budge SP, McQuilken MP, Fenlon P, Whipps JM (1995) Use of *Coniothyrium minitans* and *Gliocladium virens* for biological control of *Sclerotinia sclerotiorum* in glass house lettuce. Biol Control 5:513–522

Czajkowski R, Maciag T, Krzyzanowska DM, Jafra S (2020) Biological control based on microbial consortia–from theory to commercial products. In: How research can stimulate the development of commercial biological control against plant diseases. Springer, Cham, pp 183–202

Dandurand LM, Kundsen GR (1993) Influence of *Pseudomonas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma harizianum* in the spremosphere and rhizosphere of pea. Phytopathology 83:265–270

Datnoff LE, Nemec S, Pernezny K (1995) Biological control of *Fusarium* crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Gloums intraradices*. Biol Control 5:427–431

de Boer M, Van der Siluis I, van Loon LC, Baker PAHM (1997) In vitro compatibility between Pseudomand fluorescens Spp. Strain can increase affectivity of Fusarium wilt control by combination of these strains. In: Ogoshi A, Kobayashi K, Homma Y, Komadma F, Kondo N, Akino S (eds) In plant growth promoting rhizobacteria –present status and future prospects. Prock 4th Int. Workshop on Plant growth promoting rhizobacteria. Nakanishi Printing Sapporo, Japan, pp 380–382

de Boer M, van der Sluis I, van Loon LC, Bakker PAHM (1999) Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of fusarium wilt of radish. Eur J Plant Pathol 105: 201–210

de Boer M, Bom P, Kindt F, Keurentjes JB, van Der Sluis I, van Lunch LC, Bakker PAHM (2003) Control of Fusarium wilt of radish by combining *Pseudomonas putida* strains that have different diseases suppressive mechanisms. Phytopathology 93:626–632

De Vrieze M, Germanier F, Vuille N, Weisskopf L (2018) Combining different potato-associated *Pseudomon*as strains for improved biocontrol of *Pytophthora infestans*. Front Microbiol 9:2573

Duffy BK, Weller DM (1995) Use of *Gaeumannwmyes graminis* var. graminis alone and in combination with fluorescent *Pseudomonas* spp. to suppress take all of wheat. Plant Dis 79: 907–911

Duffy BK, Simon A, Weller DM (1996) Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology 86:188–194

Duijff BJ, Recorvet G, Bakker PAHM, Lopper JE, Lemanceau P (1999) Microbial antagonism at the root level is involved in the suppression of fusarial wilt by the combination of non-pathogenic *Fusarium oxysporum* Fo 7 and *Pseudomonas putida* WCS358. Phytopathology 89:1073–1079

Dunne C, Moenne-Loccoz Y, Mccarthy J, Higgins P, Powell J, Dowling DN, O'Gara F (1998) Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of *Pythium* mediated damping off of sugar beet. Plant Pathol 47:299–307

Dutta S, Mishra AK, Kumar BD (2008) Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. Soil Biol Biochem 40(2):452–461

El-Komy HMA (2005) Coimmobilization of *Azospirllum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. Food Technol Biotechnol 43:19–27

Eze MO, Hose GC, George SC, Daniel R (2021) Diversity and metagenome analysis of a hydrocarbon-degrading bacterial consortium from asphalt lakes located in Wietze, Germany. AMB Express 11(1):1–12

Fukui R, Fukui H, Avarez AN (1999) Comparison of single versus multiple bacterial species on biological control of *Anthurium* blight. Phyopathology 89:366–373

Garret SD (1965) Toward biological control of soil-borne plant pathogens Prelude to Biological control. (eds. Baker, K.F. and Synder, W.C.) California University Press, Berkeley 4-17

Gera R, Sharma PK (2004) Formulation and testing of biomix: Compatibility of different microoganisms in biomix. Association of microbiologists of India. 45th Annual conference, National Dairy Research Institute, Carnal (India). 11

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 47:109–117

Guetsky R, Shtienberg D, Elad Y, Dinoor A (2002) Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology 92: 967–985

Hass D, Defago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbial 3:307–319

Hassan DG, Zarjar M, Beigh GM (1997) Biological control of *Fusarium* root rot in common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosae* and *Rhizobium leguminosarum*. Mol Ecol 34:74–80

Hogan DA, Kolter R (2002) *Pseudomonas-Candida* interactions: an ecological role for virulence factors. Science 296(5576):2229–2232

Hubbard WJ, Harman GE, Hadar Y (1983) Effect of soil borne *Pseudomonas* spp. on the biological agent, *Trichoderma harzianum* on pea seeds. Phytopathology 73:655–659

Jain A, Singh A, Singh S, Singh HB (2015) Phenols enhancement effect of microbial consortium in pea plants restrains *Sclerotinia sclerotiorum*. Biol Control 89:23–32

Janisiewicz WJ (1988) Biocontrol of postharvest disease of apple with antagonist mixtures. Phytopathology 78:473–479

Janisiewicz WJ (1996) Ecological diversity, niche overlap and coexistence of antagonists used in developing mixtures for biocontrol of post harvest diseases of apples. Phytopathology 86:473–479

Janisiewicz WJ, Bors B (1995) Development of a microbial community of bacterial and yeast antagonists to control wound –invading post harvest pathogens of fruits. Appl Environ Microbiol 63:3261–3267

Jensenm CE, Perich JA, Graham PH (2002) Integrated management strategies of bean root rot with *Bacillus sublitis* and *Rhizobium* in Minnesota. Field Crops Res 74:107–115

Jetiyanon K, Kloepper JW (2002) Mixture of plant growth promoting rhizobacteria for induction of resistance against multiple plant disease. Biol Control 24:285–291

Jetiyanon K, Fowler WD, Kloepper JW (2003) Broad-spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. Plant Dis 87:1390–1394

Kamilova F, Kravchenko LV, Al S, Azarova T, Makarova N, Lugtenberg BJJ (2006) Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of activities of rhizosphere bacteria. Mol Plant Microbe Interact 19:250–256

Katiyar V, Goel R (2003) Solublization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. Microbiol Res 158:163–168

Keel C, Schinider U, Maurhofer M, Voisard C, Levelle J, Burger U, Wirthner P, Hass D, Defago G (1992) Supperession of root diseases by *Pseudomonas fluorescens* CHA0: Importance of bacterial secondary metabolites 2,4 diacetylphloroglucinol. Mol Plant Microb Interact 5:4–13

Kempf HJ, Baur PH, Schroth MN (1993) Herbicolin A associated with crown and roots of wheat after seed treatment with *Erwinia herbicola* B247. Phytopathology 83:213–216

Kloepper JW (1996) Host specificity in microbe-microbe interactions. Bioscience 46:406–409

Krzyzanowska DM, Maciag T, Siwinska J, Krychowiak M, Jafra S, Czajkowski R (2019) Compatible mixture of bacterial antagonists developed to protect potato tubers from soft rot caused by *Pectobacterium* spp. and *Dickeya* spp. Plant Dis 103:1374–1382

Leeman M, den Ouden FM, van Pelt JA, Cornelissen C, Matamala-Garros A, Bakker PAHM, Schippers B (1996) Suppression of Fusarium wilt of redish by co-inoculation of fluorescent *Pseudomonas* spp. and root colonizing fungi. Eur J Plant Pathol 102:21–31

Leibinger W, Beuker B, Hahn M, Mendgen K (1997) Control of post-harvest pathogens and colonization of apple surface by antagonistic microorganisms in the field. Phytopathology 87: 1103–1110

Lemanceau P, Alabouvette C (1991) Biological control of *Fusarium* diseases by fluorescence by *Pseudomonas* and non-pathogenic *Fusarium*. Crop Protect 10:279–286

Lorito M, Peterbauer C, Hayes CK, Harman GE (1994) Synergistic interaction between fungal cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore germination. Microbiology 140:623–629

Maciag T, Krzyzanowska DM, Jafra S, Siwinska J, Czajkowski R (2020) The Great Five—An artificial bacterial consortium with antagonistic activity towards *Pectobacterium* spp. and

Dickeya spp.: formulation, shelf life, and the ability to prevent soft rot of potato in storage. Appl Microbial Biotechnol 104(10):4547–4561

Maciag T, Krzyzanowska DM, Rabalski L, Jafra S, Czajkowski R (2022) Complete genome sequences of five gram-negative bacterial strains comprising synthetic bacterial consortium "The Great Five" with antagonistic activity against plant-pathogenic *Pectobacterium* spp. and *Dickeya* spp. Mol Plant Microbe Interact:MPMI-01

Maheshwari DK, Saraf M, Aeron A (2013) Bacteria in agrobiology. Disease management. Springer Publisher, Berlin

Mazzola M, Fugimato DK, Thomshow LS, Cook RJ (1995) Variation insensitivity of *Gaeumanmomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take all of wheat. Appl Environ Microbial 61:2554–2559

Mc Donald BA, Miles AJ, Nelson LR, Pettway RE (1994) Genetic variability in nuclear DNA in field population of *Stagonospora nodorum*. Phytopathology 84:250–255

Miller RH, May S (1991) Legume inoculation: Success and failure. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht, pp 123–134

Minchev Z, Kostenko O, Soler R, Pozo MJ (2021) Microbial consortia for effective biocontrol of root and foliar diseases in tomato. Front Plant Sci 12:756368. https://doi.org/10.3389/fpls.2021.756368

Mondal S, Halder SK, Yadav AN, Mondal KC (2020) Microbial consortium with multifunctional plant growth-promoting attributes: future perspective in agriculture. Adv Plant Microb Sust Agric 219–258

Nandakumar R, Baby S, Viswanathan R, Sheela J, Raghu Chandar T, Samiypan R (2001) A new bioformulation containing plant growth-promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield of rice. Bio-conl 46:493–510

Nautiyal CS, Mehta S, Singh HB, Pushpangadan P (2005) Synergistic bioinoculant composition comprising bacterial strains of *Bacillus subtilis* or *B. lentimorbus* from cow milk. (PCT WO 03/020038A1)

Nautiyal CS, Mehta S, Singh HB, Pushpangadan P (2006) U.S. Patent No. 7,097,830. Washington, DC: U.S. Patent and Trademark Office

Niu B, Wang W, Yuan Z, Sederoff RR, Sederoff H, Chiang VL, Borriss R (2020) Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. Front Microbiol 11:585404

Noel TC, Sheng C, York CK, Pharis RP, Hynes MF (1996) *Rhizobium leguminosorum* as a plant growth-promoting rhizobacterium: direct growth promotion of canola and lettuce. Can J Microbiol 42:279–283

Palmieri D, Vitullo D, De Curtis F, Lima G (2017) A microbial consortium in the rhizosphere as a new biocontrol approach against fusarium decline of chickpea. Plant Soil 412(1):425–439

Park C, Paulitz TC, Bakker R (1988) Biocontrol of Fusarium wilt of cucumbers resulting from interactions between *Pseudomonas putida* and non-pathogenic isolates of *Fusarium oxysporum*. Phytopathology 78:190–194

Pierson EA, Weller DM (1994) Use of mixtures of fluorescent pseudomonads to suppress take all and improve the growth of wheat. Phytopathology 84:940–947

Raaijmakers JM, van Der Sluis I, Koster M, Bakker PAHM, Weisbeek PJ, Schippers B (1995) Utilization of heterologous siderophores and rhizosphere competence of fluorescent Pseudomonas spp. Can J Microbiol 41:126–135

Raupach GS, Kloepper JW (1998) Mixture of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogen. Phytopathology 88:1158–1164

Roberts DE, Dery PD, Mao W, Herbrar PK (1997) Use of a colonization deficient strain of *Escherichia coli* in strain combination of biocontrol of cucumber seedling diseases. J Phytopathol 145:461–463

Rokni N, Alizadeh HS, Bazgir E, Darvishnia M, Mirzaei-Najafgholi H (2021) The tripartite consortium of Serendipita indica, Trichoderma simmonsii, and bell pepper (*Capsicum annum*). Biol Control 158:104608

Romano JD, Kolter R (2005) *Pseudomonas-Saccharomyces* interactions: influence of fungal metabolism on bacterial physiology and survival. J Bacteriol 187(3):940–948

Sarma BK, Yadav SK, Singh S, Singh HB (2015) Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. Soil Biol Biochem 87:25–33

Saxena AK, Tilak KVBR (1994) Interaction among beneficial soil microorganisms. Ind J Microbiol 34:91–106

Scheuring I, Yu DW (2012) How to assemble a beneficial microbiome in three easy steps. Ecol Lett 15:1300–1307

Schippers B (1992) Prospects for management of natural suppressive ness to control soil borne pathogens. In: Tjamos EC, Papavizas GC, Cook RJ (eds) Biological control of plant diseases, progress and challengers for the future, NATO ASI Series A-Life Sciences, vol 230. Plenum Press, New York, pp 21–34

Schisler DA, Slininger PJ, Botahst RJ (1997) Effects of antagonist cell concentrations and two strain mixtures on biological control of *Fusarium* dry rot of potatoes. Phytopathology 25:339–358

Shanmugam V, Senthil N, Raguchander T, Ramanathan A, Samiyappan R (2002) Interaction of *Pseudomonas* with *Rhizobium* for their effect on the management of peanut root rot. Phytoparasitica 30:169–176

Shtienberg D, Elad Y (1997) Incorporation of weather forecasting in integrated biological-chemical management of *Botrytis cinerea*. Phytopathology 87:332–340

Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of *Pseudomonas* wilt of cucumber by chitinolytic bacteria. Phytopathology 89:92–99

Sneh B, Dupler M, Elad Y, Banker R (1984) Chlamydospore germination of *Fusarium oxysporum* f.sp. cucumerinum as affected by fluorescent and lytic bacteria from a *Fusarium* suppress soil. Phytopathology 74:1115–1124

Sung KC, Chung YR (1997a) Enhanced suppression of rice sheath blight using combination of bacteria which produce chitinases or antibiotics. In: Plant Growth-promoting rhizobacteria-Present status and future prospects. Proc Int. Workshop on Plant growth-promoting rhizobacteria, 4th. A. Ogosi, K. Obiayashi Printing, Sapporo, Japan. 370–372

Sung KC, Chung YR (1997b) Enhanced suppression of rice sheath blight using combination of bacteria which produce chitinases or antibiotics. In: Plant growth-promoting rhizobacteria-Present status and future prospects. Proc Int. Workshop on Plant growth-promoting rhizobacteria, 4th A. Ogosi, K.obiayashi Printing, Sapporo, Japan, pp. 370–372

Vishwakarma K, Kumar N, Shandilya C, Mohapatra S, Bhayana S, Varma A (2020) Revisiting plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture: A review. Front Microbiol 11:1–21

Wang Z, Sui Y, Li J, Tian X, Wang Q (2022) Biological control of postharvest fungal decays in citrus: a review. Crit Rev Food Sci Nutr 62(4):861–870

Wei G, Kloepper JW, Tuzun S (1991) Induced systemic resistance of cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86:221–224

Xavier LJC, Germida JJ (2002) Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy. Soil Biol Biochem 34(2): 181–188

Chapter 3
Rhizobacterial-Mediated Interactions for Enhanced Symbiotic Performance of the Root Nodule Rhizobia in Legumes

Ahmed Idris Hassen, Olubukola Oluranti Babalola, and Rene Carlson

Abstract The rhizospheres of several plant species including legumes harbour rhizobacteria that are endowed with various beneficial traits and which act as direct plant growth promoters, biological control agents or soil health enhancers due to their versatile enzymes and genes. In the symbiotic interaction between legumes and the rhizobia, free-living rhizobacteria play tremendous roles in facilitating the process of nodulation and nitrogen fixation, biological processes highly susceptible to soil abiotic factors in the rhizosphere. The rhizobacteria often referred to as plant growth-promoting rhizobacteria (PGPR) mediate significant synergistic interaction that involves the legume and the rhizobia resulting in improved functioning of symbiotic nitrogen fixation (SNF). One aspect of applied microbiology in agriculture involves the co-inoculation of legumes with rhizobia and PGPR that results in an improved symbiotic performance of several field-grown legumes. This occurs due to several PGPR traits that trigger essential interactions between the legume and its micro-symbiont rhizobia. PGPR traits including ACC deaminase activity, siderophore secretion, phosphate solubilization and the presence of abiotic stress genes are crucially essential in the rhizobium-legume interaction. This review highlights the mechanisms by which free-living rhizobacteria mediate the successful symbiotic performances in the legume-rhizobium interactions resulting in increased yield and quality of various economically important legumes globally.

A. I. Hassen (✉)
Agricultural Research Council, Plant Health and Protection, Pretoria, South Africa

Department of Plant and Soil Sciences, Faculty of Sciences, Engineering and Agriculture,
University of Venda, Thohoyandou, South Africa
e-mail: hassena@arc.agric.za

O. O. Babalola
Food Security and Safety Focus Area, Faculty of Natural & Agricultural Sciences, North-West University, Mmabatho, South Africa
e-mail: olubukola.babalola@nwu.ac.za

R. Carlson
Faculty of Natural and Agricultural Sciences, Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa

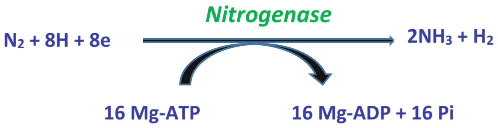
D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms
for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_3

Keywords Rhizobacteria · Rhizobia · Symbiosis · Nodulation · Nitrogen fixation · Legume · Abiotic stress

3.1 Introduction

Fertilizer N has had a tremendous impact on the global agricultural system for several decades, and it is typically remembered for what is called the Green Revolution of the 1970s and 1980s. This led to more than double increase in cereal production including that of rice, maize and wheat in most developing nations during that period (Conway 1998). Despite this, the prolonged usage of fertilizer N resulted in higher level of water pollution by increasing the concentration of toxic nitrates in drinking water supplies and eutrophication of lakes and rivers. Moreover, fertilizer N is not cost-effective as it is costly and 50% of the applied fertilizer N is quite often lost through leaching (Sprent and Sprent 1990; Zahran 1999; Subramanian et al. 2014; Amoo et al. 2021). The relatively high cost of fertilizer N as well as its impact on the environment that put pressure on world food production resulted in a current international emphasis on environmentally sustainable development that involves the use of renewable resources.

Biological nitrogen fixation (BNF), the process by which the atmospheric nitrogen (N_2) is converted into ammonia (NH_3), is one of the best examples of the application of renewable resources in sustainable agriculture. It is also one of the most extensively exploited and studied aspects of plant- microbe interactions in the soil due to its importance as the primary source of nitrogen (N) in agriculture (Zahran 1999). It is a process whereby many species of free-living and symbiotic bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) which will then be incorporated into organic compounds (e.g. protein, nucleic acid) of the bacteria and the associated plants (Amoo and Babalola 2017). The actual reduction of dinitrogen gas (N_2) in the atmosphere to the fixed nitrogen, which is ammonia (NH_3), that can be assimilated into amino acids is represented in the following equation, in which unreactive N_2 enters into the biologically active part of the global nitrogen cycle (Unkovich et al. 2008).



Both free-living and symbiotic bacteria undergo the above reaction using their complex enzyme system, the *nitrogenase complex* absent in all eukaryotic organisms. Many plants are therefore dependent on these nitrogen-fixing bacteria for their nitrogen nutrition requirement. The host plants supply carbon and energy source to the bacteria, while they receive ammonia (fixed nitrogen) to use it for their protein and nucleic acid metabolism. However, the nitrogen fixation by the free-living bacteria accounts only a few per cent of the amount fixed by symbiotic nitrogen fixation (SNF) in legumes which can yield as much as 100–300 kg N^{-1} ha^{-1} y^{-1} depending on the type of the legume host. Generally, inputs of BNF into the terrestrial ecosystem from SNF by rhizobia and their legume partners have been estimated to be at least 70 million tons of N per year (Zahran 1999).

3.2 Rhizobacterial Interaction in the Initiation of Symbiotic Nitrogen-Fixing Systems

The rhizosphere is the area about 1–2 mm around the roots and rich in nutrients and provides niches different from those in the bulk soil for the microorganisms to thrive (Babalola et al. 2021). Due to this, there occurs a lot of interactions between the free-living rhizobacteria, symbiotic rhizobia and their host legumes (Van Loon and Bakker 2003). The symbiotic relationship between rhizobia and the free-living plant growth-promoting rhizobacteria (PGPR) is well documented (Table 3.1). PGPR possess an array of traits that enhance the interaction between rhizobia and legumes including creation of additional infection sites for the rhizobia, secretion of plant growth-promoting substances and solubilization and uptake of nutrients (Mehboob et al. 2013). In the preceding sections, we will be looking into the different aspects of rhizobacterial-mediated interactions that can result in effective nodulation, nitrogen fixation and plant growth promotion.

3.2.1 Initiation of Nodule Formation and Development

Several reports in the past indicated that the symbiotic interaction between legumes and their micro-symbiont rhizobia can be enhanced by a group of ‘helper’ bacteria that reside near the roots in the rhizosphere (Vessey 2003). Many species of free-living plant growth-promoting rhizobacteria (PGPR) are involved in enhancing nodule formation by the symbiotic rhizobia (Adeleke et al. 2021). Some of the mechanisms include production of the phytohormone IAA and gibberellic acid that stimulate root elongation and increased density of root hairs that results in more colonization sites for the rhizobia (Cassan et al. 2009; Fox et al. 2011). This interaction is very crucial as legume nodules are initiated from root hairs. The effect of such interaction mediated by *Azospirillum* has been reported for several legume-rhizobium symbioses including chickpea, lentils, soybeans, common bean and alfalfa. In all cases, co-inoculation of *Rhizobium* and *Azospirillum* resulted in stimulation of root hair production, increased nodulation and nitrogen fixation (Hungria et al. 2013).

Table 3.1 Selected examples of rhizobacterial (PGPR)-mediated interactions that led to improved symbiotic performance and promotion of growth in the legume-rhizobium association

Legume crop	Associated PGPR	Symbiotic and growth promotion benefit	PGPR mode of action	References
Trifolium spp.	*Bacillus aryabhattai*, *Azotobacter vinelandii*	Increased nodulation, nitrogen fixation, leghaemoglobin content	Phosphate solubilization	Matse et al. (2020)
Phaseolus vulgaris	*Paenibacillus polymyxa*, *Bacillus megaterium*	Increased nodulation, nitrogen fixation, leghaemoglobin content	P solubilization, not measured but deduced	Korir et al. (2017)
Cicer arietinum	*Pseudomonas* spp., *Stenotrophomonas* sp., *Burkholderia* sp., *Sphingomonas* sp.	Increased nodulation	P solubilization, IAA production	Laabas et al. (2017)
Lens culinaris and *Cicer arietinum*	*Pseudomonas fluorescens*	Increased N fixation and leghaemoglobin content	Increased dehydrogenase activity and signalling	Babu et al. (2015)
Cicer arietinum	*Pseudomonas* sp.	Increased nodulation and leghaemoglobin content	P solubilization, siderophores, catalase, HCN, IAA	Kaur et al. (2015)
Lens culinaris	*Bacillus* sp., *Pseudomonas* sp.	Psychrotolerance	P solubilization, siderophores, HCN, IAA	Kaur et al. (2015)
Lens culinaris	*Pseudomonas* sp.	Increased nodulation	P solubilization	Khanna and Sharma (2011)
Lens culinaris	*Pseudomonas* sp., *Serratia* sp.	Increased nodulation	IAA production, ACC deaminase activity and P solubilization	Zahir et al. (2009)
Medicago sativa	*Burkholderia* sp.	Increased nodulation	P solubilization, siderophore secretion, ACC deaminase	Hassen et al. (2021)
Vigna radiata	*Pseudomonas putida*, *Pseudomonas fluorescens*	Increased nodulation, increased nodule biomass	ACC deaminase	Shaharoona et al. (2006)
Glycine max	*Pseudomonas* sp.	Enhanced plant growth	ACC deaminase	Husen et al. (2011)
Arachis hypogaea	*Pseudomonas fluorescens*, *Pseudomonas* sp.	Enhanced growth, yield and nutrient uptake	Siderophore, IAA, P solubilization	Dey et al. (2004), Saravankumar and Samiyappan (2006)

(continued)

Table 3.1 (continued)

Legume crop	Associated PGPR	Symbiotic and growth promotion benefit	PGPR mode of action	References
Vigna unguiculata	*Pseudomonas* sp.	Improved growth under salt stress	ACC deaminase	Trung and Thuam (2016)
Glycine max	*Pseudomonas putida* TSAU1	Improved root length, number of nodules per plant	IAA production P accumulation	Egamberdieva et al. (2017)
Beans *(Phaseolus vulgaris)*	*Azospirillum brasilense* Sp245	Increased nodule number and enhanced dry weight	IAA production	Remans et al. (2007)

3.2.2 *Induction of Flavonoid Secretion and Symbiotic Effectiveness*

The nodulation process starts with a signal exchange between the legume host and the bacterium with the secretion of *flavonoids*, specialized plant metabolites secreted from the cells just behind the roots under N stress condition, thereby attracting the bacteria to the roots (Lum and Hirsh 2003). Once secreted, the flavonoids interact with the rhizobial protein, the *Nod D*, that activates the transcription of the nodulation genes (nod genes) which in turn code the biosynthesis of the *Nod factors* (lipochitooligosaccharides). These Nod factors are the ones that elicit the deformation of the plant root hairs and support the rhizobial entry via infection threads (Perez-Montano et al. 2011) (Fig. 3.1).

Several beneficial free-living rhizobacteria have been found to induce the production of flavonoids by the legume roots in the rhizosphere. Parmer and Dadarwal (1999) reported that chickpea plants inoculated with the *Pseudomonas* strains induced the synthesis of flavonoids in the roots showing marked increase in the flavonoid level as compared to un-inoculated controls. In related experiments, the rhizobacteria *Azospirillum brasilense* promoted root branching in bean seedling roots and increased and persistent exudation of flavonoids by the bean roots that resulted in effective nodulation by the symbiotic strains *Rhizobium tropici* CIAT899 and *Rhizobium elti* ISP42 (Dardanelli et al. 2008).

An interesting example of symbiotic effectiveness from a multitrophic interaction point of view is described in a study that investigated the impact of co-inoculating *Medicago truncata* with its micro-symbiont *Sinorhizobium medicae* WSM419 and the PGPR *Pseudomonas fluorescens* WSM3457 (Fox et al. 2011). The result indicated that there was a significant increase in the rate of root nodule development with a large number of crown nodules as compared to control treatments not co-inoculated with *Pseudomonas*. Such beneficial interaction between the legume, their micro-symbionts and a free-living rhizobacteria has also been reported for several other legumes. For instance, in common bean, co-inoculation of *Rhizobium*

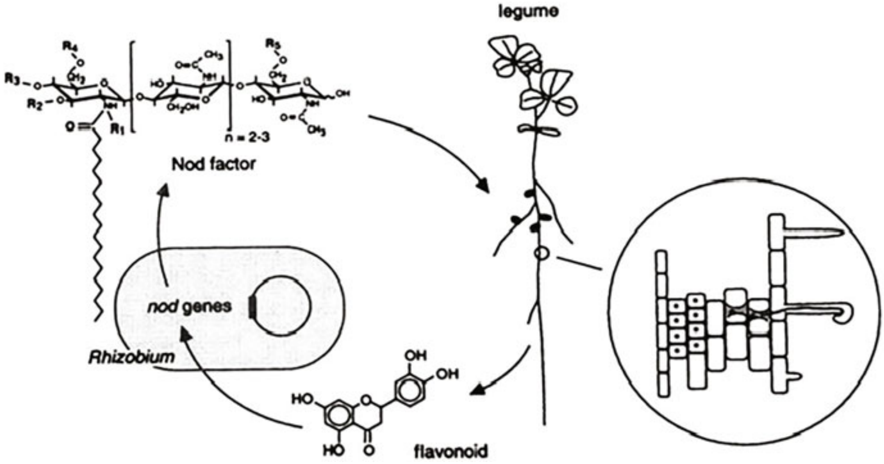


Fig. 3.1 Root nodule initiation following signal exchange in the rhizobium-legume symbiosis. Secretion of flavonoids induces rhizobial nod genes, which result in the production of the Nod factors. The Nod factors result in root hair deformation and rhizobial entry. Source: Shultze and Kondorosi (1998) with permission

tropici CIAT899 with the rhizobacteria *Azospirillum brasilense* promoted seedling branching and resulted in longer and persistent exudation of nod gene inducing flavonoids that enhance nodule formation and development (Gomez-Sagasti and Marino 2015). Several other investigations revealed that beneficial free-living rhizobacteria are involved in synergistic interactions in the rhizosphere of legumes that positively impact the metabolism of the host plants including the production of flavonoids. The PGPR *Chryseobacterium balustinum* strain Aur9, for instance, promotes nitrogen fixation by rhizobia in *Lupinus albus* and soybeans where various flavonoids were detected in the roots (Sugiyama and Yazaki 2014). Several other examples of rhizobacterial-mediated interactions that resulted in improved nodulation and promotion of growth in various legume species are indicated in Table 3.1.

3.3 Nutrient Acquisition and Abiotic Stress Tolerance

The presence of both the symbiotic root nodule rhizobia and the free-living indigenous soil microbial communities in the soil plays crucial roles in the growth and normal functioning of both leguminous and non-leguminous crops. In legumes, the symbiotic rhizobia mainly control the key functioning of nitrogen nutrition. At the same time, free-living microorganisms influence all other functions in the ecosystem including nutrient acquisition, carbon cycling, soil structure maintenance, biological control and abiotic stress tolerance. In this section, we concentrate on the role of these synergistic interactions in nutrient acquisition as well as abiotic stress tolerance in the legume-rhizobium symbiosis.

3.3.1 Induced Systemic Tolerance (IST) in the Legume-Rhizobium Symbiosis

The legume-rhizobium symbiosis and nodule formation are very sensitive to abiotic stresses including drought, salinity, acidity and osmotic stress as these inhibit the initial steps of the symbiotic interaction (Zahran 1999). For instance, the number of nodules, amount of nitrogen fixed and plant biomass of legumes are found to be negatively affected to a greater extent when the pH of the soil drops to as low as ≤4 (Vassilieva et al. 1997). Drought stress is also known to have a negative effect on nitrogen fixation activity of nodules, thus resulting in a reduced growth in legumes (Streeter 2003). However, it is not only the legumes that are directly affected by these abiotic stresses but also the rhizobia micro-symbionts of the host legumes which are sensitive to drought and other abiotic stresses resulting in a significant decrease in nitrogen fixation (Yang et al. 2009). Other abiotic stresses including soil nitrate, temperature and heavy metals such as aluminium (Al^+) toxicity are also known to affect nodulation and inhibit the actual nitrogen fixation process (Walsh 1995). Generally, these abiotic factors affect nodule structure, impair nodule functioning and induce drastic metabolic and molecular modifications leading to a stress-induced senescence (SIS) which ultimately affects the entire process of symbiotic nitrogen fixation (Dupont 2012). To counteract the negative impacts of these abiotic stresses, certain PGPR trigger a system of defence by plants known as *induced systemic tolerance* (IST). The term IST was initially proposed by Yang et al. (2009) to describe PGPR-induced physical and chemical changes in plants that result in increased tolerance to various abiotic stresses.

Most plants require ethylene biosynthesis for the purpose of breaking seed dormancy. However, higher level of ethylene following germination is quite often inhibitory to root elongation. The synthesis of a high level of ethylene from the precursor 1-aminocyclopropane-1-carboxylic acid (ACC) during biotic and abiotic stress conditions would result in retarded root growth and cause senescence in the plant (Saravankumar and Samiyappan 2006). Interactions mediated by beneficial rhizobacteria play significant roles in alleviating abiotic stress in various legumes. Several *Pseudomonas* spp. have significant ACC deaminase activity that results in a decreased level of ethylene in many legume roots, which in turn results in increased root elongation and enhanced nodulation by the rhizobium strains (Gomez-Sagasti and Marino 2015). Inoculating legumes with mixed cultures of rhizobium and ACC deaminase-positive PGPR promotes nodulation through inhibition of ethylene biosynthesis thereby enhancing nodulation and nitrogen fixation. A few examples include early growth and promotion of nodulation in *Glycine max* by ACC deaminase rhizobacteria and enhanced nodulation in *Pisum sativum* by ACC deaminase *Rhizobium leguminosarum* bv. viciae 128C53K (Cattelane et al. 1999). The mechanisms by which plants develop an IST to various abiotic stresses including drought, salinity and nutrient deficiency as a result of the interaction mediated by plant growth-promoting rhizobacteria are indicated in Fig. 3.2. Interactions with plant growth-promoting bacteria have also been reported to alleviate abiotic stresses and

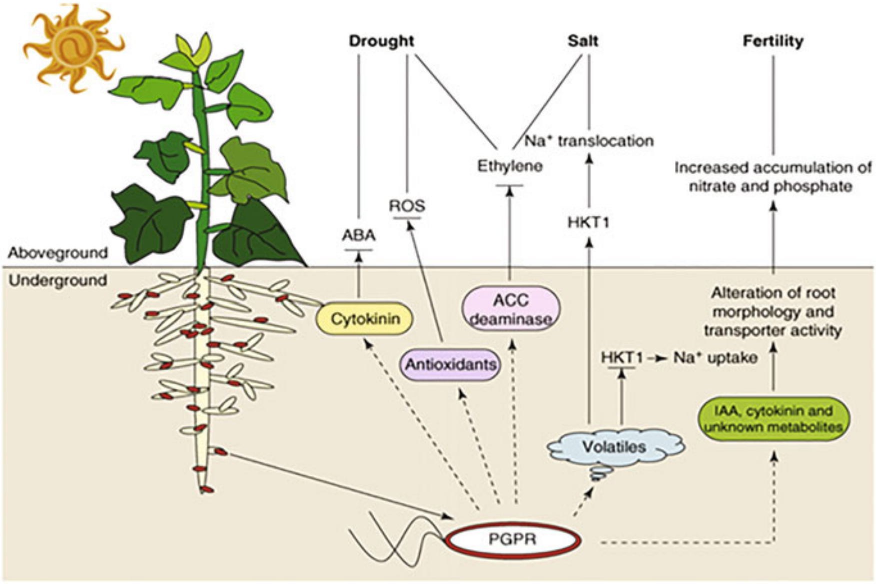


Fig. 3.2 A schematic illustration of the mechanisms by which PGPR mitigate various abiotic stresses such as drought, salinity and nutrient deficiency (fertility) stress and elicit induced systemic tolerance (IST) in plants. Source: Yang et al. (2009), *Trends in Plant Science*, with permission from J. Kloepper

improve growth of non-leguminous plants. For instance, improved growth and tolerance to drought stress were observed in maize plants inoculated with two ACC deaminase and IAA-producing actinomycete strains, *Arthrobacter arilaitensis* and *Streptomyces pseudovenezuelae* (Chukwuneme et al. 2020).

3.3.2 *Rhizosphere Interaction for Iron (Fe^{+3}) Acquisition*

Iron (Fe^{+3}) acquisition by the symbiotic nitrogen-fixing rhizobia is essential, and it is required in a relatively high amount by the nitrogen-fixing bacteroides in the root nodules to support nitrogen fixation. This is because of the nitrogenase enzyme system which has a high iron requirement for the synthesis of the iron sulphur centre in the enzyme complex (Fig. 3.3). Iron is also required for the synthesis of leghaemoglobin, a structure in the nodules used to deliver oxygen to the nodules, and that gives the characteristic pink to red colour to effective nodules that fix nitrogen (Crowley 2006). However, although iron is the fourth most abundant element in the earth's crust, it is essentially unavailable under aerobic environment at biological pH as it tends to form Fe^{+3} oxyhydroxide complex (Geetha and Joshi 2013; Olanrewaju and Babalola 2019).

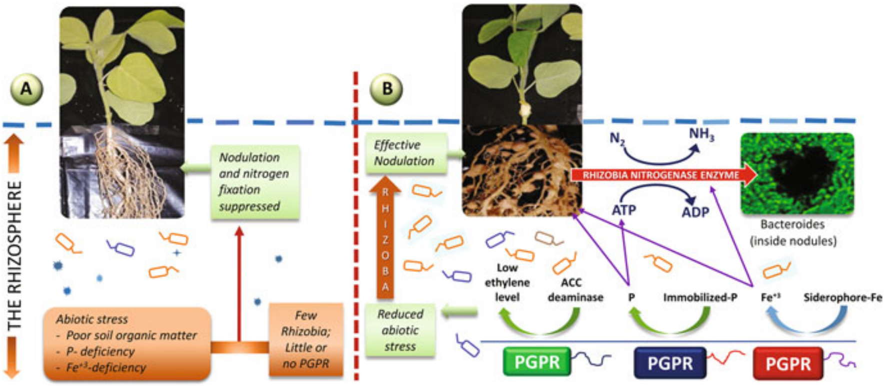


Fig. 3.3 Schematic illustration indicating the beneficial interaction between plant growth-promoting rhizobacteria (PGPR) and symbiotic rhizobia. The rhizosphere soil in compartment A is poor in soil organic matter and available phosphorus (P), iron (Fe^{+3}) and other essential nutrients with very few ineffective rhizobia and little or no PGPR. The overall effect results in suppression of root nodule formation and nitrogen fixation. In compartment B, the immobilized nutrients such as P and Fe are easily available to the plants and the rhizobia due to the presence of several PGPR strains with phosphate solubilization and siderophore production ability. The abiotic stress on the roots caused by high ethylene level is also reduced due to the ACC deaminase activity of the PGPR. The solubilized P will be used by the legume plant, and part of it is used as the source of ATP by the energy-demanding nitrogenase enzyme of the rhizobia that converts atmospheric nitrogen (N_2) into ammonia (NH_3) in the nodule bacteroides. Similarly, the chelated iron by the siderophores of the PGPR will be directly used by the legume and as part of the iron-sulphur centre of the rhizobia nitrogenase enzyme complex. All these traits contributed to effective nodule formation and hence nitrogen fixation

Under the above-mentioned condition of iron deficiency in the soil, microorganisms produce *siderophores*, structurally diverse low molecular weight compounds that bind ferric iron (Fe^{+3}) with high affinity. Bacterial siderophores are used by many plants as iron source for growth as they have direct benefits to plant growth promotion by acting as a direct source of iron and making it available to plants (Yehunda et al. 1996; Vansuyt et al. 2007; Olanrewaju et al. 2017). Iron (Fe^{+3}) and molybdenum (Mo) are very much required both by the free-living and symbiotic nitrogen-fixing diazotrophic bacteria not only for the electron shuttle reactions but also as a component of the nitrogenase complex. Symbiotic rhizobia require iron to grow in the rhizosphere and for optimum nodulation and development of the bacteroides. Thus, siderophore-producing rhizobia have an added advantage as it helps the bacteria by incorporating the iron and molybdenum into the nitrogenase enzyme complex. This suggests that siderophores are required for adequate nitrogen fixation by the symbiotic rhizobia (Tang et al. 1992). Rhizobia that are not capable of producing their own siderophores in iron-limiting soils have less competitive advantage than those producing siderophores (Adeleke et al. 2021). However, such rhizobia are able to use heterologous siderophores, the iron-bound siderophore complex, produced by other non-symbiotic rhizobacteria living in their vicinity.

Pseudomonas fluorescens are found to be efficient providers of heterologous siderophores to enhance plant nutrition and improve the legume-rhizobium symbiosis in many instances (Sharma et al. 2003; Geetha and Joshi 2013). Such heterologous siderophore utilization by the symbiotic rhizobia is widespread in various legumes. In the report by Hassen et al. (2012) that investigated rhizobia associated with the nodulation of the indigenous beverage rooibos (*Aspalathus linearis* Burm. f.), several endophytic rhizobacteria such as *Burkholderia* and *Herbaspirillum* spp. have been found to be associated with the nodules of this native legume. The production of siderophore by *Herbaspirillum seropedicae* SARCC-619 and SARCC-636 isolated from the nodules of *Aspalathus linearis* as non-rhizobial endophytes (NRE) is considered as an added advantage for the nitrogen-fixing symbionts. This is because the iron (Fe^{+3}) chelated by the siderophores of the non-rhizobial endophytes could be utilized by the rhizobia-legume symbiotic process (unpublished data from author's lab). The presence of bacteria other than the nitrogen-fixing *Rhizobia* spp. within the nodule microbiome has been discussed in a recent review by Martinez-Hidalgo and Hirsh (2017), which elaborated that nitrogen-fixing rhizobia do not live alone within the nodules and many of these endophytes enhance the process of biological nitrogen fixation. In another study by Hassen et al. (2021), improved nodulation and plant biomass have been reported after co-inoculation of *Medicago sativa* L. with its rhizobium symbiont *Sinorhizobium meliloti* strain RF14 and the PGPR *Burkholderia* sp. strain Nafp2/4-1b. The PGPR strain, previously screened for its significant growth promotion effect on maize (Khambani et al. 2019), possesses several plant growth-promoting traits such as the production of siderophores, P solubilization, IAA acid and ACC deaminase all of which are essential for effective nodulation by the rhizobia and increased growth in the legume (Fig. 3.4).

3.3.3 Phosphorus Acquisition for SNF

Phosphorus (P) is the other major essential element required for both the process of nodulation and symbiotic nitrogen fixation. Although P is considered as the second most essential nutrient after nitrogen and the most abundant in agricultural soils, it is not easily available to plants as the majority of it is insoluble (Oteino et al. 2015). The main reason for the unavailability of P to plants despite its abundance in the soil is that it is fixed by free oxides and hydroxides of aluminium (Al) and iron (Fe) in acidic soil and by calcium (Ca) in alkaline soils (Rodriguez and Fraga 1999). Natural rock phosphates are also good sources of P despite that most of the P in rock phosphate exist as non-exchangeable form and hence not directly available for uptake by plants (Yu et al. 2012). Therefore, this net unavailability of P in many soils makes it the major growth-limiting factor for several plants.

In the legume-rhizobium symbiosis, the nodule bacteria require adequate concentrations of phosphorus (P) along with other essential nutrients as P is very crucial for the energy-demanding nitrogenase enzyme that converts atmospheric nitrogen

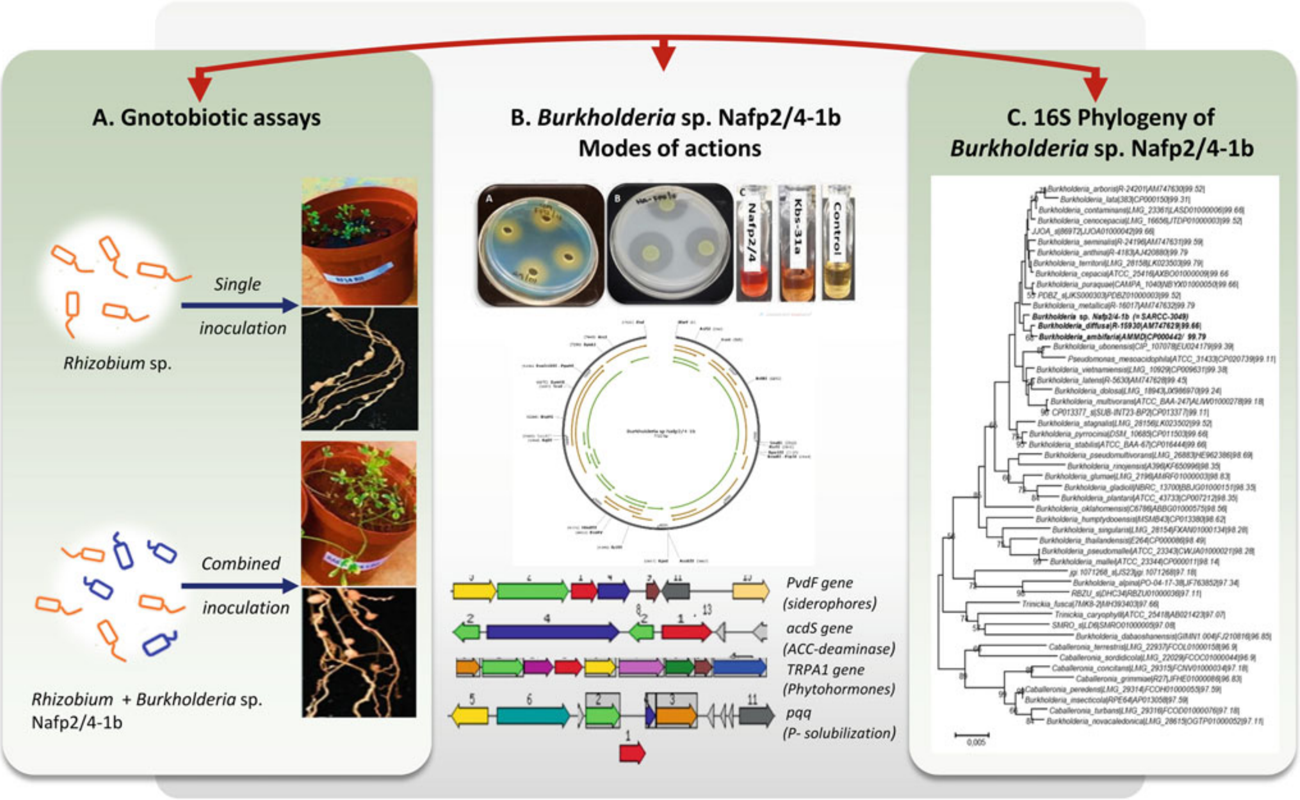


Fig. 3.4 Rhizobacterial-mediated interaction between a plant growth-promoting rhizobacteria *Burkholderia* sp. SARCC-3049 and a rhizobium micro-symbiont of the legume *Medicago sativa* in a study that involved gnotobiotic nodulation test (**a**), in vitro mode of action and genomic characterization (**b**) and phylogeny of the PGPR (**c**). Source: Graphic abstract in Hassen et al. (2021)

(N_2) to plant-available form of ammonia (NH_3) (Schulze et al. 2011; O'Hara 2001). It is therefore apparent that phosphorus limitation can reduce legume productivity not only by its effect on nitrogenase activity of the rhizobium but also by negatively affecting nodule development and function. Phosphorus is therefore one of the principal yield-limiting nutrients in the legume-rhizobium interaction. A synergistic interaction occurs between certain phosphate-solubilizing free-living bacteria and the legume-rhizobium. Co-inoculation of rhizobia with phosphate solubilizing bacteria could have a positive effect on symbiotic parameters such as increasing nodule number and plant biomass which results in grain yield of legumes (Saharan and Nehra 2011). In the report by Kumar et al. (2011), co-inoculation of groundnut with a consortium of bacteria comprising *Rhizobium* strain Tt 9 with the PGPR *Bacillus megaterium* var. phosphaticum resulted in fulfilling about 50% of the phosphatic fertilizer requirement of the groundnut thereby improving nodulation, plant growth and yield. Similar study indicated that inoculation of common bean with *R. leguminosarum* and P-solubilizing *Bacillus megaterium* increased N and P solubility that resulted in increased nodulation and improvement in growth and yield (Elkoka et al. 2010). In general, there is a promising trend of the practice of co-inoculation of rhizobia and PGPR in the development of sustainable agriculture in the future.

3.4 Concluding Summary

Rhizobacteria including *Azospirillum* and *Pseudomonas* species assist the symbiotic rhizobia to thrive under various abiotic stresses in the rhizosphere and to increase their symbiotic performance. The rhizobacteria assist the symbiotic rhizobia and enhance SNF through promotion of root hair formation and root development, flavonoid secretion and creating more potential colonization sites for the rhizobia infection. Acquisition of essential but usually unavailable nutrients such as iron (Fe^{+3}) and phosphorus (P) that are crucially needed by the symbiotic rhizobia is made possible by the synergistic interaction. The rhizobia in turn fix the non-reactive, unavailable atmospheric nitrogen (N_2) which would be used by all other organisms not capable of fixing nitrogen. Generally, synergistic interaction exists between legumes, their rhizobial micro-symbionts and certain free-living plant growth-promoting rhizobacteria (PGPR) which ultimately results in improving symbiotic nitrogen fixation (SNF) in several legume crops.

Acknowledgement Not applicable.

Conflict of Interest Authors declare no conflict of interest.

References

Adeleke BS, Babalola OO, Glick BR (2021) Plant growth-promoting root-colonizing bacterial endophytes. Rhizosphere 20:100433

Amoo AE, Babalola OO (2017) Ammonia-oxidizing microorganisms: key players in the promotion of plant growth. J Soil Sci Plant Nutr 17:935–947

Amoo AE, Enagbonma BJ, Ayangbenro AS, Babalola OO (2021) Biofertilizer: an eco-friendly approach for sustainable crop production. In: Babalola OO (ed) Food security and safety. Springer, Cham. https://doi.org/10.1007/978-3-030-50672-8_32

Babalola OO, Emmanuel OC, Adeleke BS, Odelade KA, Nwachukwu BC, Ayiti OE, Adegboyega TT, Igiehon NO (2021) Rhizosphere microbiome cooperations: strategies for sustainable crop production. Curr Microbiol 78(4):1069–1085

Babu S, Prasanna R, Bidyarani N, Nain L, Shivay YS (2015) Synergistic action of PGP agents and *Rhizobium* spp. for improved plant growth, nutrient mobilization and yields in different leguminous crops. Biocat Agric Biotechnol 4(4):456–464

Cassan F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009) *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (Glycine max L.). Eur J Soil Biol 45:28–35

Cattelane AJ, Hartela PG, Fuhrman JJ (1999) Screening for plant growth promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 34:635–648

Chukwuneme CF, Babalola OO, Kutu FR, Ojuederie OB (2020) Characterization of actinomycetes isolates for plant-growth promoting traits and their effects on drought tolerance in maize. J Plant Interact 15:93–105

Conway G (1998) The doubly green revolution: food for all in the twenty-first century. Comstock Pub, Ithaca, NY. ISBN 0-8014-8610-6

Crowley DE (2006) Microbial siderophores in the plant rhizosphere. In: Barton LL, Abadia J (eds) Iron nutrition in plants and rhizospheric microorganism. Springer, Netherland, pp 169–198

Dardanelli MS, de Cordoba FJF, Espuny MR, Carvajal MAR, Diaz MES, Serrano AMG, Okon Y, Megías M (2008) Effect of *Azospirillum brasilense* co-inoculated with Rhizobium on *Phaseolus vulgaris* flavonoids and nod factor production under salt stress. Soil Biol Biochem 40:2713–2721

Dey R, Pal KK, Bhat DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. Microbiol Res 159:371–394

Dupont L, Geneviève Alloing, Olivier Pierre, Sarra El Msehli, Julie Hopkins, Didier Hérouart and Pierre Frendo (2012). In: Tetsuji N (ed) The legume root nodule: from symbiotic nitrogen fixation to senescence. In Tech, http://www.intechopen.com/books/senescence/the-legume-root-nodule-from-symbiotic-nitrogen-fixation-tosenescence. Accessed 2021

Egamberdieva D, Wirth S, Jabborova D, Räsänen LA, Liao H (2017) Coordination between Bradyrhizobium and Pseudomonas alleviates salt stress in soybean through altering root system architecture. J Plant Interact 12:100–107

Elkoka E, Turan M, Donmez MF (2010) Effects of single, dual and multiple inoculation with *Bacillus subtilis*, *Bacillus megaterium* and *Rhizobium leguminosarum* bv. phaseoli on nodulation, nutrient uptake, yield and yield parameters of common bean (*Phaseolus vulgaris*) l. cv. elkora 05. J Plant Nutr 33:2104–2119

Fox SL, O'Hara GW, Brau L (2011) Enhanced nodulation and symbiotic effectiveness of *Medicago truncatula* when co-inoculated with *Pseudomonas fluorescens* WSM3457 and *Ensifer* (*Sinorhizobium) medicae* WSM419. Plant Soil 348:245–254

Geetha SJ, Joshi SJ (2013) Engineering rhizobacterial bioinoculants: a strategy to improve iron nutrition. Sci World J 2013:1–15

Gomez-Sagasti MT, Marino D (2015) PGPR and nitrogen-fixing legumes: a perfect team for efficient cd phytoremediation. Front Plant Sci 6:1–9

Hassen AI, Bopape FL, Lamprecht SC, Habig J (2012) Nodulation of rooibos (*Aspalathus linearis* burm. f.), an indigenous South African legume by members of both the α-proteobacteria and β-proteobacteria. Biol Fert Soils 48:295–303

Hassen AI, Khambani LS, Swanevelder ZH, MtswenI NP, Bopape FP, van Vuuren A, van der Linde EJ, Morey L (2021) Elucidating key plant growth–promoting (PGPR) traits in *Burkholderia* sp. Nafp2/4-1b (=SARCC-3049) using gnotobiotic assays and whole-genome-sequence analysis. Lett Appl Microbiol 73(5):658–671. https://doi.org/10.1111/lam.13556

Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and Azospirilla: strategies to improve sustainability. Biol Fert Soils 49:791–801

Husen E, Wahyudi AT, Suwanto A (2011) Growth enhancement and disease reduction of soybean by 1-aminocyclopropane-1-carboxylate deaminase producing Pseudomonas. Am J Appl Sci 8: 1073–1080

Kaur N, Sharma P, Sharma S (2015) Co-inoculation of *Mesorhizobium* sp. and plant growth promoting rhizobacteria Pseudomonas sp. as bio-enhancer and bio-fertilizer in chickpea (*Cicer arietinum* L.). Legume Res Int J 38:367–374

Khambani LS, Hassen AI, Regnier T (2019) Rhizospheric bacteria from pristine grassland have beneficial traits for plant growth promotion in maize (*Zea mays* L.). Cog. Biol 5:1630972. https://doi.org/10.1080/23312025.2019.1630972

Khanna V, Sharma P (2011) Potential for enhancing lentil (*Lens culinaris*) productivity by co-inoculation with PSB, plant growth-promoting rhizobacteria and Rhizobium. Indian J Agric Sci 81(10):932

Korir H, Mungai NW, Thuita M, Hamba Y, Masso C (2017) Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front Plant Sci 8:141

Kumar A, Prakash A, Johri BN (2011) *Bacillus* as PGPR in crop ecosystem. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer, Berlin, pp 37–58

Laabas S, Boukhatem ZF, Bouchiba Z, Benkritly S, Abed NE, Yahiaoui H, Tsaki H (2017) Impact of single and co-inoculations with Rhizobial and PGPR isolates on chickpea (*Cicer arietinum*) in cereal-growing zone soil. J Plant Nutr 40:1616–1626

Lum MR, Hirsh AM (2003) Roots and their symbiotic microbes: strategies to obtain nitrogen and phosphorus in a nutrient limiting environment. J Plant Growth Regul 21:368–382

Martinez-Hidalgo P, Hirsh AM (2017) The nodule microbiome: N_2-fixing rhizobia do not live alone. Phytobiomes 1:70–82

Matse DT, Huang CH, Huang YM, Yen MY (2020) Effects of coinoculation of Rhizobium with plant growth promoting rhizobacteria on the nitrogen fixation and nutrient uptake of Trifolium repens in low phosphorus soil. J Plant Nutr 43:739–752

Mehboob I, Naveed M, Zahir ZA, Sessitsch A (2013) Potential of rhizosphere bacteria for improving rhizobium-legume symbiosis. In: Plant microbe symbiosis: fundamentals and advances. Springer, New Delhi, pp 305–349

O'Hara GW (2001) Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review. Aust J Exp Agric 41:417–433

Olanrewaju OS, Babalola OO (2019) Streptomyces: implications and interactions in plant growth promotion. App Microbiol Biotechnol 103:1179–1188

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. W J Microbiol Biotechnol 33:1–16

Oteino N, Lally RD, Kiwamaka S, Lloyd A, Ryan D, Germaine KJ, Dowling D (2015) Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. Front Microbiol 6:745

Parmer N, Dadarwal KR (1999) Stimulation of nitrogen fixation and induction of flavonoid like compounds by rhizobacteria. J Appl Microbiol 86:36–44

Perez-Montano F, Guasch-Vidal B, Gonzalez-Barroso S, Lopez-Baena FJ, Cubo T, Ollero FJ, Gil-Serrano AM, Rodriguez-Carvajal MA, Bellogin RA, Espuny MR (2011) Nodulation gene inducing flavonoids increase overall production of autoinducers and expression of N-acyl homoserine lactone synthesis genes in rhizobia. Res Microbiol 162:715–772

Remans R, Croononborghs A, Gutierrez RT, Michiels J, Vanderleyden J (2007) Effects of plant growth promoting rhizobacteria on nodulation of Phaseolus vulgaris L. are dependent on P nutrition. Eur J Plant Pathol 119:341–351

Rodriguez H, Fraga R (1999) Phosphate solubilising bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res LSMR-21:1–2

Saravankumar D, Samiyappan R (2006) ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in ground nut (*Arachis hypogea*) plants. J Appl Microbiol 102:1283–1292

Schulze J, Mohamed MAN, Carlsson G, Drevon JJ (2011) Phosphorus deficiency decreases nitrogenase activity but increases proton efflux in N2-fixing *Medicago truncatula*. Plant Physiol Biochem 49:458–460

Shaharoona B, Arshad M, Zahir ZA (2006) Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (Zea mays L.). Lett Appl Microbiol 42:155–159

Sharma A, John BN, Sharma AK, Glick BR (2003) Plant growth promoting bacterium *Pseudomonas* sp. GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35:887–894

Shultze M, Kondorosi A (1998) Regulation of symbiotic root nodule development. Ann Rev Genet 32:33–57

Sprent JI, Sprent P (1990) Nitrogen-fixing organisms. Pure and applied aspects. Chapman and Hall, London

Streeter JG (2003) Effects of drought on nitrogen fixation in soybean roots. Plant Cell Environ 26: 1199–1204

Subramanian G, Sathya A, Vijayabharathi R, Varshney RK, Gowda CLL, Krishnamurthy L (2014) Plant growth promoting rhizobia: challenges and opportunities

Sugiyama A, Yazaki K (2014) Flavonoids in plant rhizosphere: secretion, fate and their effects on biological communication. Plant Biotechnol 31(5):431–443. https://doi.org/10.5511/plantbiotechnology.14.0917a

Tang C, Robinson AD, Dilworth MJ (1992) The role of iron in the (Brady) rhizobium legume symbiosis. J Plant Nutr 15:2235–2252

Trung NT, Thuam NH (2016) Screening of strong 1-aminocyclopropane-1-carboxylate deaminase producing bacteria for improving the salinity tolerance of cowpea. Appl Microbiol 2:100111

Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey B, Giller K, Giller K, Alves B, Chalk P (2008) Measuring plant associated nitrogen fixation in agricultural systems ACIAR Monograph No 136, pp 258

Van Loon LC, Bakker PAHM (2003) Signaling in rhizobacteria-plant interactions. In: de Kroon H, Visser EWJ (eds) Ecological studies, vol 168. Springer, Berlin, p 1

Vansuyt G, Robin A, Briat JF, Curie C, Lemanceau (2007) Iron acquisition from Fe-Pyoverdine by *Arabidopsis thaliana*. Mol Plant Micr Inter 20:441–447

Vassilieva V, Milanove G, Ignatov G, Nikolov B (1997) Effect of low pH on nitrogen fixation of common bean growth at various calcium and nitrate levels. J Plant Interact 20:279–294

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Walsh KB (1995) Physiology of the legume nodule and its response to stress. Soil Biol Biochem 27: 637–655

Yang Y, Kloepper JW, Ryu C-M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yehunda Z, Shenker M, Romheld V, Marshner H, Hadar Y, Chen Y (1996) The role of ligand exchange in the uptake of iron from microbial siderophores by gramineous plants. Plant Physiol 112:1273–1281

Yu X, Liu X, Zhu T-H, Liu G-H, Mao C (2012) Co-inoculation with phosphate-solubilizing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut. Eur J Soil Biol 50:112–117

Zahir ZA, Ghani U, Naveed M, Nadeem SM, Asghar HN (2009) Comparative effectiveness of Pseudomonas and Serratia spp. containing ACC deaminase for improving growth and yield of wheat (Triticum aestivum) under salt stressed conditions. Arch Microbiol 191:415–424

Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in arid climate. Microbil Mol Biol Rev 63:968–989

Chapter 4
Plant Growth-Promoting Bacterial Consortia Render Biological Control of Plant Pathogens: A Review

Marika Pellegrini, Rihab Djebaili, Giancarlo Pagnani, Daniela M. Spera, and Maddalena Del Gallo

Abstract Plant disease management via biocontrol of phytopathogens is one of the major approaches to the imagination of sustainable agriculture. Besides agrochemicals, using inoculants of beneficial bacteria is a common tool to control fungal and bacterial phytopathogens; they are known as biological control agents (BCAs) or biopesticides. This chapter focuses on using PGPB (plant growth-promoting bacteria) in consortia to control bacterial and fungal pathogens. The most relevant publications on biocontrol brought by PGPB in consortia are reviewed, casting insight into the core mechanisms used to achieve effective biocontrol and disease management. In this review, the compatibility and diversity of beneficial PGPB strains, their stability in bioformulations, environmental safety, and association with various plants have been covered with special emphasis on the mechanisms of biocontrol. There is an urgent requirement for optimization and adjustment of effective PGPB in consortia that can help to improve the biocontrol of phytopathogens in the rhizosphere and increase crop productivity while safeguarding human health and environmental integrity.

Keywords PGPB · Bacterial consortia · Biocontrol · Fungal and bacterial phytopathogens

---

M. Pellegrini (✉) · R. Djebaili · D. M. Spera · M. Del Gallo
Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy
e-mail: marika.pellegrini@univaq.it; rihab.djebaili@guest.univaq.it; maddalena.delgallo@univaq.it

G. Pagnani
Faculty of Bioscience and Technologies for Food, Agriculture and Environment, University of Teramo, Teramo, Italy
e-mail: gpagnani@unite.it

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_4

4.1 Introduction

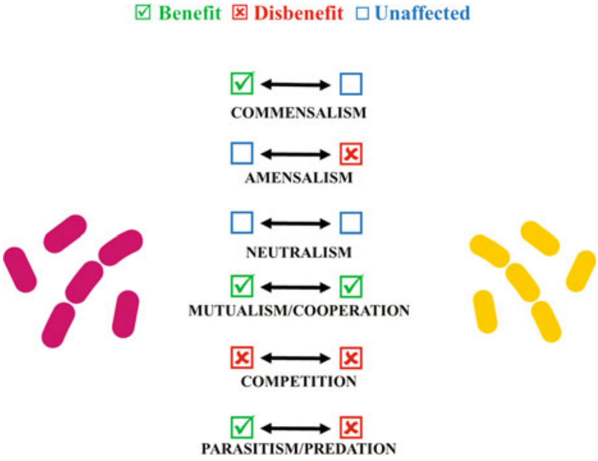
Plant growth-promoting bacteria (PGPB) are a broad group of microbes that promote plant growth directly and indirectly (Glick 2012; Jain et al. 2022). Direct activity includes nitrogen fixation, mineral solubilization, phytohormone production or modulation, and siderophore release (Chandran et al. 2021). PGPB also indirectly reduce or prevent the negative impacts of one or more plant diseases (Massa et al. 2022). This indirect activity can produce harmful compounds or enhance plant resistance to biotic and abiotic stresses (Beneduzi et al. 2012). Since their first description (Kloepper and Schroth 1978), many PGPB strains have been selected and tested for their biostimulant abilities. Many workers have also investigated biocontrol activity against a wide range of phytopathogens. Findings so far revealed the suitability of synthetic microbial consortia for many agricultural applications (Gupta et al. 2020; Liang et al. 2022). Generally, consortia are most efficient than single-strain formulations (Deter and Lu 2022). Thanks to synergistic associations among the different strains, consortia boost plant growth and protect it from phytopathogens (Ram et al. 2022). Furthermore, the effectiveness of microbial consortia is high due to the existence of multiple strains which exhibit superior performance in functional behaviors to tolerate environmental changes (Compant et al. 2019; Hussain et al. 2022). In the last few decades, microbial consortia have primarily been used as plant or soil inoculants to improve agricultural productivity, soil nutrient status, and crop quality and as biocontrol agents to control the growth of devastating phytopathogens during harvest or postharvest storage (Gupta et al. 2022; Jiao et al. 2021; Saeed et al. 2021; Seenivasagan and Babalola 2021; Verma et al. 2022). PGPB consortia use completely complies with the United Nations' 2030 Agenda for Sustainable Development Goals, and many products are already available on the markets. Still, more research is needed to meet the market's global demand for biostimulant and biocontrol agents. Biological, technological, regulatory, infrastructural, financial, marketing, field-level, quality, carrier, and biosafety restrictions affect microbial-based products' use, industrialization, and commercialization (Basu et al. 2021). These numerous limits make creating PGPB formulations and their usage in sustainable agriculture a big task. Scientific, industrial, and agricultural experiments and a high degree of knowledge are linked to this market. The biofertilizer trend is already rising (Joshi and Gauraha 2022). Without active collaboration from the scientific community, industries, and farms, the market risks being unable to meet the growing demand for biofertilizers (Joshi and Gauraha 2022; Pellegrini et al. 2020b). The scientific community and industry should promote the use of PGPB consortia through scientifically sound research. From this perspective, this book chapter aimed to report PGPB (plant growth-promoting bacteria) consortia use to manage bacterial and fungal diseases. The most relevant publications on PGPB consortia biocontrol are examined, providing insight into PGPB's processes and experiments that resulted in successful biocontrol against bacterial and fungal diseases.

4.2 Plant Growth-Promoting Bacterial Consortia

In nature, microbe does not live isolated. Like all organisms, their association with other microorganisms/organisms is obvious and has been studied widely. Synergistic associations allow microorganisms to carry out essential and complex tasks in several natural processes (Asmamaw and Fassil 2014). Inspired by natural microbial consortia, the scientific community has developed artificial microbial consortia such as in Fig. 4.1.

Many interactions occur in the formulation of microbial consortia. Microbial interactions are essential for creating and maintaining a microbial population in a variety of settings and on a variety of hosts (Braga et al. 2016). Many years of coevolution between the species resulted in interdependent adaptation and specialization, as well as a variety of symbiotic connections (Braga et al. 2016). Figure 4.1 depicts various sorts of interactions that might arise during the establishment of microbial consortia. Commensalism is described as a relationship in which one partner benefits while the other is unaffected. When one of the partners is suppressed or destroyed while the other remains undamaged, this is known as amensalism. There is interaction in neutralism, but it does not affect the partners engaged. Mutualism and collaboration characterize a scenario in which both parties benefit. In contrast, both partners lose out in a competition. The other partner benefits from parasitism and predation. Microbial consortia are resilient to biotic and abiotic stressors and have multiple functions because of synergistic interactions that achieve what individual strains cannot realize. Microbial interactions can involve a wide range of processes, dependencies, and dynamical features, in addition to being simply positive or negative, helpful, or suppressive. This complexity of interactions requires further research into cutting-edge methodologies that allow for the understanding of multidimensional and dynamic relationships among microorganisms and between bacteria and the host (Weiland-Bräuer 2021). As the number of partners

Fig. 4.1 Graphical representation of the interactions that occur during microbial consortia formation



increases, the community complexity grows as a result of the combinatorial growth in the number of pairwise interactions and the formation of higher-order interactions (Duncker et al. 2021; Wootton 2002). A third partner, for example, can alter the strength or nature of a paired interaction, and a fourth can then alter that interaction (Bairey et al. 2016). The types and intensity of these interactions can affect a community's stability, and computer modeling can be used to forecast community composition over time (Bairey et al. 2016; Blanchard and Lu 2015; Coyte et al. 2015). The presence of numerous strains reduces the metabolic burden, optimizes the division process of labor, and broadens the substrate spectrum (Giri et al. 2020; Qian et al. 2020). In agriculture, there is a need for this multi-functional biofertilizer and biocontrol agents to achieve sustainable production and biotic and abiotic stress control (Kour et al. 2020). The technology involved in producing bacterial consortia is pretty simple and cheap, and enormous progress has been made recently. The knowledge of plant physiology, biochemistry, and biology, the application of several bioassays and investigation techniques, and the integration of the data recovered from this knowledge are mandatory to establish and validate the activities and synergies within PGPB microbial formulations (Povero 2020). Many authors have studied the technologies and commercial aspects of consortia production and the diversity of PGPB (Arora et al. 2011). PGPB consortia formulations should have:

1. Safety – it must be safe for the environment (not a pollutant, biodegradable) and animal and human health.
2. Economic bioprocess and low-cost, accessible, and sufficient raw material supply and pH adjustments.
3. Stable formulation and long shelf life and be able to maintain metabolically viable high numbers under unfavorable conditions as desirable characteristics (Herrmann and Lesueur 2013).

Different commercial microbial consortia formulations are available (liquid, peat, granules, and powders). There are many examples of commercial microbial consortia formulations. Root Life Microbes by Key To Life (USA) contains *Bacillus firmus*, *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. azotoformans*, *B. coagulans*, *Paenibacillus polymyxa*, and *Paenibacillus durum*. Luxowo® by Luoxiaowang (China) is a *Bacillus* spp.-based product. Arka Microbial Consortium by Natura Crop Care (India) contains N_2-fixing, phosphate- and zinc-solubilizing, and plant growth-promoting microbes. MICOSAT line by CCS (Italy) contains multi-microbe consortia. MICOSAT F TAB Plus, for example, contains symbiotic fungi (*Glomus* spp. GB 67, *G. mosseae* GP 11, *G. viscosum* GC 41), rhizosphere bacteria (*Agrobacterium radiobacter* AR 39, *Bacillus subtilis* BA 41, and *Streptomyces* spp. SB 14), saprophytic fungi (*Pochonia chlamydosporia* PC 50 and *Trichoderma harzianum* TH 01), and yeasts (*Pichia pastoris* PP 59). MIKAR line products (MIKAR®, MIKAR® T, MIKAR® L, MIKAR® Seed Liquid) by SPPA (Italy) contain rhizosphere bacteria and mycorrhizal fungi. Among formulations, liquid ones are easy to handle and apply and offer many advantages over conventional solid carrier-based inoculants. Liquid formulations have a long shelf life (up to 2 years) and higher purity. They allow the manufacturer to add an adequate amount of nutrients, cell protectants, and inducers (for the formation of the cells, spores, or cysts, further ensuring longer shelf life) and are easier to commercialize (Satinder 2012). Recently, O'Callaghan et al. (2022) summarized many single-strain-based inoculants for phytopathogens' control. However, a single strain might have limited activity against diverse phytopathogens and pedoclimatic conditions' sensitivity (Saravanakumar et al. 2009). The combination of multiple strains is more effective in inducing tolerance and decreasing the damage of phytopathogenic attacks (Bharathi et al. 2004; Thilagavathi et al. 2007; Thomloudi et al. 2019; Pellegrini et al. 2020a, 2021; Djebaili et al. 2021). The induction of the plants' defenses following an application of a bacterial consortium is more effective than a single microbial inoculum (Santoyo et al. 2021). The steps to follow to produce biocontrol PGPB consortia can be different and depend on the type of strains used. Isolation and purification of strains followed by characterization of biocontrol traits (i.e., enzymatic activities and in vitro inhibition); consortium formulation; experiments to test *in planta* biocontrol activity under gnotobiotic, greenhouse, and open field conditions (carried out for many crops and pedoclimatic conditions); and optimization of product formulation for commercialization are the key steps. Before commercialization, the product must follow all the registration and approval, including the human and environmental safety evaluation. The regulatory procedures to register and commercialize a product differ depending on the state of registration and commercialization (Backer et al. 2018).

4.3 Plant Growth-Promoting Bacterial Consortia-Mediated Biocontrol Mechanisms

Bacterial consortia counteract the detrimental effects of one or more phytopathogenic organisms through the involvement of different mechanisms (Choudhary et al. 2011). The mechanisms used by microbial consortia to control microbial plant pathogenic attacks include synthesis of antimicrobial agents; competition for nutrients, iron, and space; predation; and induced systemic resistance (ISR) (Fig. 4.2). The most recognized direct mechanisms for biological control are the suppression of pathogens through the synthesis of anti-pathogenic compounds (e.g., peptides with antimicrobial activity and chitinolytic enzymes) (Raaijmakers et al. 2002; Compant et al. 2005). Different microorganisms produce different antimicrobial activities. But in some cases, the BCAs have the ability to control a wide range of pathogens due to inhabiting multiple attributes that are involved in controlling the phytopathogens' (Lahlali et al. 2022) activities. The genus *Bacillus* produces effective peptides as antibiotics such as iturins, mycosubtilin, bacillomycin D, surfactin, fengycin, and zwittermicin A, whereas *Pseudomonas* produces 2,4-diacetylphloroglucinol, phenazines, pyoluteorin, oomycin A, pyrrolnitrin, viscosin, and massetolide A (Kenawy et al. 2019). Reactive oxygen species (ROS) modulate the defensive enzymes, phytoalexins, and pathogenesis-related protein synthesis, which reduce pathogens'

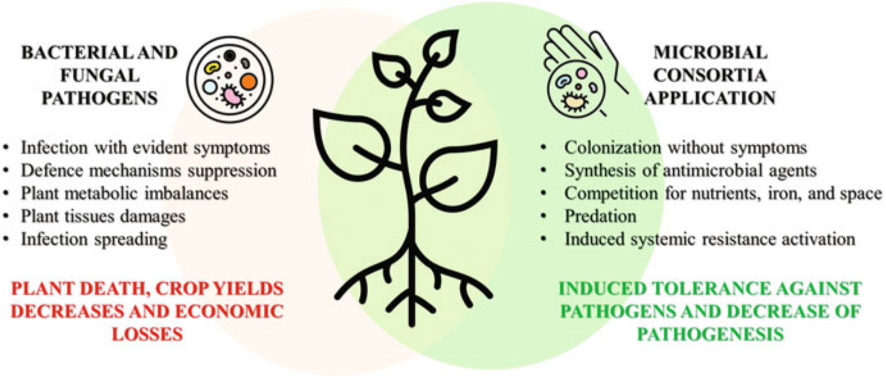


Fig. 4.2 Summary of the mechanisms used by microbial consortia for the control of microbial plant pathogenic attacks

propagation (Maurhofer et al. 1994; Silva et al. 2004; Van Loon and Bakker 2005; Saravanakumar et al. 2009). Interactions between microbial consortia and phytopathogens are also crucial for plant disease control. Different interactions can be described for biocontrol, such as predation and competition for resources (i.e., for carbon sources and iron, with siderophore production) and space (niche occupation) (Haas and Défago 2005). Microbial consortia can improve the plant's defensive capacity toward pathogens by ISR in the host plant (Pozo et al. 2002; Srivastava et al. 2010), increasing nutrient uptake and root architecture (Wehner et al. 2010), and synthesizing signaling compounds (Lyu et al. 2020). Resistance mechanisms induced by microbial consortia enhance plant protection, suppressing disease severity and incidence (Ramamoorthy et al. 2001). Plant resistance that involves the contribution of PGPB is often referred to as induced systemic resistance. ISR has been identified and illustrated in many plant species, including *Arabidopsis thaliana*, *Cucumis sativus*, *Dianthus caryophyllus*, *Raphanus sativus*, *Solanum lycopersicum*, *Nicotiana tabacum*, and *Phaseolus vulgaris* (Pieterse et al. 1998). ISR guards plants against bacteria, fungi, viruses, and insects (Bhattacharyya and Jha 2012) and plants' parasitic nematodes. Microbial consortia-induced ISR is comparable to systemic acquired resistance (SAR) induced by pathogens, making plants more resistant to consecutive pathogenic attacks (Van Wees et al. 1997). However, ISR and SAR involve different signaling pathways. ISR entails signaling pathways of jasmonic acid and ethylene without symptoms in the host plant (Bakker et al. 2003). SAR involves salicylic acid pathways, causing visible symptoms (De Vleesschauwer and Höfte 2009; Van der Ent et al. 2009). The signaling molecules coordinate defense responses and, if applied exogenously, induce resistance (Ryals et al. 1996). Molecular studies on *Arabidopsis* showed that both SAR and ISR are linked via the NPR1 gene and act in synergy (Van Wees et al. 2000). Among signaling compounds, phytohormones involved exist for both plant-to-microbe and microbe-to-plant communications (Lyu et al. 2020). This close relationship in the phytomicrobiome regulates aspects of growth and metabolism in both elements of the holobiont (plant and phytomicrobiome). Thuricin 17 and lipo-chitooligosaccharides (LCOs) are two microbial plant signals that increase stress tolerance in many plant species (Smith et al. 2015; Subramanian et al. 2016; Lyu et al. 2020). Plant hormones, such as auxins (Kazan and Manners, 2009), brassinosteroids, gibberellins, and cytokinins (Nakashita et al. 2003; Navarro et al. 2008; Giron et al. 2013; Lee et al. 2010; Mukherjee et al. 2022), also showed to function as modulators of the plant immune signaling network. Phytohormonal crosstalk allows plants to regulate immune responses for their protection and growth (Pieterse et al. 2014).

4.4 Plant Growth-Promoting Bacterial Consortia Against Bacterial Pathogens

Bacterial pathogens are another causal agent of phytopathogenic diseases. They induce massive economic losses in agriculture because of the low efficacy of agrochemicals, plant resistance or immunity, and wide latency and spreading of diseases and survival strategies (Van der Wolf and De Boer 2015; Martins et al. 2018). PGPB achieve pathogenic bacteria control mainly through antimicrobial molecules and competition for nutrients and space. Among antimicrobial molecules, producing low-molecular-weight compounds (antibiotics) by other microorganisms is the most common (Duffy et al. 2003). Bacteriocins are peptides secreted by bacteria with toxic effects on closely related bacteria (Riley and Wertz 2002; Abriouel et al. 2011; Nazari and Smith 2020). Bacteria produce over one bacteriocin, and some have broader inhibition spectra (Abriouel et al. 2011). Limited studies report microbial consortia application as biocontrol agents against bacterial diseases. Many reports concern the application of single PGPB strains or their cell-free supernatants (Kwak et al. 2018; Pellegrini et al. 2020b) in biocontrol of fungal pathogen. However, the findings so far underlined this tool’s effectiveness (Niu et al. 2020; Wang et al. 2021). In a recent study, excellent biocontrol activity of the consortium of *Nocardiopsis aegyptica* and *Streptomyces albidoflavus* was observed against *S. lycopersicum* and *Daucus carota* bacterial pathogens (*P. syringae*, *P. corrugate*, and *Pectobacterium carotovorum*) (Djebaili et al. 2021). *Xanthomonas oryzae* pv. *oryzicola* in rice was controlled by *S. shenzhenesis* and *Streptomyces* sp. consortium (Hata et al. 2021). The recent report of Maciag and collaborators explained the use of the consortium formed by *Serratia plymuthica*, *Enterobacter amnigenus*, *Rahnella aquatilis*, and two strains of *Serratia rubidaea* against *Pectobacterium* spp. and *Dickeya* spp. (Maciag et al. 2020). Other studies showed the effectiveness of microbial consortia against the pathogen *Ralstonia solanacearum*, using a consortium of non-virulent *Ralstonia* spp. (Wei et al. 2015), *Pseudomonas* spp. (Hu et al. 2016), *Bacillus* spp., and *Chryseobacterium* sp. (Liu et al. 2014). There are various examples illustrating the beneficial impact of consortia of PGPB rendering biological control of phytopathogens (Table 4.1).

Table 4.1 PGPB consortia for biological control of bacterial pathogens of various plants

Consortia	Bacterial pathogens	Plants	References
N. aegyptica and *S. albidoflavus*	*P. syringae*, *P. corrugate*, and *P. carotovorum*	*S. lycopersicum* and *D. carota*	Djebaili et al. (2021)
Streptomyces shenzhenesis and *Streptomyces* sp.	*X. oryzae* pv. *oryzicola*	*Oryza sativa*	Hata et al. (2021)
S. plymuthica, *E. amnigenus*, *R. aquatilis*, and two strains of *S. rubidaea*	*Pectobacterium* spp. and *Dickeya* spp.	*S. tuberosum*	Maciag et al. (2020)
Non-virulent *Ralstonia* spp.	*R. solanacearum*	*S. lycopersicum*	Wei et al. (2015)
Pseudomonas spp.		*S. lycopersicum*	Hu et al. (2016)
Bacillus spp. and *Chryseobacterium* sp.		*Capsicum annuum*	Liu et al. (2014)

4.5 Plant Growth-Promoting Bacterial Consortia Against Fungal Pathogens

Fungal pathogens are the principal agent of over 70% of agricultural crop diseases (Kumar et al. 2018), with over 10,000 species that induce epidemics in plants and cause serious losses in yields (Agrios 2005; Suprapta 2012). Pathogenic fungi biocontrol is mainly ascribed to the activity of hydrolytic enzymes (e.g., chitinase, glucanase, protease, and cellulase) (Compant et al. 2005). Competition for nutrients and space and the production of antifungal metabolites are also effective against fungal pathogens (Karkachi et al. 2010; Akhtar et al. 2016). Microbial cyclic lipopeptides, for example, act on fungal pathogens and facilitate the PGPB root colonization and the stimulation of host defense mechanisms (Raaijmakers et al. 2010; Tsolakidou et al. 2019). Table 4.2 summarizes the PGPB consortia against fungal pathogens. Among the beneficial rhizospheric microflora, fluorescent pseudomonads are relevant biological control agents. They can colonize plant roots and secrete various antifungal secondary metabolites (Erdogan and Benlioglu 2010; Akhtar et al. 2016), compete for space and nutrients, produce lytic enzymes, and induce systemic resistance in plants (Dunne et al. 1998; Ramamoorthy et al. 2002). The creation of barriers beyond the infection sites, by the accumulation of callose, lignin, and phenolic compounds on cortical and epidermal cell walls, was also described (M'piga et al. 1997). The effective application of *Pseudomonas* strain consortia has been described for the control of *Gaeumannomyces graminis* var. *tritici* (Sivasithamparam and Parker 1978), *Fusarium oxysporum* (De Boer et al. 1999), *Sarocladium oryzae* (Saravanakumar et al. 2009), and *Sclerotium rolfsii* (Senthilraja et al. 2010). *Bacillus* is another genus well known and described for the wide range of enzymatic activities and genetic markers linked to biological control activities (Tsolakidou et al. 2019). Multiple *Bacillus* strain consortia showed effective biocontrol of *F. oxysporum* f. sp. *lycopersici* (Myresiotis et al. 2012;

Table 4.2 PGPB consortia for biological control of fungal pathogens of various plants

Consortia	Fungal pathogens	Plants	References
Pseudomonas fluorescens spp.	*Gaeumannomyces graminis* var. *tritici*	*Triticum*	Sivasithamparam and Parker (1978)
	Fusarium oxysporum	*R. sativus*	De Boer et al. (1999)
	Sarocladium oryzae	*O. sativa*	Saravanakumar et al. (2009)
	S. rolfsii	*A. hypogaea*	Senthilraja et al. (2010)
Pseudomonas	*F. oxysporum* f. sp. *lycopersici*	*S. lycopersicum*	Myresiotis et al. (2012)
Bacillus	*F. oxysporum* f. sp. *lycopersici*		Tsolakidou et al. (2019)
Bacillus	*F. oxysporum* f. sp. *lycopersici*		Arif et al. (2020)
Three *S. griseus*, *S. africanus*, and *S. coelicolor*	*Fusarium oxysporum* f. sp. *ciceri*	*Cicer arietinum*	Ankati et al. (2021)
B. ambifaria, *G. diazotrophicus*, and *H. seropedicae*	*R. solani* and *F. oxysporum* f. sp. *radicis-lycopersici*	*S. lycopersicum* *S. tuberosum*	Pellegrini et al. (2020a)
	F. oxysporum f. sp. *cannabis*	*Cannabis sativa*	Pellegrini et al. (2021)
N. aegyptica and *S. albidoflavus*	*Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*	*S. tuberosum* and *D. carota*	Djebaili et al. (2021)
P. putida and *F. oxysporum*	*Fusarium*	*D. caryophyllus*	Van Peer et al. (1991)
Pseudomonas, *Bacillus*, and *Enterobacter*	*Aspergillus niger*, *A. flavus*, and *Fusarium oxysporum*	*A. hypogaea*	Syed et al. (2020)
Chitinophaga sp. and *Flavobacterium* sp.	*Rhizoctonia solani*	*Beta vulgaris*	Carrión et al. (2019)
P. aeruginosa and *Azospirillum* sp.	*Rhizoctonia bataticola*	*Gossypium*	Marimuthu et al. (2013)
Xanthomonas spp., *Stenotrophomonas* spp., and *Microbacterium* spp.	*Hyaloperonospora arabidopsidis*	*A. thaliana*	Berendsen et al. (2018)

Tsolakidou et al. 2019; Arif et al. 2020). With these genera, interesting antifungal activity was also shown by *Streptomyces* consortia to control *F. oxysporum* f. sp. *ciceri* (Ankati et al. 2021). Consortia can also be formed by multiple strains that belong to different genera. Our recent works showed that the consortium of *Burkholderia ambifaria*, *Gluconacetobacter diazotrophicus*, and *Herbaspirillum seropedicae* has biocontrol ability against *Rhizoctonia solani* and *Fusarium oxysporum* spp. in *Solanum tuberosum*, *S. lycopersicum*, and *Cannabis sativa* (Pellegrini et al. 2020a, 2021). The consortium of *N. aegyptica* and *S. albidoflavus* is used to control *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* in *S. tuberosum* and *Daucus carota* (Djebaili et al. 2021). Many other authors have reported the multi-bacterial genera exhibited genus biocontrol. The suppression of *Fusarium* wilt in *D. caryophyllus* is controlled by *P. putida* WCS358 and *F. oxysporum* Fo47 (Van Peer et al. 1991). In *Arachis hypogaea*, the combination of *Pseudomonas*, *Bacillus*, and *Enterobacter* consortia performed consortium limited damages for *Aspergillus niger*, *A. flavus*, and *Fusarium oxysporum* (Syed et al. 2020). *Rhizoctonia* spp. have been effectively controlled by the application of *Chitinophaga* sp. and *Flavobacterium* sp. (Carrión et al. 2019) and *P. aeruginosa* and *Azospirillum* sp. (Marimuthu et al. 2013). Similarly, *Hyaloperonospora arabidopsidis* in *A. thaliana* has been controlled by *Xanthomonas* spp., *Stenotrophomonas* spp., and *Microbacterium* spp. (Berendsen et al. 2018). Enriching PGPB consortia with PGPF (plant growth-promoting fungi) is also a valid strategy for effective biocontrol. The literature contains many reports that underline the positive effects of the association of *Trichoderma* spp. with many PGPB on biocontrol activity, especially with *Bacillus* spp. (Brewer and Larkin 2005; Morsy et al. 2009; Yobo et al. 2011; Thakkar and Saraf 2015; Ali et al. 2017; Wu et al. 2018; Alamri et al. 2019; Thomloudi et al. 2019; Izquierdo-García et al. 2020). *T. viride* was also effectively combined with *B. subtilis* and *P. putida* that showed biocontrol of *Macrophomina phaseolina* (Thilagavathi et al. 2007). *T. harzianum* with *P. aeruginosa* and *Mesorhizobium* controlled *S. rolfsii* (Singh et al. 2014); *Rhizobium*, *B. subtilis*, and *P. fluorescens* against *Fusarium* wilt (Rajasekhar et al. 2016); and *B. subtilis* and *P. aeruginosa* to that of *Sclerotinia sclerotiorum* (Jain et al. 2012, 2013). Consortia of plant growth-promoting bacteria and plant growth-promoting fungi contribute to biocontrol of fungal pathogens in the rhizosphere as evidenced and supported by various studies in the past (Table 4.3).

4.6 Conclusions and Future Perspectives

Microbial consortia-based biocontrol agents are some of the emerging tools in sustainable agriculture. The present book chapter highlighted microbial consortia could represent new frontiers of formulations used in agriculture to control microbial phytopathogens. The biocontrol activities and mode of action, environment and human safety, commercial suitability, and stability of formulations are mandatory in creating and producing a microbial consortium to be used as a biocontrol agent. Within this context, the scientific community and the industrial sector should promote the use of microbial consortia, and the studies on this subject are carried out with rigorous and high scientific soundness. These goals will produce strong scientific bases and industrialized technologies able to compete with agrochemicals. Using the latter is already inducing several problems within the environment, threatening human health and earth ecosystems.

Table 4.3 PGPB and PGPF consortia for biological control of bacterial pathogens of various plants

PGPF strain	PGPB	Fungal pathogen	Plant	References
T. harzianum	*B. subtilis*	*R. solani*	*S. tuberosum*	Brewer and Larkin (2005)
T. viride		*F. solani*	*S. lycopersicum*	Morsy et al. (2009)
T. atroviride, *T. harzianum*, *T. pseudokoningii*, and *Trichoderma* sp.		*R. solani*	*P. vulgaris*	Yobo et al. (2011)
T. citrinoviride	*P. aeruginosa*, *B. cereus*, and *B. amyloliquefaciens*	*M. phaseolina* and *S. sclerotiorum*	*Glycine max*	Thakkar and Saraf (2015)
T. koningii and *T. harzianum*	*B. subtilis*	*R. solani*	*S. tuberosum*	Ali et al. (2017)
T. asperellum	*B. amyloliquefaciens*	*Botrytis cinerea*	In vitro	Wu et al. (2018)
T. harzianum	*B. subtilis*	*Exserohilum rostratum* and *F. oxysporum*	*Lactuca sativa*	Alamri et al. (2019)
T. virens	*B. velezensis*	*F. oxysporum*	*Physalis peruviana*	Izquierdo-García et al. (2020)
T. viride fungal pathogens	*B. subtilis* and *P. putida*	*M. phaseolina*	*Vigna radiata*	Thilagavathi et al. (2007)
T. harzianum	*P. aeruginosa* and *Mesorhizobium*	*S. rolfsii*	*C. arietinum*	Singh et al. (2014)
	P. fluorescens, *B. subtilis*, and *Rhizobium* spp.	*F. oxysporum* and *S. rolfsii*	*Cajanus cajan*	Rajasekhar et al. (2016)
	B. subtilis and *P. aeruginosa*	*S. sclerotiorum*	*Pisum sativum*	Jain et al. (2012, 2013)

Acknowledgment We acknowledge the SPAA s.r.l. (Italy) for the support in the retrieval of industrial and commercial contents.

Conflict of Interest The authors declare no conflict of interest.

References

Abriouel H, Franz CMAP, Omar NB, Gálvez A (2011) Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiol Rev 35:201–232. https://doi.org/10.1111/j.1574-6976.2010.00244.x

Agrios GN (2005) Plant pathology, 5th edn. Elsevier Academic Press, Burlington, MA, pp 79–103

Akhtar N, Naveed M, Iqbal MZ et al (2016) Effect of consortium of plant growth promoting and compost inhabiting bacteria on physicochemical changes and defense response of maize in fungus infested soil. Pak J Agri Sci 53:59–68. https://doi.org/10.21162/PAKJAS/16.4700

Alamri SA, Hashem M, Mostafa YS et al (2019) Biological control of root rot in lettuce caused by *Exserohilum rostratum* and *Fusarium oxysporum* via induction of the defense mechanism. Biol Control 128:76–84. https://doi.org/10.1016/j.biocontrol.2018.09.014

Ali AA, Abf El-Kader A, Ghoneem K et al (2017) Two *Trichoderma* species and *Bacillus subtilis* as biocontrol agents against *Rhizoctonia* disease and their influence on potato productivity. Egypt J Agric Res 95:527–541

Ankati S, Srinivas V, Pratyusha S, Gopalakrishnan S (2021) *Streptomyces* consortia-mediated plant defense against *Fusarium* wilt and plant growth-promotion in chickpea. Microb Pathog 157: 104961. https://doi.org/10.1016/j.micpath.2021.104961

Arif I, Batool M, Schenk PM (2020) Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trends Biotechnol 38:1385–1396. https://doi.org/10.1016/j.tibtech.2020.04.015

Arora NK, Khare E, Maheshwari DK (2011) In: Maheshwari DK (ed) Plant growth promoting rhizobacteria: constraints in bioformulation, commercialization, and future strategies BT—plant growth and health promoting bacteria. Springer, Berlin, pp 97–116

Asmamaw T, Fassil A (2014) Co-culture: a great promising method in single cell protein production. Biotechnol Mol Biol Rev 9:12–20. https://doi.org/10.5897/BMBR2014.0223

Backer R, Rokem JS, Ilangumaran G et al (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 871:1–17. https://doi.org/10.3389/fpls.2018.01473

Bairey E, Kelsic ED, Kishony R (2016) High-order species interactions shape ecosystem diversity. Nat Commun 7:12285. https://doi.org/10.1038/ncomms12285

Bakker PAHM, Ran LX, Pieterse CMJ, van Loon LC (2003) Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. Can J Plant Pathol 25:5–9. https://doi.org/10.1080/07060660309507043

Basu A, Prasad P, Das SN et al (2021) Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustainability 13:1140. https://doi.org/10.3390/su13031140

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051. https://doi.org/10.1590/s1415-47572012000600020

Berendsen RL, Vismans G, Yu K et al (2018) Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J 2:1496–1507. https://doi.org/10.1038/s41396-018-0093-1

Bharathi R, Vivekananthan R, Harish S et al (2004) Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Prot 23:835–843. https://doi.org/10.1016/j.cropro.2004.01.007

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350. https://doi.org/10.1007/s11274-011-0979-9

Blanchard AE, Lu T (2015) Bacterial social interactions drive the emergence of differential spatial colony structures. BMC Syst Biol 9:59. https://doi.org/10.1186/s12918-015-0188-5

Braga RM, Dourado MN, Araújo WL (2016) Microbial interactions: ecology in a molecular perspective. Braz J Microbiol 47:86–98. https://doi.org/10.1016/j.bjm.2016.10.005

Brewer MT, Larkin RP (2005) Efficacy of several potential biocontrol organisms against Rhizoctonia solani on potato. Crop Prot 24:939–950. https://doi.org/10.1016/j.cropro.2005.01.012

Carrión VJ, Perez-Jaramillo J, Cordovez V et al (2019) Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science 366:606–612. https://doi.org/10.1126/science.aaw9285

Chandran H, Meena M, Swapnil P (2021) Plant growth-promoting rhizobacteria as a green alternative for sustainable agriculture. Sustainability 13:10986. https://doi.org/10.3390/su131910986

Choudhary DK, Sharma KP, Gaur RK (2011) Biotechnological perspectives of microbes in agro-ecosystems. Biotechnol Lett 33:1905–1910. https://doi.org/10.1007/s10529-011-0662-0

Compant S, Duffy B, Nowak J et al (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005

Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J Adv Res 19:29–37. https://doi.org/10.1016/j.jare.2019.03.004

Coyte KZ, Schluter J, Foster KR (2015) The ecology of the microbiome: networks, competition, and stability. Science 350:663–666. https://doi.org/10.1126/science.aad2602

De Boer M, van der Sluis I, van Loon LC, Bakker PA (1999) Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of *Fusarium* wilt of radish. Eur J Plant Pathol 105:201–210. https://doi.org/10.1023/A:1008761729073

De Vleesschauwer D, Höfte M (2009) Chapter 6 rhizobacteria-induced systemic resistance. Adv Bot Res:223–281. https://doi.org/10.1016/S0065-2296(09)51006-3

Deter HS, Lu T (2022) Engineering microbial consortia with rationally designed cellular interactions. Curr Opin Biotechnol 76:102730. https://doi.org/10.1016/j.copbio.2022.102730

Djebaili R, Pellegrini M, Ercole C et al (2021) Biocontrol of soil-borne pathogens of *Solanum lycopersicum* L. and *Daucus carota* L. by plant growth-promoting actinomycetes: in vitro and in planta antagonistic activity. Pathogens 10:1305. https://doi.org/10.3390/pathogens10101305

Duffy B, Schouten A, Raaijmakers JM (2003) Pathogen self-defence: mechanisms to counteract microbial antagonism. Annu Rev Phytopathol 41:501–538. https://doi.org/10.1146/annurev.phyto.41.052002.095606

Duncker KE, Holmes ZA, You L (2021) Engineered microbial consortia: strategies and applications. Microb Cell Factories 20:211. https://doi.org/10.1186/s12934-021-01699-9

Dunne C, Moënne-Loccoz Y, McCarthy J et al (1998) Combining proteolytic and phloroglucinol-producing bacteria for improved biocontrol of *Pythium*-mediated damping-off of sugar beet. Plant Pathol 47:299–307. https://doi.org/10.1046/j.1365-3059.1998.00233.x

Erdogan O, Benlioglu K (2010) Biological control of *Verticillium* wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. Biol Control 53:39–45. https://doi.org/10.1016/j.biocontrol.2009.11.011

Giri S, Shitut S, Kost C (2020) Harnessing ecological and evolutionary principles to guide the design of microbial production consortia. Curr Opin Biotechnol 62:228–238. https://doi.org/10.1016/j.copbio.2019.12.012

Giron D, Frago E, Glevarec G et al (2013) Cytokinins as key regulators in plant–microbe–insect interactions: connecting plant growth and defence. Funct Ecol 27:599–609. https://doi.org/10.1111/1365-2435.12042

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:963401. https://doi.org/10.6064/2012/963401

Gupta S, Ross TD, Gomez MM et al (2020) Investigating the dynamics of microbial consortia in spatially structured environments. Nat Commun 11:2418. https://doi.org/10.1038/s41467-020-16200-0

Gupta A, Singh UB, Sahu PK et al (2022) Linking soil microbial diversity to modern agriculture practices: a review. Int J Environ Res Public Health 19:3141. https://doi.org/10.3390/ijerph19053141

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319. https://doi.org/10.1038/nrmicro1129

Hata EM, Yusof MT, Zulperi D (2021) Induction of systemic resistance against bacterial leaf streak disease and growth promotion in rice plant by *Streptomyces shenzhenesis* TKSC3 and *Streptomyces* sp. SS8. Plant Pathol J 37:173. https://doi.org/10.5423/ppj.oa.05.2020.0083

Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97:8859–8873. https://doi.org/10.1007/s00253-013-5228-8

Hu J, Wei Z, Friman V-P et al (2016) Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. mBio 7:e01790–e01716. https://doi.org/10.1128/mBio.01790-16

Hussain MH, Mohsin MZ, Zaman WQ et al (2022) Multiscale engineering of microbial cell factories: a step forward towards sustainable natural products industry. Synth Syst Biotechnol 7:586–601. https://linkinghub.elsevier.com/retrieve/pii/S2405805X21001010

Izquierdo-García LF, González-Almario A, Cotes AM, Moreno-Velandia CA (2020) *Trichoderma virens* Gl006 and *Bacillus velezensis* Bs006: a compatible interaction controlling *Fusarium* wilt of cape gooseberry. Sci Rep 10:6857. https://doi.org/10.1038/s41598-020-63689-y

Jain A, Singh A, Singh S, Singh HB (2013) Microbial consortium-induced changes in oxidative stress markers in pea plants challenged with *Sclerotinia sclerotiorum*. J Plant Growth Regul 32: 388–398. https://doi.org/10.1007/s00344-012-9307-3

Jain A, Singh S, Kumar Sarma B, Bahadur Singh H (2012) Microbial consortium–mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. J Appl Microbiol 112:537–550. https://doi.org/10.1111/j.1365-2672.2011.05220.x

Jain P, Gupta A, Chandukar P (2022) Sustainable agriculture with rhizobacteria (PGPR). Int J Curr Res Rev 05:1834–1838. https://doi.org/10.47191/ijcsrr/V5-i6-02

Jiao X, Takishita Y, Zhou G, Smith DL (2021) Plant associated rhizobacteria for biocontrol and plant growth enhancement. Front Plant Sci 12:634796. https://doi.org/10.3389/fpls.2021.634796

Joshi SK, Gauraha AK (2022) Global biofertilizer market: emerging trends and opportunities. In: Soni RG, Suyal DC, Yadav AN, Reeta BT (eds) Developments in applied microbiology and biotechnology—trends of applied microbiology for sustainable economy. Academic Press, London, pp 689–697. https://www.sciencedirect.com/science/article/pii/B9780323915953000240

Karkachi NE, Gharbi S, Kihal M, Henni JE (2010) Biological control of *Fusarium oxysporum* f. sp. *lycopersici* isolated from Algerian tomato by *Pseudomonas fluorescens, Bacillus cereus, Serratia marcescens* and *Trichoderma harzianum*. Int J Agron 4:31–34. https://doi.org/10.3923/rjagr.2010.31.34

Kazan K, Manners JM (2009) Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci 14:373–382. https://doi.org/10.1016/j.tplants.2009.04.005

Kenawy A, Dailin DJ, Abo-Zaid GA et al (2019) Biosynthesis of antibiotics by PGPR and their roles in biocontrol of plant diseases. In: Sayyed R (ed) Plant Growth Promoting Rhizobacteria for Sustainable Stress management, Microorganisms for sustainability, vol 13. Springer, Singapore, pp 1–35. https://doi.org/10.1007/978-981-13-6986-5_1

Kloepper JW, Schroth MN (1978) Plant growth promoting rhizobacteria on radishes. In: Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria. Station de Pathologie Vegetale et Phytobacteriologie, Angers. pp. 879–892

Kour D, Rana KL, Kaur T et al (2020) Microbial biofilms: functional annotation and potential applications in agriculture and allied sectors. In: Yadav MK, Singh BP (eds) New and future developments in microbial biotechnology and bioengineering: microbial biofilms. Elsevier, Amsterdam, pp 283–301. https://doi.org/10.1016/B978-0-444-64279-0.00018-9

Kumar M, Brar A, Yadav M et al (2018) Chitinases—potential candidates for enhanced plant resistance towards fungal pathogens. Agriculture 8:88. https://doi.org/10.3390/agriculture8070088

Kwak M-J, Kong HG, Choi K et al (2018) Rhizosphere microbiome structure alters to enable wilt resistance in tomato. Nat Biotechnol 36:1100–1109. https://doi.org/10.1038/nbt.4232

Lahlali R, Ezrari S, Radouane N et al (2022) Biological control of plant pathogens: a global perspective. Microorganisms 10:596. https://doi.org/10.3390/microorganisms10030596

Lee ZH, Hirakawa T, Yamaguchi N, Ito T (2010) The roles of plant hormones and their interactions with regulatory genes in determining meristem activity. Int J Mol Sci 20:4065. https://doi.org/10.3390/ijms20164065

Liang Y, Ma A, Zhuang G (2022) Construction of environmental synthetic microbial consortia: based on engineering and ecological principles. Front Microbiol 13:829717. https://doi.org/10.3389/fmicb.2022.829717

Liu H-X, Li S-M, Luo Y-M et al (2014) Biological control of *Ralstonia* wilt, *Phytophthora* blight, Meloidogyne root-knot on bell pepper by the combination of *Bacillus subtilis* AR12, *Bacillus subtilis* SM21 and *Chryseobacterium* sp. R89. Eur J Plant Pathol 139:107–116. https://doi.org/10.1007/s10658-013-0369-2

Lyu D, Backer R, Subramanian S, Smith DL (2020) Phytomicrobiome coordination signals hold potential for climate change-resilient agriculture. Front Plant Sci 11:634. https://doi.org/10.3389/fpls.2020.00634

M'piga P, Belanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. Physiol Mol 50:301–320. https://doi.org/10.1006/pmpp.1997.0088

Maciag T, Krzyzanowska DM, Jafra S et al (2020) The great five—an artificial bacterial consortium with antagonistic activity towards *Pectobacterium* spp. and *Dickeya* spp.: formulation, shelf life, and the ability to prevent soft rot of potato in storage. Appl Microbiol Biotechnol 104:4547–4561. https://doi.org/10.1007/s00253-020-10550-x

Marimuthu S, Ramamoorthy V, Samiyappan R, Subbian P (2013) Intercropping system with combined application of *Azospirillum* and *Pseudomonas fluorescens* reduces root rot incidence caused by *Rhizoctonia bataticola* and increases seed cotton yield. J Phytopathol 161:405–411. https://doi.org/10.1111/Fjph.12084

Martins PMM, Merfa MV, Takita MA, De Souza AA (2018) Persistence in phytopathogenic bacteria: do we know enough? Front Microbiol 9:1–14. https://doi.org/10.3389/fmicb.2018.01099

Massa F, Defez R, Bianco C (2022) Exploitation of plant growth promoting bacteria for sustainable agriculture: hierarchical approach to link laboratory and field experiments. Microorganisms 10:865. https://doi.org/10.3390/microorganisms10050865

Maurhofer M, Hase C, Meuwly P et al (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the gacA gene and of pyoverdine production. Phytopathology 84:139–146. https://doi.org/10.1094/Phyto-84-139

Morsy EM, Abdel-Kawi KA, Khalil MNA (2009) Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants. Egypt J Phytopathol 37:47–57. https://doi.org/10.21608/ejar.2019.111021

Mukherjee A, Gaurav AK, Singh S et al (2022) The bioactive potential of phytohormones: a review. Biotechnol Rep 35:e00748. https://doi.org/10.1016/j.btre.2022.e00748

Myresiotis CK, Karaoglanidis GS, Vryzas Z, Papadopoulou-Mourkidou E (2012) Evaluation of plant-growth-promoting rhizobacteria, acibenzolar-S-methyl and hymexazol for integrated control of *Fusarium* crown and root rot on tomato. Pest Manag Sci 68:404–411. https://doi.org/10.1002/ps.2277

Nakashita H, Yasuda M, Nitta T et al (2003) Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. Plant J 33:887–898. https://doi.org/10.1046/j.1365-313X.2003.01675.x

Navarro L, Bari R, Achard P et al (2008) DELLAs Control plant immune responses by modulating the balance of Jasmonic acid and salicylic acid signaling. Curr Biol 18:650–655. https://doi.org/10.1016/j.cub.2008.03.060

Nazari M, Smith DL (2020) A PGPR-produced bacteriocin for sustainable agriculture: a review of thuricin 17 characteristics and applications. Front Plant Sci 11:916. https://doi.org/10.3389/fpls.2020.00916

Niu B, Wang W, Yuan Z et al (2020) Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. Front Microbiol 11:585404. https://doi.org/10.3389/fmicb.2020.585404

O'Callaghan M, Ballard RA, Wright D (2022) Soil microbial inoculants for sustainable agriculture: limitations and opportunities. Soil Use Manag 38:1340–1369. https://doi.org/10.1111/sum.12811

Pellegrini M, Ercole C, Di Zio C et al (2020a) In vitro and in planta antagonistic effects of plant growth-promoting rhizobacteria consortium against soilborne plant pathogens of *Solanum tuberosum* and *Solanum lycopersicum*. FEMS Microbiol Lett 367:fnaa099. https://doi.org/10.1093/femsle/fnaa099

Pellegrini M, Ercole C, Gianchino C et al (2021) *Fusarium Oxysporum* f. sp. *Cannabis* isolated from Cannabis sativa L.: in vitro and in planta biocontrol by a plant growth promoting-bacteria consortium. Plants 10:2436. https://doi.org/10.3390/plants10112436

Pellegrini M, Pagnani G, Bernardi M et al (2020b) Cell-free supernatants of plant growth-promoting bacteria: a review of their use as biostimulant and microbial biocontrol agents in sustainable agriculture. Sustainability 12:9917. https://doi.org/10.3390/su12239917

Pieterse CM, Van Wees SC, Van Pelt JA et al (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10:1571–1580. https://doi.org/10.1105/tpc.10.9.1571

Pieterse CMJ, Zamioudis C, Berendsen RL et al (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Povero G (2020) Exploring natural resources for biostimulants. In: Geelen D, Xu L (eds) The chemical biology of plant biostimulants. Wiley, Hoboken, NJ, pp 181–204. https://doi.org/10.1002/9781119357254.ch7

Pozo MJ, Cordier C, Dumas-Gaudot E et al (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J Exp Bot 53:525–534. https://doi.org/10.1093/jexbot/53.368.525

Qian X, Chen L, Sui Y et al (2020) Biotechnological potential and applications of microbial consortia. Biotechnol Adv 40:107500. https://doi.org/10.1016/j.biotechadv.2019.107500

Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M (2010) Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiol Rev 34:1037–1062. https://doi.org/10.1111/j.1574-6976.2010.00221.x

Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Leeuwenhoek 81:537–547. https://doi.org/10.1023/A:1020501420831

Rajasekhar L, Satish KS, Divya J (2016) Evaluation of microbial consortium for plant health management pigeon pea. Int J Plant Anim Env Sci 6:107–113

Ram RM, Debnath A, Negi S, Singh HB (2022) Use of microbial consortia for broad spectrum protection of plant pathogens. In: Rakshit A, Meena VS, Singh AK et al (eds) . Elsevier, Biopesticides, pp 319–335. https://doi.org/10.1016/B978-0-12-823355-9.00017-1

Ramamoorthy V, Raguchander T, Samiyappan R (2002) Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici*. Plant Soil 239:55–68. https://doi.org/10.1023/A:1014904815352

Ramamoorthy V, Viswanathan R, Raguchander T et al (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot 20:1–11. https://doi.org/10.1016/S0261-2194(00)00056-9

Riley MA, Wertz JE (2002) Bacteriocins: evolution, ecology, and application. Annu Rev Microbiol 56:117–137. https://doi.org/10.1146/annurev.micro.56.012302.161024

Ryals JA, Neuenschwander UH, Willits MG et al (1996) Systemic acquired resistance. Plant Cell 8(10):1809–1819. https://doi.org/10.1105/tpc.8.10.1809

Saeed Q, Xiukang W, Haider FU et al (2021) Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. Int J Mol Sci 22:10529. https://doi.org/10.3390/ijms221910529

Santoyo G, Guzmán-Guzmán P, Parra-Cota FI et al (2021) Plant growth stimulation by microbial consortia. Agronomy 11:219. https://doi.org/10.3390/agronomy11020219

Saravanakumar D, Lavanya N, Muthumeena K et al (2009) Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease. BioControl 54:273–286. https://doi.org/10.1007/s10526-008-9166-9

Satinder KB (2012) Shelf-life of biofertilizers: an accord between formulations and genetics. J Biofertil Biopestic 03. https://doi.org/10.4172/2155-6202.1000e109

Seenivasagan R, Babalola OO (2021) Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product. Biology 10:1111. https://doi.org/10.3390/biology10111111

Senthilraja G, Anand T, Durairaj C et al (2010) A new microbial consortia containing entomopathogenic fungus, *Beauveria bassiana* and plant growth promoting rhizobacteria, *Pseudomonas fluorescens* for simultaneous management of leafminers and collar rot disease in groundnut. Biocontrol Sci Tech 20:449–464. https://doi.org/10.1080/09583150903576949

Silva HSA, da Silva Romeiro R, Macagnan D et al (2004) Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. Biol Control 29:288–295

Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB (2014) Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during Sclerotium rolfsii infection. Microbiol Res 169:353–360. https://doi.org/10.1016/j.micres.2013.09.014

Sivasithamparam K, Parker CA (1978) Effects of certain isolates of bacteria and actinomycetes on *Gaeumannomyces graminis* var. *tritici* and take-all of wheat. Aust J Bot 26:773–782. https://doi.org/10.1016/S1049-9644(03)00163-4

Smith DL, Praslickova D, Ilangumaran G (2015) Inter-organismal signaling and management of the phytomicrobiome. Front Plant Sci 6:722. https://doi.org/10.3389/fpls.2015.00722

Srivastava R, Khalid A, Singh US, Sharma AK (2010) Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biol Control 53:24–31. https://doi.org/10.1016/j.biocontrol.2009.11.012

Subramanian S, Souleimanov A, Smith DL (2016) Proteomic studies on the effects of lipo-Chitooligosaccharide and Thuricin 17 under unstressed and salt stressed conditions in Arabidopsis thaliana. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01314

Suprapta DN (2012) Potential of microbial antagonists as biocontrol agents against plant fungal pathogens. J ISSAAS 18:1–8

Syed S, Tollamadugu NP, Lian B (2020) *Aspergillus* and *Fusarium* control in the early stages of *Arachis hypogaea* (groundnut crop) by plant growth-promoting rhizobacteria (PGPR) consortium. Microbiol Res 240:126562. https://doi.org/10.1016/j.micres.2020.126562

Thakkar A, Saraf M (2015) Development of microbial consortia as a biocontrol agent for effective management of fungal diseases in *Glycine max* L. Arch Phytopathol Plant Prot 48:459–474. https://doi.org/10.1080/03235408.2014.893638

Thilagavathi R, Saravanakumar D, Ragupathi N, Samiyappan R (2007) A combination of biocontrol agents improves the management of dry root rot (*Macrophomina phaseolina*) in greengram. Phytopathol Mediterr 46:157–167. https://doi.org/10.14601/Phytopathol_Mediterr-2147

Thomloudi E-E, Tsalgatidou PC, Douka D et al (2019) Multistrain versus single-strain plant growth promoting microbial inoculants-the compatibility issue. Hell Plant Prot J 12:61–77. https://doi.org/10.2478/hppj-2019-0007

Tsolakidou M-D, Stringlis IA, Fanega-Sleziak N et al (2019) Rhizosphere-enriched microbes as a pool to design synthetic communities for reproducible beneficial outputs. FEMS Microbiol Ecol 95:fiz138. https://doi.org/10.1093/femsec/fiz138

Van der Ent S, Van Wees SCM, Pieterse CMJ (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70:1581–1588. https://doi.org/10.1016/j.phytochem.2009.06.009

Van der Wolf J, De Boer SH (2015) Phytopathogenic bacteria. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Cham, pp 65–77

Van Loon LC, Bakker P (2005) Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht. https://doi.org/10.1007/1-4020-4152-7_2

Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81:728–734

Van Wees SCM, de Swart EAM, van Pelt JA et al (2000) Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. PNAS 97:8711–8716. https://doi.org/10.1073/pnas.130425197

Van Wees SCM, Pieterse CMJ, Trijssenaar A et al (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol Plant-Microbe Interact 10:716–724. https://doi.org/10.1094/MPMI.1997.10.6.716

Verma H, Kant C, Singh SK et al (2022) Biocontrol potential of microbial consortia: approaches in food security and disease management. In: Kumar A (ed) Microbial biocontrol: sustainable agriculture and phytopathogen management. Springer, Cham, pp 187–203. https://doi.org/10.1007/978-3-030-87512-1_7

Wang H, Liu R, You MP et al (2021) Pathogen biocontrol using plant growth-promoting bacteria (PGPR): role of bacterial diversity. Microorganisms 9:1988. https://doi.org/10.3390/microorganisms9091988

Wehner J, Antunes PM, Powell JR et al (2010) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia 53:197–201. https://doi.org/10.1016/j.pedobi.2009.10.002

Wei Z, Yang T, Friman V-P et al (2015) Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nat Commun 6:8413. https://doi.org/10.1038/ncomms9413

Weiland-Bräuer N (2021) Friends or foes—microbial interactions in nature. Biology 10:496. https://doi.org/10.3390/biology10060496

Wootton JT (2002) Indirect effects in complex ecosystems: recent progress and future challenges. J Sea Res 48:157–172. https://doi.org/10.1016/S1385-1101(02)00149-1

Wu Q, Ni M, Dou K et al (2018) Co-culture of *Bacillus amyloliquefaciens* ACCC11060 and *Trichoderma asperellum* GDFS1009 enhanced pathogen-inhibition and amino acid yield. Microb Cell Factories 17:155. https://doi.org/10.1186/s12934-018-1004-x

Yobo KS, Laing MD, Hunter CH (2011) Effects of single and combined inoculations of selected *Trichoderma* and *Bacillus* isolates on growth of dry bean and biological control of *Rhizoctonia solani* damping-off. Afr J Biotechnol 10:8746–8756. https://doi.org/10.5897/AJB10.2213

Chapter 5
Phytohormonal Role of Microorganisms Involved in Bioinoculants

Rubén Bottini, Federico J. Berli, M. Victoria Salomon, and Patricia N. Piccoli

Abstract The world population is supported by food availability that depends on cultivation. Agriculture, however, comports environmental threats related with global warming and nature contamination. Therefore, more friendly techniques that are less damaging for the environment without resignation of yield have gained attention. Among them, bioinoculants containing microbial species (bacteria and fungi) emerge in the farming market as possible contributors. This review revises the scientific basis of such products as biofertilizers, biostimulators, stress regulators, biopesticides, and bioremediation agents. More than 40 years of research suggests that bioinoculants' efficiency relies on root growth promotion that enhances soil exploration and root capability for superior nutrient and water uptake, and such growth promotion is mostly dependent (directly and/or indirectly) on production of plant growth regulators (phytohormones) by microorganisms. Notwithstanding, in keeping yield, bioinoculants are not enough by themselves, and they should be combined with other technologies, namely, plants genetically designed for more efficient use of resources, direct sowing, moderate and specifically oriented fertilization, green soil coverage, and crop rotation.

Keywords Bioformulations · PGPB · Plant growth-promoting bacteria · Plant hormones · Root efficiency · Sustainable agriculture

---

R. Bottini
Instituto Argentino de Veterinaria Ambiente y Salud, Universidad Juan A. Maza, Guaymallén, Argentina

F. J. Berli · M. V. Salomon · P. N. Piccoli (✉)
Grupo de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo, Chacras de Coria, Argentina
e-mail: ppiccoli@fca.uncu.edu.ar

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_5

Abbreviations

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
AMF	Arbuscular mycorrhizal fungi
BCAs	Biological control agents
BRs	Brassinosteroids
Cyt	Cytokinins
Et	Ethylene
GAs	Gibberellins
IAA	Indole-3-acetic acid
ISR	Induced systemic resistance
JA	Jasmonic acid
MeJA	Methyl ester jasmonic acid
PGRs	Plant growth regulators
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SL	Strigolactones

5.1 Introduction

Estimations regarding world population (https://www.worldometers.info/world-population/world-population-by-year/) indicate that the number of inhabitants on planet earth tripled in a span of 1800 years, from year 1 AD to the early nineteenth century. After that, the numbers grew eightfold in (approximately) the last 200 years. Such rapid increase in latter times was coincidental with the establishment of the Industrial Revolutions (Lucas 2004). The reasons for this are related to various causes correlated with the industrial era and the times afterward, namely, increases of per capita income, improvement of sanitary conditions, use of vaccines and antibiotics, and advances in medical technology. However, food production has been at the base of all. Indeed, a growing number of people demanded sufficient food supply to attend the more basic life necessity (Tilman and Clark 2015).

Food availability is essentially dependent on cultivation. Agriculture has developed some 12,000 years ago with domestication of higher plants, when mankind began to handpick the best seeds to plant next season, which allowed harvest to be stored, and, by consequence, the food availability permitted people to settle permanently in villages first and then cities (Tauger 2010). This activity was improved by plant breeding and development of new farming techniques, which in turn admitted agriculture expansion in extended areas. Therefore, more food allowed more people. In fact, the regulation of plant growth and development, and of the physiological processes that alleviate the negative effects of environmental stresses (biotic and abiotic), determines the productivity of crops (Yakhin et al. 2017). In the last six decades, a two steps' green revolution happened (Pimentel 1996; von der Goltz et al. 2020). The first step occurred during the 1960s with the insertion of dwarf genes that allowed wheat to be heavily fertilized (Appleford et al. 2007; Gale et al. 1985; Gooding et al. 2012) and the development of hybrid rice (Bai et al. 2018; Li et al. 2018; Liao et al. 2021; Wang and Deng 2018). Both types of cereals were known as high-yielding varieties and permitted substantially higher productions than the traditional cultivars. In the 1990s, a second step materialized because of the use of transgenic plants tolerant to pests (Koziel et al. 1993) and pesticides (Padgette et al. 1995) and the practice of no tilling (direct sowing; Nouri et al. 2019; Scopel et al. 2005); thus, the overall yield increased hugely. More recently, a second "green revolution" seems to be started, which is called "urban agriculture" (Armanda et al. 2019). However, although all these developments permitted to feed the increased number of people, they comported environmental threats because of high consumption of fossil combustible that increased CO_2 emissions (11% is due to agriculture expenditure, according to the US Environmental Protection Agency), extensive use of agrochemicals (Benbrook 2012; Bonny 2008), and anthropic (Vega et al. 2009) or geological (Funes Pinter et al. 2018) contamination with heavy metals. Deforestation has also contributed to the rise in CO_2 atmospheric levels, which augmented worldwide average temperatures (Mgbemene et al. 2016). Moreover, the soil water systems and rhizosphere microorganisms are polluted by accumulation of chemicals; consequently, the interaction between host plants and microbes may be also impacted (Compant et al. 2010). Also, drought covers approximately 41% of earth's land surface (Reynolds et al. 2007), so crops are in permanent relationship with dry environments that restrict crop yield.

Therefore, to use more friendly approaches without resignation of yield (quantitatively but also qualitatively), the exploration of techniques less injurious for the environment has gained attention (Phalan et al. 2011; Saad et al. 2020). Among them, the use of biostimulants specially containing microbial species, that is, bioinoculants (Woo and Pepe 2018), is profusely offered in the farming market in the last years. This review revises the scientific basis of such products.

5.2 Bioinoculant: Uses and Practices

Diverse concepts and terminologies are used for biostimulants in plant science (reviewed by Yakhin et al. 2017). Generally, the term is used to denominate products that are applied in low doses, different vias, and timings, with the aim to improve productivity (plant growth and crop yield). Their main mode of action is through changes in the plant hormonal status that leads to more efficient nutrition and activation of metabolic processes, which includes growth stimulation and/or production of protective compounds. Biostimulants present in the market are mostly products of biological origin and diverse sources (microbes, seaweeds, plants, animals, and humate-containing materials) with incomplete identified composition.

Therefore, their functional components and their mode of action are commonly unclear and vary over time and among batches.

Within the biostimulants, bioinoculants or bioformulations are those based on microbial species (bacteria or fungi; Rani and Kumar 2019). Bioinoculants are applied to the seeds, plants (roots or leaves), or soils, at implantation/sowing time and/or afterward, to influence the rhizospheric microflora. They show different types of relationships within the plant root system, as free-living or symbionts, and so promoting root and, by consequence, plant growth. Generally, fungal and bacterial microbial inoculation to the host plants may serve as biofertilizer (N_2 fixing and P solubilization), biostimulator by plant growth regulators' (PGRs; phytohormones) production, stress regulator (tolerance to drought and salinity), and biological control agents (BCAs) against phytopathogens (biopesticides; Mendes et al. 2011). Also, they may be used in bioremediation since heavy metals and organic contaminants can be controlled by consortiums of microbes and plants (Chaudhary and Shukla 2019; Funes Pinter et al. 2017).

The association between N_2-fixing microorganisms and plants has been investigated for many years, especially regarding the successful symbiotic relations between Leguminosae and bacteria of the genera *Rhizobium*, *Sinorhizobium*, and *Bradyrhizobium*. Notwithstanding, since this is a rather specific well-known technique, it will not be covered in this review, and the focus will be in the role of PGRs, mainly phytohormones, associated with the microorganisms used as bioinoculants. Some of them are bacteria that have the capability to fix N_2, so they are named diazotrophs, which include the genera *Rhizobia*, *Azotobacter*, *Gluconacetobacter*, *Azospirillum*, and *Azoarcus* (Morel and Castro-Sowinski 2013).

Microbial inoculants must colonize the root system and interact and compete with native microorganisms through plant-soil-microbe interactions. Thereby, different signaling pathways from plant microorganism and between microbes are involved in the establishment of the inoculant in the root region (dos Santos and Olivares 2021). For example, microbia sense environmental signals, produce phytohormones, and transmit such information to key regulatory elements allowing the plants to fine tune their responses to precise situations (Sparks et al. 2013). Changes in the plant microbiome are also related to agricultural practices, mainly by nutritional variations (Cai et al. 2017), and to evaluate the effectiveness of microbe inoculation by their root colonization and persistence over time, various methodological approaches are used (Romano et al. 2020).

The effects of bioinoculants on plant growth promotion and crop yield can be classified as direct when the inoculum acts on plant metabolism, for example, by phytostimulation through microorganism PGR production (Glick 1995; Piccoli et al. 2011) and/or by plant's elicitation of PGRs (Cohen et al. 2008; Gaiero et al. 2013) and also by biofertilization as microorganism N_2 fixation (Boddey and Dobereiner 1995), phosphate solubilization, and siderophore production (Glick 1995) as well as by improving the distribution of roots in the soil, increasing the sites of colonization by the microbiota and water and nutrient uptake (Ambrosini et al. 2016). As mentioned, phytostimulation is related to plant growth and is activated by PGRs, produced by the microorganism and/or by eliciting their synthesis in plant tissues.

PGRs may include abscisic acid (ABA), gibberellins (GAs), auxins (mainly indole-3-acetic acid, IAA), cytokinins (Cyt), ethylene (Et), jasmonic acid (JA), salicylic acid (SA), strigolactones (SL), and brassinosteroids (BRs; Fahad et al. 2015a; Ruyter-Spira et al. 2013; Stringlis et al. 2018).

The bioinoculants may also act as biofertilizers, providing the plant with nitrogen and phosphorus, for example, through symbiotic associations between nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and arbuscular mycorrhizal fungi (AMF; Müller et al. 2016). The bacteria solubilize phosphorus ions, while the AMF can translocate them to the plant. In this sense, it has been shown that phytohormones also interact to regulate the establishment and functioning of the AMF symbiosis (Bucher et al. 2014; Foo et al. 2013; Gutjahr 2014).

On the other hand, there are indirect effects of bioinoculants through promotive mechanisms in plant's growth by biocontrol, inhibiting the growth of plant pathogens (Glick and Bashan 1997), by producing hydrogen cyanide (Lorck 1948), and also by increases in plant's tolerance to damaging effects of environmental stresses like drought (Cohen et al. 2016), salinity (Shahzad et al. 2017), nutrient deficiency (Chanway and Holl 1994), and heavy metal contamination (Funes Pinter et al. 2017). Reductions of Et levels were found in the plant due to bacterial secretion of the aminocyclopropane-1-carboxylate (ACC) deaminase enzyme, production of exopolysaccharides, and increased activity of antioxidant enzymes (Glick et al. 2007). Additionally, the bioinoculants may enhance the plant defense system by a mechanism known as induced systemic resistance (ISR) that is activated by PGRs (Pieterse et al. 2014). Also, filamentous bacteria that oxidize sulfides have been described, reducing toxic levels and suppressing methane emissions (Pfeffer et al. 2012). They have been associated with roots of aquatic plants, importantly rice, although evidence suggests an ample distribution in plant-bacteria relationships (Scholz et al. 2021).

Commercial bioinoculants or bioformulations are based on microbes (bacteria or fungi) and are compounds of a single strain or mixed cultures or in co-inoculation with other microbes (consortia-based bioinoculants; Mendes et al. 2011). The success of these formulations is based on their performance under field condition, market price, ease of handling, and application (Fravel et al. 1998). Generally, they are formulated as solid or liquids for application such as sprays, seed treatments, and/or incorporation into soil, having poor shelf life and irregular performance in field conditions. The active ingredient is a viable organism (microbe or spore) immobilized or trapped on various types of carrier materials such as inert (inactive) substrates, liquids, and soils (Bashan et al. 2014). Suitable carrier material may increase the shelf life of the product and also assure that the active ingredients are easily established in the root region (Burges 2012). Commonly used inert carrier materials are fine clay, peat, vermiculite, alginate, and polyacrylamide beads (Digat 1989). Adjuvants or additives such as anticaking agents, ultraviolet protectants, and adsorption materials, including xanthan gums, silica gel, carboxymethyl cellulose, and starch, are used, to protect the active ingredient and to improve physicochemical and nutritional properties (Hynes and Boyetchko 2006; Schisler et al. 2004). It is important to note that the potency of the formulations is based on the capability of the microorganism to survive and proliferate in field condition, primarily dependent on the strain of microorganism used, and also based on the stabilization and protection of the microorganisms from damaging factors and its survival during production, storage, distribution, and application (Mishra and Arora 2016). Broadly, two types of formulations are available in the market for bioinoculants: solids and liquids (Burges 2012). Solid formulations include granules, microgranules, wettable powders, wettable/water-dispersible granules, and dust (Keswani et al. 2016). Liquid formulations are flowable and are suspensions in water, oils, or both (emulsions).

The most frequently used plant growth-promoting fungi genera are *Aspergillus*, *Fusarium*, *Penicillium*, *Piriformospora*, *Phoma*, and *Trichoderma* (Hossain et al. 2017). Among PGPB genera are *Rhizobium* spp., *Bradyrhizobium* spp., *Mesorhizobium* spp., *Pseudomona*s spp., *Bacillus* spp., *Azotobacter* spp., and *Azospirillum* spp. (Mishra and Arora 2016). The BCAs mostly used in the biopesticides include fungi – *Trichoderma* spp., *Ampelomyces quisqualis*, *Coniothyrium minitans*, *Aspergillus niger*, and nonpathogenic *Fusarium* spp. – and bacteria *Pseudomonas fluorescens*, *Bacillus* spp., and *Agrobacterium radiobacter* (Keswani et al. 2016).

5.3 Phytohormones Produced by Soil Microorganisms

Some of the mechanisms proposed to account for PGPB, like N_2 fixation, have been of lower agronomic impact of what was originally predicted and require complementary chemical or organic fertilization (Glick 2012; James and Olivares 1998). Contrariwise, the demonstrated capacity of most PGPB to produce plant hormones has gained importance (Bashan and de-Bashan 2005; Okon and Labandera-González 1994; Zaman et al. 2015). It has been established that about 80% of the bacteria isolated from the plant's rhizosphere zone are able to produce auxins (Cheryl and Glick 1996; Costacurta and Vanderleyden 1995; Patten and Glick 1996). However, the bacteria also produce GAs (Bastián et al. 1998; Bottini et al. 1989; Janzen et al. 1992; Rademacher 1994), ABA (Cohen et al. 2008; Salomon et al. 2014), Cyt, Et (Iosipenko and Ignatov 1995; Strzelczyk et al. 1994), and JA (Forchetti et al. 2007). Also, AMF capacity to biosynthesize phytohormones and therefore promote root growth and tissues' defensive responses to several stresses is well documented (Bucher et al. 2014; Foo et al. 2013; Goddard et al. 2021; Gutjahr 2014; Pozo et al. 2015).

Historically, the first report of a plant growth regulator-like substance produced by microorganisms goes back to the 1920s in Japan, when Kurosawa (1926; cited in Tamura 1991) reported that the filtrates of cultures of the fungus *Fusarium moniliforme* provoked the rice disease known as *bakanae* (foolish plant). This is characterized by unusually long internodes, which make plants to fall down with the consequent loss in yield because of grain deterioration. As the sexual form of *F. moniliforme* is *Gibberella fujikuroi*, the name gibberellic acid was coined to denominate a substance of acidic nature produced by cultures of such genus.

Afterward, Yabuta and Sumiki (1938; cited in Tamura 1991) elucidated its chemical structure by spectroscopic methods. Notwithstanding, the rest of the world has noticed GAs' existence at the beginning of the 1950s, when the former structure proposed for gibberellic acid was confirmed as a mixture of the now known GA_1 and GA_3 (Curtis and Cross 1954). Although GAs' production by plants was genetically predicted by maize mutants (Phinney 1956) and spectroscopically identified by MacMillan and Suter (1958) from *Phaseolus* sp., these phytohormones are produced not only by higher plants and fungi but also by bacteria (Bastián et al. 1998; Bottini et al. 1989, 2004; Janzen et al. 1992). Gibberellins are diterpenoids involved in various physiological processes in plants (Crozier et al. 2000; Davies 2004; Takahashi et al. 1991), including germination of seeds, seedling elongation, stem and leaf growth, floral induction, and flower and fruit growth and development (King and Evans 2003; Pharis and King 1985). Gibberellins also promote root growth, profusion of root hairs, impairment of floral bud differentiation in woody angiosperms, regulating vegetative and reproductive bud dormancy, and delaying senescence in organs of various plant species (Bottini and Luna 1993; Fulchieri et al. 1993; Reinoso et al. 2002; Rood et al. 1989; Tanimoto 1991, 2005). In much of such processes, GAs do not work alone but in combination with other plant hormones and regulatory elements, and therefore, the signaling network becomes highly intricate (Trewavas 2000). To date, 136 different chemical structures have been characterized as GAs (http://www.plant-hormones.info/gainfo.asp). Also, an unknown number of glucose conjugates may work as biosynthetic and catabolic intermediates (Schneider and Schliemann 1994). However, of the 136 free forms, only the 3β-hydroxylated, C19 GAs GA_1, GA_3, GA_4, GA_7, and purportedly GA_{32} (Bottini et al. 1985) have been reported as directly effective in shoot length promotion (Crozier et al. 2000). Metabolism of GAs differentiates in fungus and higher plants (Hedden et al. 2001), and bacterial GAs' biosynthesis resembles that of plant tissues (Lemke et al. 2019; Nett et al. 2017; Salazar-Cerezo et al. 2018).

The concept of the chemical (hormonal) control of tissues' growth and development in plants (first) and sensitive organisms (later) was developed in the second half of the nineteenth century by pioneering work of several botanists whose results were applied to study the phenomena of photo- and geotropism by Charles and Francis Darwin (Crozier et al. 2000). Such studies led to the isolation by Went (1926) of the first so-called hormone, named auxin that is derived from the Greek word "auxein" for growth. Auxin is the generic term denominating the group of indole compounds that induce cell enlargement (generally in the subapical region of plant shoots), which is the physiological action that characterizes IAA, which is the main auxin in plant tissues that was first spectroscopically characterized by Haagen-Smith et al. (1942). The auxin mode of action has been related with shoot and root growth orientation in response to light and gravity (Yang et al. 2020), apical dominance (Barbier et al. 2017), differentiation of vascular tissues (Aloni 2001), rooting (Pacurar et al. 2014), cell division, and general elongation of shoots and roots (Wang and Ruan 2013). The sources that regulate the "pool" of endogenous auxin available for the plant include de novo synthesis, catabolism, reversible (synthesis and hydrolysis) conjugation with glucose and irreversible with amino acids (Park et al. 2007), as well as bacterial synthesis (Spaepen and Vanderleyden 2011). Production of IAA by bacteria has been extensively studied from two standpoints: (i) the physiological effect on the plant (Bar and Okon 1992; Fallik et al. 1989) and (ii) the plant-microorganism interaction (Costacurta and Vanderleyden 1995).

Abscisic acid is a terpenic (C15) plant hormone chemically identified for the first time in young cotton fruits by Ohkuma et al. (1963) and in sycamore buds by Cornforth et al. (1965), and the name “abscisin” was proposed since the substance was correlated with abscission of the organs. ABA is produced by higher plants, algae, and fungi (Zeevaart 1999) and bacteria (Cohen et al. 2008) and even (purportedly) in human tissues (Bruzzone et al. 2007; Le Page-Degivry et al. 1986). In higher plants, its synthesis takes place in plastids via the carotene pathway in chloroplasts (Finkelstein and Rock 2002; Kushiro et al. 2004; Li and Walton 1990; Lichtenthaler 1999; Nambara and Marion-Poll 2005; Xu et al. 2002). Notwithstanding, ABA catabolism happens in cytosol, and glycosylation is associated with endoplasmic reticulum (Ma et al. 2018), and ABA site of synthesis correlates with its role in regulating stomata closure since occlusive cells are the only epidermal ones having chloroplasts. In fungi, ABA synthesis proceeds directly from farnesyl diphosphate (Takino et al. 2018), while for ABA-producing bacteria, the mechanisms are mostly speculative. The conspicuous effect that characterizes ABA’s role in plants is the closure of stomata (Davies 2004; Leung and Giraudat 1998), and it is known that plants growing in dry soils close their stomata (Huang and Zhu 2004; Tardieu et al. 1991; Tardieu and Davies 1992; Zhang and Davies 1989). Other purported functions are the promotion of protein storage in seeds and general defensive roles toward environmental stresses (Davies and Zhang 1991; Dodd and Davies 2004). In addition to this, growth as dry matter increase is the result not only of CO_2 fixation and reduction, but it also depends on carbon transport to different plant sinks. As sink strength may depend on the cell turgidity, its improvement should account for an increased carbon allocation in plant components of interest for human use and/or important for plant’s survival and prevalence, like roots, reserve parts, and reproductive organs. In correlation, grain yield has been improved in field-grown wheat (Travaglia et al. 2007, 2010) and soybean (Travaglia et al. 2009) after ABA application. The concept is reinforced by the finding that ABA enhances phloem area and expression of sugar transporters, stimulating carbohydrate mobility (Murcia et al. 2015).

The search of promoters of plant’s cell division in culture media leads to the accidental (as degraded nucleic acid) discovery of Cyt by Skoog and Miller (1957). They are N^6-substituted adenine derivatives, which usually contain an isoprenoid in the side chain. Zeatin, 6-(4-hydroxy-3-methylbut-trans-2-enylamino)purine, is the natural Cyt in higher plants, and it is also produced by some bacteria. Its chemical structure was established by Letham et al. (1964) from immature maize grains, so it was called zeatin. In plants, Cyt are present as both the free base and the corresponding nucleosides and nucleotides, which are suggested to be derived from *t*RNA. Their synthesis shares similar enzymes and intermediates in both plants and bacteria but originated from ADP/ATP in plants, while AMP is the initial compound in bacteria (Mok and Mok 2001). These hormones modulate various aspects of plant growth and development, like apical dominance, differentiation of chloroplast, seedling de-etiolation, flower and fruit development, organs' (specially leaves) senescence, influence on the plant-pathogen interactions, and seed germination (Crozier et al. 2000). They act as intermediates in the plant's response to various environmental signals (Arkhipova et al. 2007) and in an integrated manner with other phytohormones (Khan et al. 2020). In fact, several microorganisms have been reported as producing different hormones (Bastián et al. 1998; Karadeniz et al. 2006).

Ethylene (ethene) is a single and symmetric molecule, composed of two C united by a double bond and four atoms of H. There exist numerous publications related with its synthesis in higher plants (Mattoo and Suttle 1991), but very few are related with microbial synthesis (Arshad and Frankenberger 2002). Lieberman et al. (1965) revealed that methionine is the precursor of Et in plants, which has been also proposed for bacteria (Fukuda et al. 1993). It is a true phytohormone associated with high respiration rates, typically in senescent or damaged tissues. High auxin levels stimulate Et synthesis (Morgan and Drew 1997; Morgan and Hall 1964). In general, Et formation is directly linked with stresses (Beyer 1984; Grichko and Glick 2001; Hyodo 1991; Jian-Jun et al. 2015; Morgan and Drew 1997), like low temperatures, heat, flooding, wounding, and drought, in an integrated net of responses with ABA, JA, auxins, and BRs (Zhao et al. 2021). Pathogen attacks are related with Et evolution in the affected tissues (Reinhardt et al. 1991), and some Et-producing bacteria are related to their pathogenicity. Also, several bacteria promote plant growth indirectly via the ACC deaminase, a key enzyme in Et metabolism that hydrolyzes ACC, which is an immediate precursor (Glick 1995).

Jasmonic acid and its methyl ester (MeJA) are fatty acid-derived cyclopentanones ubiquitously present in plants and insects. They affect an ample variety of physiological processes, acting as potent signals for the expression of defensive proteinase inhibitors active in plant defense (Howe 2010). Soil PGPB produce JA in chemically defined medium (Forchetti et al. 2007). Jasmonates are involved in defense responses to multiple biotic and abiotic stresses (Santino et al. 2013; Taniguchi et al. 2013; Yamada et al. 2012). However, it has been also established that JA favors the entrance of pathogens through wounded tissues (Antúnez-Lamas et al. 2009) and even triggers infection (Giménez-Ibánez et al. 2014) via bacterial effectors that activate the JA signaling (Jiang et al. 2013), increasing the pathogen virulence (Katsir et al. 2008). It has been reported that JA stimulates production of secondary metabolites (Cappellari et al. 2019). The JA and SA signaling also maintain crosstalk in defense against pathogens (Giménez-Ibánez and Solano 2013).

Although the presence of SA (2-hydroxybenzoic acid) in plants, especially from *Salix* cortex, has been recognized for a long time (Raskin 1992a), it was just proposed as having a hormonal role in the plants themselves in 1992 (Raskin 1992b). It participates in several physiological and biochemical plant processes (Hayat et al. 2007), mainly related with plant defense against pathogens via the systemic acquired resistance (SAR; Kim and Hwang 2014) and abiotic stresses (Khan et al. 2015). Its biosynthesis in plants apparently proceeds via an isochorismate-utilizing pathway in the chloroplast (Lefevere et al. 2020). However, even though SA production by bacteria has been reported (Lin et al. 2014), its role from the microorganism standpoint is mostly ignored.

Strigolactones are a relatively new class of phytohormones derived from carotenoids (Alder et al. 2012) with a tricyclic lactone structure (Al-Babili and Bouwmeester 2015), mainly involved against abiotic stresses in plants (Wajeeha et al. 2017). More specifically, SL root exudation seems to be responsible for plant responses to P/N limitation stresses (Santoro et al. 2021; Umehara et al. 2015). They mostly induce branching of AMF hyphae, but to what extent they affect the rhizosphere microbiomes is poorly understood, except that they stimulate fungal rhizosphere but not bacterial communities (Carvalhais et al. 2019). Notwithstanding their active role in molecular signaling between plant roots and AMF, SL seems to be only produced by the host and not microbes.

Brassinosteroids, first reported by Mitchell et al. (1970), are steroidal phytohormones present in most plant species (algae, ferns, gymnosperms, and angiosperms; Fujioka and Yokota 2003; Sasse 2003). Their synthesis in plant cells comes from cycloartenol, which is derived from the cyclized triterpene squalene (Bishop and Yokota 2001). There exists ca. 70 different BR-like structure-based triterpenes of C27, C28, and C29 (Wang et al. 2014). The BRs affect stem elongation, stimulate tracheary element differentiation and cell division, accelerate senescence and membrane hyperpolarization, and have been shown to mediate abiotic and biotic stresses, including salinity and drought, temperature extremes, and pathogen attack (Clouse 2011). They also regulate timing of flowering (Domagalska et al. 2010), pollen development (Ye et al. 2010), and organ differentiation (González-García et al. 2011). Participation of BRs in the plant interactions with pathogens has been reported, although the effects are dependent on the pathogen's types and the plant species. In some cases, they induce resistance, but in others, they induce susceptibility (Yu et al. 2018).

5.4 Role of Phytohormones in the PGPB-Plant Relationship

Plants are subjected to a variety of abiotic and biotic stresses that cause significant losses in crop productivity. This is because they must adapt to stress in a physiologically costly way resulting in reduced resources to produce biomass, seeds, and thus yield (Dresselhaus and Hückelhoven 2018). Phytohormones play a crucial role during the response of plants to that stressful condition as they regulate cellular activities and signal transduction pathways (Pieterse et al. 2009). The mode of action of phytohormones involves a crosstalk between them (synergistically or antagonistically), which is crucial for plant development as well as the responses to biotic and abiotic stresses (Khan et al. 2020). Therefore, the ability of PGPB to produce phytohormones or to regulate their content in plant tissues is a characteristic that becomes important when formulating a bioinoculant for plants growing in stressful conditions.

Environmental factors, often related to global warming, that negatively affect plant's establishment and growth include drought, salinity, ultraviolet B radiation, and heavy metals' contamination. Drought is one of the most widespread stresses affecting plants (Farooq et al. 2012). There are zones of the earth where water availability is naturally restricted, but also the increase in atmospheric CO_2 concentration and temperature caused by climate change leads to a decrease in soil water content with the concomitant augment of areas with drought problems (Cohen et al. 2016; Mishra and Singh 2011). PGPB showed a positive effect on plants submitted to water restriction. The ABA producer *Azospirillum brasilense* can enhance ABA content in *Arabidopsis thaliana*, and that was correlated with higher relative water content in leaves and a delay in wilting, thus alleviating water-deficit stress (Cohen et al. 2014). It is known that in this plant many drought-regulated genes are ABA-responsive genes (Huang et al. 2008). In grapevine, the application of *Bacillus licheniformis* and *Pseudomonas fluorescens* decreased water loss rate since plants inoculated with these bacteria lost 4% and 10% less water than controls, in correlation with an increase of 70-fold and 40-fold, respectively, in leaf ABA content (Salomon et al. 2014). Also, PGPB increase tolerance to drought and salinity of plants like wheat, rice, and mung bean by enhancing antioxidant enzymes' synthesis and/or activity (Chandra et al. 2018; Filgueiras et al. 2020; Sarma and Saikia 2014), a response that is regulated by ABA (Berli et al. 2010; Xu et al. 2018). Although ABA is the major stress hormone studied under drought stress due to its function in stomata closure, the tolerance to drought induced by bacteria is also associated with the augment of other phytohormones. In cucumber, inoculation with PGPB reduces adverse effects of salinity and osmotic stress by increasing GAs and SA content (Kang et al. 2014). Gibberellin production by bacteria has been related to increases in growth and yield in several plants (Bottini et al. 2004), purportedly via stimulation of root growth and root hair density (Fulchieri et al. 1993). Gutiérrez-Mañero et al. (2001) showed that applications of extracts from medium incubated with the GAs' producers *Bacillus pumilus* and *Bacillus licheniformis* reversed the dwarf phenotype in alder (*Alnus glutinosa* L.) seedlings. Kang et al. (2012) reported that *Promicromonospora* sp. SE188 inoculation significantly activated GAs' biosynthesis pathways in tomato plants, in correlation with significantly higher shoot length and biomass as compared to controls. On the contrary, ABA content was decreased in these plants. Thus, the increment of GAs' content in plants induced by the inoculation of PGPB may also be involved in the alleviation of drought indirectly by augmenting the root ability of water and mineral uptake (Fulchieri et al. 1993). Also, it has been reported that IAA produced by PGPB improved the responses to drought and salt stresses due to its effect in elongation and formation of lateral roots and root hairs, improving the absorption of water in alfalfa and wheat (Chandra et al. 2018; Defez et al. 2017; Li et al. 2020). In salinity stress, the ionic imbalance derives in hyperosmotic stress that provokes ion toxicity and water deficit in plants, increasing reactive oxygen species (ROS) accumulation (Fahad et al. 2015b). In this context, root systems are crucial in crop salt tolerance due to their importance in water and nutrient acquisition and restriction of salt accumulation.

Heavy metal pollution has recently emerged as a stress factor affecting growth and agricultural productivity worldwide (Ma et al. 2018). Besides natural sources, industrialization and intensive agriculture are the major causes of the global heavy metal pollution problems (Funes Pinter et al. 2017; Islam et al. 2017). Like the effect of drought, plants exposed to heavy metals show physiological and metabolic anomalies ranging from chlorosis of leaves to protein degradation, lipid peroxidation, and increase of ROS; thus, growth is severely affected (Emamverdian et al. 2015; Nazli et al. 2020). The ACC deaminase enzyme present in several PGPB has been proposed as one of the main mechanisms by which bacteria help plants grow in heavy metal-contaminated environments. This enzyme sequesters and cleaves the Et precursor ACC, causing a decrease of Et levels in the plants and helping them in the formation of longer roots (increasing growth) and enhancing seedling survival (Glick 2005; Nazli et al. 2020). Also, the IAA production by bacteria may act in improving even more root length (Spaepen and Vanderleyden 2011). This has been reported in bacteria strains of the genera *Pseudomonas*, *Serratia*, *Klebsiella*, *Bacillus*, *Rahnella*, and *Achromobacter* (Gontia-Mishra et al. 2016; He et al. 2013; Khan et al. 2017; Ma et al. 2008; Mendoza-Hernández et al. 2016). Furthermore, plants submitted to heavy metal stress increased their antioxidant enzyme activities after PGPB inoculation, alleviating the toxic effect of ROS and increasing plant biomass (Islam et al. 2014). Funes Pinter et al. (2017, 2018) reported that *Micrococcus luteus*, *B. licheniformis*, and *P. fluorescens* increased arsenic tolerance and growth of grape plants by eliciting an augment of catalase, ascorbate peroxidase, and peroxidase activity. Similar results were found in inoculated maize subjected to chromium contamination (Islam et al. 2017). As it was mentioned above, there is evidence that the activation of antioxidant response in plant is regulated by the phytohormone ABA (Berli et al. 2010; Xu et al. 2018).

Rhizogenic PGPB and fungi also help plants to cope with biotic stresses caused by pests. They can inhibit growth of pathogens by production of inhibitory compounds like antibiotics and lytic enzymes (Kenawy et al. 2019; Meena et al. 2020), bacteriocins (Nazari and Smith 2020), and volatiles (Fernando et al. 2005; Sang et al. 2011). Another mechanism is to induce a defense response in the plant called ISR, in which microorganisms help the plant to reduce the incidence of diseases by eliciting modification of physical and biochemical properties of the host plant that enhances its defensive capacity against pathogenic bacteria, viruses, and fungi (Kloepper et al. 2004). Different bacterial molecules have been identified acting as elicitors. Investigation on the signaling pathway of ISR showed that JA and Et play a central role in the regulation of the response (Mhlongo et al. 2018). It was demonstrated in *Arabidopsis*, tomato, rice, and cucumber using JA and Et signaling mutants (or inhibitory compounds), showing that signaling of these phytohormones was blocked and thus the ability to express ISR upon colonization of the roots by the PGPB (Van der Ent et al. 2009). Other evidence of JA involvement in ISR was evaluated at gene level; Martínez-Hidalgo et al. (2015) showed that the bacteria *Micromonospora* reduce the fungal pathogen *Botrytis cinerea* infection in tomato-inoculated plants, and the gene expression analysis revealed that JA-dependent defense was strongly induced in the presence of the bacteria. Different strains of

Pseudomonas also showed the ability to induce an ISR in plants by priming JA- and Et-dependent gene expression (Van Wees et al. 2008). The response in plants usually derives in an enhancement of defense enzyme activity and pathogen-related proteins and synthesis of secondary metabolites such as terpenes and phenols (Heil and Bostock 2002; Magnin-Robert et al. 2007; Salomon et al. 2014; Chakraborty et al. 2006).

5.5 Yield Increase and Environmental Advantages: Phytohormonal Bioinoculants

Many efforts have been made to test the ability of bioinoculants, basically composed by consortia of PGPB and/or mycorrhizal fungi-bacteria, with the idea of improving rhizosphere biodiversity and growth/yield of several cash crops, like wheat (Dal Cortivo et al. 2020). However, positive results obtained at the lab/greenhouse level are rarely reproducible in the open field as significant yield increases on a yearly basis, and even in the case of increments, they are far away from those obtained with chemical fertilization (Dobbelaere et al. 2003; Glick 2012; James and Olivares 1998). A possible explanation for such ineffectiveness may be competition with the indigenous population of microorganisms (Bacilio-Jiménez et al. 2001), although it is also possible that the resident rhizobiome is effective enough by itself. That is, the microorganism/microbial complex (holobioma; Bordenstein and Theis 2015) is in equilibrium under "normal" non-stressful conditions (Hibbing et al. 2010). As matter of fact, plant bacterization shows more effective when the system is under traumatic environmental situations (Egamberdieva and Adesemoye 2016). This scenario resembles what happens in human beings (Hibbing et al. 2010; Kho and Lal 2018), where disturbance of gut microbiome is a health issue, but alteration may be counteracted by enrichment of the microflora with probiotics (Baumgartner et al. 2020). There have been reported, however, direct growth and yield stimulation in some species and particular situations (Bashan and de-Bashan 2005), like organically grown strawberries (Esitken et al. 2010), although in such cases what happened is an enrichment of rather gnotobiotic systems with beneficial microorganisms. Notwithstanding, replacement of chemical fertilization with bioinoculants is poorly effective, unless they are accompanied (at least) by organic amendments and compost (Faisal et al. 2021; Ullah et al. 2021).

As it has been mentioned above, several mechanisms (direct and indirect) have been claimed as (partially) responsible for plant's growth and yield promotion by bioinoculants, namely, inhibition of plant pathogens, production of plant hormones, fixation of atmospheric nitrogen, mineral solubilization, siderophore production, lowering of stress-induced Et by impairment of ACC synthetase, production of exopolysaccharides, enhancement of antioxidant enzymes' activity, and elicitation of tolerance to drought and heavy metal contamination.

Having in mind the evidence accumulated throughout approximately 40 years of experimental work, the beneficial effects of PGPB consortia may be (generally) explained because of enhancement in the roots' ability to water absorption and nutrient uptake, which in turn improves cell expansion by amelioration of the plant water status (cell turgor), dry matter accumulation, and increase of metabolism. In fact, the concurrence of many of the precited mechanisms is related to upgrading water and nutrient availability, which would explain the better hydric and mineral condition. Such holistic approach may be sustained by the general concept that the hidden part of the plant accounts for what happens in the upper visible one, particularly under restrictive environmental conditions. In summary, a more developed and functionally active root system makes plants to grow and produce better.

However, most mechanisms deal (directly or indirectly) with phytohormonal production by microorganisms or elicitation of their biosynthesis by cell tissues because of bacterial action (Kurniawan and Chuang 2022). For instances, inhibition of plant pathogens (Olanrewaju et al. 2017) may result in an improved proliferation of beneficial (by phytohormonal production) PGPB that increases root growth. But PGPB may in turn elicit via ABA the synthesis of volatile defense substances against pathogens (Salomon et al. 2017b) as well as induce systemic resistance against biotic stresses by enhancement of antioxidant enzymes' activity (Salomon et al. 2016, 2017a), a well-known effect of ABA (Berli et al. 2010, 2015). Alternatively (or complementary), elicitation of tolerance to drought (Cohen et al. 2016) and heavy metal contamination (Funes Pinter et al. 2017, 2018) may be triggered by ABA produced by rhizospheric bacteria that promote accumulation of defense compounds (like terpenes) which protect cells against abiotic agents as ROS (Salomon et al. 2016). Also, osmo-protective and anti-oxidative mechanisms related to water stress tolerance may be elicited through production of nitric oxide (NO) and ABA (Pontin et al. 2021).

Bacterial production of the plant hormones auxins (Crozier et al. 1988) may result in promotion of root growth, which is linked with augmented GA levels because of biosynthesis (Bottini et al. 1989), metabolization (Cassán et al. 2001a), and/or deconjugation (Cassán et al. 2001b). Both auxins and GAs interact in root enhancement of soil exploration (Tanimoto 2005), while GAs also increase the root surface and the absorbing (hair) zone (Fulchieri et al. 1993), both beneficial for water and mineral uptake efficiency. Besides, stimulation in the production of exopolysaccharides, related to PGPB ability to help plants to overcome drought conditions, has been linked with phytohormone production by bacteria and delivery to the host plant (Ghosh et al. 2019).

Abscisic acid production by bacteria and/or elicitation of its synthesis by the plant tissues may also induce partial stomata closure keeping a better plant's hydric condition and by consequence favoring cell expansion (Salomon et al. 2014), as well as improvement in photo-assimilate transport (Moreno et al. 2011; Murcia et al. 2015, 2018). The negative effect on the photosynthetic activity by restriction in CO_2 diffusion through partial stomata closure may be counteracted by improvement of water use efficiency (Berli et al. 2021; Salomon et al. 2014; Sansberro et al. 2004) and dry matter accumulation, especially controlling the carbon allocation (Moreno et al. 2011; Murcia et al. 2015, 2018), both dependent on ABA levels.

Several bacterial genera are known to produce Cyt – *Arthrobacter*, *Bacillus*, *Azospirillum*, and *Pseudomonas* (Naz et al. 2009) – and this ability has been related to increased plants' tolerance to abiotic stresses (Jorge et al. 2019), like salinity (Egamberdieva 2009) and drought (Liu et al. 2013). However, studies just correlated Cyt production by bacteria with stress tolerance of plants, without further explanation regarding the mechanisms purportedly involved. Therefore, the question poses on if the Cyt are cause, consequence or the beneficial effects may be attributed to a crosstalk among different phytohormones.

It is known that JA and MeJA (its volatile ester) have defense roles in the plant responses against insects and microbial pathogens (Bari and Jones 2009). Most of the research performed so far deals with the regulative effect of JA produced by plants over the soil-plant microflora (Carvalhais et al. 2013). Nevertheless, production of JA by bacteria isolates has been demonstrated (Forchetti et al. 2007; Piccoli et al. 2011), so a protective role of JA produced by PGPB over the plant's tissues may be considered.

Several soil bacteria produce Et via different pathways (Nagahama et al. 1992), and growth promotion has been achieved when Et root levels increase by ACC synthase activity (Suganuma et al. 1995), alone or in combination with auxins (Ribaudo et al. 2006). Bacterial production of Et may also accelerate breaking seed dormancy (Berner et al. 1999) allowing an earlier seedling emergence useful in the establishment of extensive crops. However, Et synthesis may be impaired by a decrease in Et levels by producing ACC deaminase, an enzyme responsible for the cleavage of the plant Et precursor ACC into ammonia and α-ketobutyrate, providing resistance to some GA-producing fungus like *Fusarium* sp. (Liu et al. 2019). Overall results suggest that the role of this volatile alkene is under tight homeostasis in the holobioma and so is the root growth and capacity for water and nutrient uptake.

Most PGPB are diazotrophs, that is, free-living (non-symbiotic or mutualistic) N_2-fixing microbes allegedly able to promote plant growth by N fixation of atmospheric nitrogen (Baldani et al. 2002; Di Benedetto et al. 2017). However, the amount of N fixed by PGPB has been questioned as being substantial on a root size basis (Glick 2012; James and Olivares 1998), although they may be capable of supplement quite efficiently the necessity of mineral forms (Di Benedetto et al. 2017; Fulchieri and Frioni 1994; Jalilian et al. 2012; Leaungvutiviroj et al. 2010; Spolaor et al. 2016). It is feasible so that increases in total N_2 availability from fixation by soil bacteria may be more a matter of increases in the rhizospheric volume because the increments in hairy and branched roots (Fulchieri et al. 1993).

Mineral solubilization, like K and/or P (Alzate Zuluaga et al. 2021; De Freitas et al. 1997; Khan et al. 2007; Liba et al. 2006), is another way PGPB may promote plant growth, which is related with a bacterial elicitation for plant roots to segregate small organic acids (Feng et al. 2004; Rodríguez et al. 2004). However, there is no information regarding the mechanism of how bacteria elicit soil solution acidification; rather, most experiences inform promotion of soil microflora after organic and/or inorganic fertilization (Zhu et al. 2020), which in turn may be promoting root growth by phytohormonal production.

Production of siderophores is an indirect mechanism that has been associated with the increase in plant growth by PGPB. Siderophores produced by bacteria purportedly increase iron availability and inhibit pathogen growth in the rhizosphere (Kloepper et al. 1980). Their production has been correlated with auxin produced by PGPB (Díaz Peralta et al. 2012), purportedly via impairment of metal-induced inhibition of auxin synthesis (Dimkpa et al. 2008).

In short, growth promotion of the “hiding part” is reflected in the mass accumulation and fruit yield of the “visible one.” Therefore, it is possible to envision a saving of fertilizers when they are combined with bioinoculants. Such supplementary effects result in augmentation of the root efficiency. The overall beneficial consequence is reduction in the negative environmental impact.

Also, PGPB stimulation of plant’s survival during water shortage and throughout periods of increased plants’ sensitivity may be of crucial importance in improving yield at harvesting. Finally, a total replacement of mineral fertilization with bioinoculants sounds chimeric, since the microbe-based biofertilizers could hardly replace the use of agrochemicals in extensive agriculture without resignation of yield, but rather may help in a more rational use of the resources that will be environmentally amiable. Of course, the problem is not simple and requires the concurrence of several ecologically friendly agriculture techniques, which may be pursued through further advancements in “agriculture of precision.”

5.6 Concluding Remarks

The efficiency of bioinoculants relies on root growth promotion that enhances soil exploration for superior nutrient and water uptake, and such growth promotion is mostly dependent (directly and/or indirectly) on production of phytohormones by microorganisms.

In keeping worldwide yield, bioinoculants are not solely efficient by themselves but through combination with other technologies, namely, plants genetically designed for more efficient use of resources, direct sowing, moderate fertilization, organic manuring, green soil coverage, and crop rotation.

Benefits are diminution of resource expenditures, less contamination of soils and water sources, more “healthy” products harvested, and protection of biodiversity.

References

Al-Babili S, Bouwmeester HJ (2015) Strigolactones, a novel carotenoid-derived plant hormone. Annu Rev Plant Biol 66:161–186. https://doi.org/10.1146/annurev-arplant-043014-114759

Alder A, Jamil M, Marzorati M, Bruno M, Vermathen M, Bigler P, Ghisla S, Bouwmeester H, Beyer P, Al-Babili S (2012) The path from β-carotene to carlactone, a strigolactone-like plant hormone. Science 335:1348–1351. https://doi.org/10.1126/science.1218094

Aloni R (2001) Foliar and axial aspects of vascular differentiation: hypotheses and evidence. J Plant Growth Regul 20:22–34. https://doi.org/10.1007/s003440010001

Alzate Zuluaga MY, Martinez de Oliveira AL, Valentinuzzi F, Tiziani R, Pii Y, Mimmo T, Cesco S (2021) Can inoculation with the bacterial biostimulant *Enterobacter* sp. strain 15S be an approach for the smarter P fertilization of maize and cucumber plants? Front Plant Sci 12: 1771. https://doi.org/10.3389/fpls.2021.719873

Ambrosini A, de Souza R, Passaglia LM (2016) Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. Plant Soil 400:193–207. https://doi.org/10.1007/s11104-015-2727-7

Antúnez-Lamas M, Cabrera E, Lopez-Solanilla E, Solano R, González-Melendi P, Chico JM, Toth I, Birch P, Pritchard L, Liu H, Rodríguez-Palenzuela P (2009) Bacterial chemoattraction towards jasmonate plays a role in the entry of *Dickeya dadantii* through wounded tissues. Mol Microbiol 74:662–671. https://doi.org/10.1111/j.1365-2958.2009.06888.x

Appleford NEJ, Wilkinson MD, Ma Q, Evans DJ, Stone MC, Pearce SP, Powers SJ, Thomas SG, Jones HD, Phillips AL, Hedden P, Lenton JR (2007) Decreased shoot stature and grain α-amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat. J Exp Bot 58:3213–3226. https://doi.org/10.1093/jxb/erm166

Arkhipova TN, Prinsen E, Veselov SU, Martinenko EV, Melentiev AI, Kudoyarova GR (2007) Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292:305–315. https://doi.org/10.1007/s11104-007-9233-5

Armanda DT, Guinée JB, Tukker A (2019) The second green revolution: innovative urban agriculture's contribution to food security and sustainability—A review. Glob Food Sec 22: 13–24. https://doi.org/10.1016/j.gfs.2019.08.002

Arshad M, Frankenberger WT (2002) Ethylene. Agricultural Sources and Applications. Kluwer Academic/Plenum Publishers, New York, NY. ISBN: 0-303-46666-X

Bacilio-Jiménez M, Aguilar-Flores S, del Valle MV, Prez A, Zepeda A, Zenteno E (2001) Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. Soil Biol Biochem 33:167–172. https://doi.org/10.1016/S0038-0717(00)00126-7

Bai S, Yu H, Wang B, Li J (2018) Retrospective and perspective of rice breeding in China. J Gen Genom 45(11):603–612. https://doi.org/10.1016/j.jgg.2018.10.002

Baldani JI, Reis VM, Baldani VLD, Dobereiner J (2002) A brief story of nitrogen fixation in sugarcane-reasons for success in Brazil. Funct Plant Biol 29:417–423. https://doi.org/10.1071/PP01083

Bar T, Okon Y (1992) Tryptophan conversion to indole-3-acetic acid via indole-3-acetamide in *Azospirillum brasilense* Sp7. Can J Microbiol 39:81–86. https://doi.org/10.1139/m93-011

Barbier FF, Dun EA, Beveridge CA (2017) Apical dominance. Curr Biol 27:864–865. https://www.cell.com/current-biology/pdf/S0960-9822(17)30560-2.pdf

Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69:473–488. https://doi.org/10.1007/s11103-008-9435-0

Bashan Y, de-Bashan LE (2005) Bacteria/plant growth-promotion. In: Hillel D (ed) Encyclopedia of soils in the environment. Elsevier, Oxford, pp 103–115

Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33. https://doi.org/10.1007/s11104-013-1956-x

Bastián F, Cohen A, Piccoli P, Luna V, Baraldi R, Bottini R (1998) Production of indole-3-acetic acid and gibberellins A_1 and A_3 by *acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. Plant Growth Regul 24:7–11. https://doi.org/10.1023/A:1005964031159

Baumgartner M, Bayer F, Pfrunder-Cardozo KR, Buckling A, Hall AR (2020) Resident microbial communities inhibit growth and antibiotic-resistance evolution of *Escherichia coli* in human gut microbiome samples. PLoS Biol 18:e3000465. https://doi.org/10.1371/journal.pbio.3000465

Benbrook CM (2012) Impacts of genetically engineered crops on pesticide use in the U.S.—the first sixteen years. Environ Sci Eur 24:24. https://doi.org/10.1186/2190-4715-24-24

Berli FJ, Moreno D, Piccoli P, Hespanhol-Viana L, Bressan-Smith R, Silva MF, Cavagnaro JB, Bottini R (2010) Abscisic acid is involved in the response of grape (*Vitis vinifera* L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. Plant Cell Environ 33:1–10. https://doi.org/10.1111/j.1365-3040.2009.02044.x

Berli FJ, Alonso R, Beltrano J, Bottini R (2015) High altitude solar UV-B and abscisic acid sprays increase grape berry antioxidant capacity. Am J Enol Vitic 66:65–72. https://doi.org/10.5344/ajev.2014.14067

Berli FJ, Piccoli PN, Bottini R (2021) Abscisic acid and fruit ripening. Its role in grapevine acclimation to the environment: a case of study. In: Gupta DK, Corpas FJ (eds) Hormones and plant response. Springer, Switzerland. https://doi.org/10.1007/978-3-030-77477-6

Berner DK, Schaad NW, Völksch B (1999) Use of ethylene-producing bacteria for stimulation of *striga* spp. seed germination. BioControl 15:274–282. https://doi.org/10.1006/bcon.1999.0718

Beyer EM (1984) In: Fuchs Y, Chalutz E (eds) Why do plants metabolize ethylene? In: Ethylene. Advances in agricultural biotechnology, vol 9. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-6178-4_7

Bishop GJ, Yokota T (2001) Plants steroid Hormones, Brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. Plant Cell Physiol 42:114–120. https://doi.org/10.1093/pcp/pce018

Boddey RM, Dobereiner J (1995) Nitrogen fixation associated with grasses and cereals: recent progress and perspectives for the future. Fert Res 42:241–250. https://doi.org/10.1007/BF02370099

Bonny S (2008) Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. Agron Sustain Dev 28:21–32. https://doi.org/10.1051/agro:2007044

Bordenstein SR, Theis KR (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol 13:e1002226. https://doi.org/10.1371/journal.pbio.1002226

Bottini R, Luna V (1993) Bud dormancy in deciduous fruit trees. Curr Top Plant Physiol 1:147–159

Bottini R, de Bottini G, Koshioka M, Pharis RP, Coombe BG (1985) Identification of gibberellins A_1, A_5, A_{29}, and A_{32} from immature seeds of apricot (*Prunus armeniaca* L.). Plant Physiol 78: 417–419. https://doi.org/10.1104/pp.78.2.417

Bottini R, Fulchieri M, Pearce D, Pharis RP (1989) Identification of gibberellins A_1, A_3, and iso-A_3 in cultures of *Azospirillum lipoferum*. Plant Physiol 90:45–47. https://doi.org/10.1104/pp.90.1.45

Bottini R, Cassán F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503. https://doi.org/10.1007/s00253-004-1696-1

Bruzzone S, Moreschi I, Usai C, Guida L, Damonte G, Salis A (2007) Abscisic acid is an endogenous cytokine in human granulocytes with cyclic ADP-ribose as second messenger. Proc Natl Acad Sci U S A 104:5759–5764. https://doi.org/10.1073/pnas.0609379104

Bucher M, Hause B, Krajinski F, Küster H (2014) Through the doors of perception to function in arbuscular mycorrhizal symbioses. New Phytol 204:833–840. https://doi.org/10.1111/nph.12862

Burges HD (2012) Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-4926-6

Cai F, Pang G, Miao Y, Li R, Li R, Shen Q, Chen W (2017) The nutrient preference of plants influences their rhizosphere microbiome. Appl Soil Ecol 110:146–150. https://doi.org/10.1016/j.apsoil.2016.11.006

Cappellari LR, Santoro MV, Schmidt A, Gershenzon J, Banchio E (2019) Induction of essential oil production in *Mentha x piperita* by plant growth promoting bacteria was correlated with an increase in jasmonate and salicylate levels and a higher density of glandular trichomes. Plant Physiol Biochem 141:142–153. https://doi.org/10.1016/j.plaphy.2019.05.030

Carvalhais LC, Dennis PG, Badri DV, Tyson GW, Vivanco JM, Schenk PM (2013) Activation of the Jasmonic acid plant Defence pathway alters the composition of rhizosphere bacterial communities. PLoS One 8:e56457. https://doi.org/10.1371/journal.pone.0056457

Carvalhais LC, Rincon-Florez VA, Brewer PB, Beveridge CA, Dennis PG, Schenk PM (2019) The ability of plants to produce strigolactones affects rhizosphere community composition of fungi but not bacteria. Rhizosphere 9:18–26. https://doi.org/10.1016/j.rhisph.2018.10.002

Cassán F, Bottini R, Schneider G, Piccoli P (2001a) *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA_{20} and metabolize the resultant aglycones to GA_1 in seedlings of Rice dwarf mutants. Plant Physiol 125:2053–2058. https://doi.org/10.1104/pp.125.4.2053

Cassán F, Lucangeli C, Bottini R, Piccoli P (2001b) *Azospirillum* spp. metabolize [17,17-$^2H_2$] gibberellin A_{20} to [17,17-$^2H_2$]gibberellin A_1 in vivo in *dy* rice mutant seedlings. Plant Cell Physiol 42:763–767. https://doi.org/10.1093/pcp/pce099

Chakraborty U, Chakraborty B, Basnet M (2006) Plant growth promotion and induction of resistance in *Camellia sinensis* by *bacillus megaterium*. J Basic Microbiol 46:185–195. https://doi.org/10.1002/jobm.200510050

Chandra D, Srivastava R, Sharma AK (2018) Influence of IAA and ACC deaminase producing fluorescent *pseudomonads* in alleviating drought stress in wheat (*Triticum aestivum*). Agric Res 7:290–299. https://doi.org/10.1007/s40003-018-0305-y

Chanway CP, Holl FB (1994) Ecological growth response specificity of two Douglas-fir ecotypes inoculated with coexistent beneficial bacteria. Can J Bot 72:582–586. https://doi.org/10.1139/b94-077

Chaudhary T, Shukla P (2019) Bioinoculants for bioremediation applications and disease resistance: innovative perspectives. Indian J Microbiol 59:129–136. https://doi.org/10.1007/s12088-019-00783-4

Cheryl P, Glick B (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220. https://doi.org/10.1139/m96-032

Clouse SD (2011) Brassinosteroids. The Arabidopsis Book 9:e0151. https://doi.org/10.1199/tab.0151

Cohen AC, Bottini R, Piccoli P (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regul 54:97–103. https://doi.org/10.1007/s10725-007-9232-9

Cohen AC, Bottini R, Pontin M, Berli FJ, Moreno D, Boccanlandro H, Travaglia CN, Piccoli PN (2014) *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153:79–90. https://doi.org/10.1111/ppl.12221

Cohen AC, Piccoli P, Bottini R, Salomon MV (2016) Plant–rhizobacteria interaction and drought stress tolerance in plants. In: Hossain MA, Wani SH, Bhattachajee S, Burritt DJ, Tran L-SP (eds) Drought tolerance in plants, Physiology and Biochemistry, vol 1. Springer, New York. https://doi.org/10.1007/978-3-319-28899-4_12

Compant E, van der Heijden MGA, Sessitsch A (2010) Climate change effects on beneficial-plant microorganism interactions. FEMS Microbiol Ecol 73:197–214. https://doi.org/10.1111/j.1574-6941.2010.00900.x

Cornforth JW, Milborrow BV, Ryback G, Wareing PF (1965) Identity of sycamore ‘dormin’ with abscisin II. Nature 205:1269–1270. https://doi.org/10.1038/2051269b0

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18. https://doi.org/10.3109/10408419509113531

Crozier A, Arruda P, Jasmim JM, Monteiro AM, Sandberg G (1988) Analysis of Indole-3-acetic acid and related indoles in culture medium from *Azospirillum lipoferum* and *Azospirillum brasilense*. Appl Environ Microbiol 54:2833–2837. https://doi.org/10.1128/aem.54.11.2833-2837.1988

Crozier A, Kamiya Y, Bishop G, Yokota T (2000) Biosynthesis of hormones and elicitor molecules. In: Buchanan B, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD. ISBN 0-943088-37-2

Curtis PJ, Cross BE (1954) Gibberellic acid. A new metabolite from the culture filtrates of *Gibberella fujikuroi*. Chem Ind 1(35):1066

Dal Cortivo C, Ferrari M, Visioli G, Lauro M, Fornasier F, Barion G, Panozzo A, Vamerali T (2020) Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (*Triticum aestivum* L.) in the field. Front Plant Sci 11:72. https://doi.org/10.3389/fpls.2020.00072

Davies PJ (2004) Plant Hormones; biosynthesis, signal transduction, action! Springer, Dordrecht, p 749. ISBN: 1402026854

Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. Annu Rev Plant Biol 42:55–76. https://doi.org/10.1146/annurev.pp.42.060191.000415

De Freitas JR, Banerjee MR, Germida JJ (1997) Phosphate-solubilising rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus*). Biol Fert Soils 24:358–364. https://doi.org/10.1007/s003740050258

Defez R, Andreozzi A, Dickinson M, Charlton A, Tadini L, Pesaresi P, Bianco C (2017) Improved drought stress response in alfalfa plants nodulated by an IAA overproducing *rhizobium* strain. Front Microbiol 8:2466. https://doi.org/10.3389/fmicb.2017.02466

Di Benedetto NA, Corbo MR, Campaniello D, Cataldi MP, Bevilacqua A, Sinigaglia M, Flagella Z (2017) The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. AIMS Microbiol 3:413–434. https://doi.org/10.3934/microbiol.2017.3.413

Díaz Peralta K, Araya T, Valenzuela S, Sossa K, Martínez M, Peña-Cortés H, Sanfuentes E (2012) Production of phytohormones, siderophores and population fluctuation of two root-promoting rhizobacteria in *Eucalyptus globulus* cuttings. World J Microbiol Biotechnol 28:2003–2014. https://doi.org/10.1007/s11274-012-1003-8

Digat B (1989) Strategies for seed bacterization. Acta Hortic 253:121–130. https://doi.org/10.17660/ActaHortic.1989.253.12

Dimkpa C, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *streptomyces* spp. Chemosphere 74:19–25. https://doi.org/10.1016/j.chemosphere.2008.09.079

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth promoting effects of diazotrophs in the rhizosphere. CRC Crit Rev Plant Sci 22:107–149. https://doi.org/10.1080/713610853

Dodd IC, Davies WJ (2004) Hormones and the regulation of water balance. In: Davies PJ (ed) Plant Hormones: biosynthesis, signal transduction, action! 3rd edn. Kluwer Academic Publishers, Dordrecht, pp 493–512. https://doi.org/10.1007/978-1-4020-2686-7_23

Domagalska MA, Sarnowska E, Nagy F, Davis SJ (2010) Genetic analyses of interactions among gibberellin, abscisic acid, and Brassinosteroids in the control of flowering time in *Arabidopsis thaliana*. PLoS One 2010:5. https://doi.org/10.1371/journal.pone.0014012

dos Santos LF, Olivares FL (2021) Plant microbiome structure and benefits for sustainable agriculture. Curr Plant Biol 26:100198. https://doi.org/10.1016/j.cpb.2021.100198

Dresselhaus T, Hückelhoven R (2018) Biotic and abiotic stress responses in crop plants. Agronomy 8:267. https://doi.org/10.3390/agronomy8110267

Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31:861–864. https://doi.org/10.1007/s11738-009-0297-0

Egamberdieva D, Adesemoye AO (2016) Improvement of crop protection and yield in hostile agroecological conditions with PGPR-based biofertilizer formulations. In: Arora NK, Mehnaz S, Balestrini R (eds) Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 199–211. https://doi.org/10.1007/978-81-322-2779-3_11

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015:756120. https://doi.org/10.1155/2015/756120

Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A (2010) Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Sci Hort 124:62–66. https://doi.org/10.1016/j.scienta.2009.12.012

Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Huang J (2015a) Crop plant hormones and environmental stress. Sustain Agric Rev:371–400. https://doi.org/10.1007/978-3-319-09132-7_10

Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J (2015b) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res Int 22:4907–4921. https://doi.org/10.1007/s11356-014-3754-2

Faisal Z, Jianjun C, Adnan Y, Zainul A, Muhammad N, Hans-Werner K, Kadambot S (2021) Biochar, compost, and biochar–compost blend applications modulate growth, photosynthesis, osmolytes, and antioxidant system of medicinal plant Alpinia zerumbet. Front Plant Sci 12: 1751. https://doi.org/10.3389/fpls.2021.707061

Fallik E, Okon Y, Epstein E, Goldman A, Fischer M (1989) Identification and quantification of IAA and IBA in *Azospirillum brasilense*-inoculated maize roots. Soil Biol Biochem 21:147–153. https://doi.org/10.1016/0038-0717(89)90024-2

Farooq M, Hussain M, Wahid A, Siddique KHM (2012) Drought stress in plants: an overview. In: Aroca R (ed) Plant responses to drought stress. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-32653-0_1

Feng K, Lu HM, Sheng HJ, Wang XL, Mao J (2004) Effect of organic ligands on biological availability of inorganic phosphorus in soils. Pedosphere 14:85–92. ISSN 1002-0160/CN 32-1315/P

Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk SC (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem 37(5): 955–964. https://doi.org/10.1016/j.soilbio.2004.10.021

Filgueiras L, Silva R, Almeida I et al (2020) *Gluconacetobacter diazotrophicus* mitigates drought stress in *Oryza sativa* L. Plant Soil 451:57–73. https://doi.org/10.1007/s11104-019-04163-1

Finkelstein RR, Rock CD (2002) Abscisic acid biosynthesis and response. Arabidopsis Book 1: e0058. https://doi.org/10.1199/tab.0058

Foo E, Ross JJ, Jones WT, Reid JB (2013) Plant hormones in arbuscular mycorrhizal symbioses: an emerging role for gibberellins. Ann Bot 111:769–779. https://doi.org/10.1093/aob/mct041

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76:1145–1152. https://doi.org/10.1007/s00253-007-1077-7

Fravel DR, Connick WJ, Lewis JA (1998) In: Burges HD (ed) Formulation of microorganisms to control plant diseases. In: Formulation of microbial biopesticides. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-4926-6_5

Fujioka S, Yokota T (2003) Biosynthesis and metabolism of Brassinosteroids. Annu Rev Plant Biol 54:137–164. https://doi.org/10.1146/annurev.arplant.54.031902.134921

Fukuda H, Ogawa T, Tanase S (1993) Ethylene production by micro-organisms. In: Rose AH (ed) Advances in microbial physiology, vol 35. Academic Press, pp 275–306. ISBN 9780120277353. https://doi.org/10.1016/S0065-2911(08)60101-0

Fulchieri M, Frioni L (1994) Azospirillum inoculation on maize (*Zea mays*): effect on yield in a field experiment in Central Argentina. Soil Biol Biochem 26:921–923. https://doi.org/10.1016/0038-0717(94)90308-5

Fulchieri M, Lucangeli C, Bottini R (1993) Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots. Plant Cell Physiol 34:1305–1309. https://doi.org/10.1093/oxfordjournals.pcp.a078554

Funes Pinter MI, Salomon MV, Berli FJ, Bottini R, Piccoli P (2017) Characterization of the as^{+3} tolerance conferred by plant growth promoting rhizobacteria to in vitro-grown grapevine. Appl Soil Ecol 109:60–68. https://doi.org/10.1016/j.apsoil.2016.10.003

Funes Pinter I, Salomon MV, Gil R, Mastrantonio LE, Bottini R, Piccoli PN (2018) Arsenic and trace elements in soil, water, grapevine and onion in Jáchal, Argentina. Sci Total Environ 615:1485–1498. https://doi.org/10.1016/j.scitotenv.2017.09.114

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750. https://doi.org/10.3732/ajb.1200572

Gale MD, Youssefian S, Russell GE (1985) Dwarfing genes in wheat. In: Russell GE (ed) Progress in plant breeding—1. Butterworths, London, pp 1–35. ISBN: 0-407-00780-6

Ghosh D, Gupta A, Mohapatra S (2019) A comparative analysis of exopolysaccharide and phytohormone secretions by four drought-tolerant rhizobacterial strains and their impact on osmotic-stress mitigation in *Arabidopsis thaliana*. World J Microbiol Biotech 35:90. https://doi.org/10.1007/s11274-019-2659-0

Giménez-Ibánez S, Solano R (2013) Nuclear jasmonate and salicylate signaling and crosstalk in defense against pathogens. Front Plant Sci 4:72. https://doi.org/10.3389/fpls.2013.00072

Giménez-Ibánez S, Boter M, Fernández-Barbero G, Chini A, Rathjen JP, Solano R (2014) The bacterial effector HopX1 targets JAZ transcriptional repressors to activate Jasmonate signaling and promote infection in Arabidopsis. PLoS Biol 12:e1001792. https://doi.org/10.1371/journal.pbio.1001792

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117. https://doi.org/10.1139/m95-015

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7. https://doi.org/10.1016/j.femsle.2005.07.030

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica (Cairo) 2012:963401. https://doi.org/10.6064/2012/963401

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. Biotechnol Adv 15:353–378. https://doi.org/10.1016/S0734-9750(97)00004-9

Glick BR, Cheng Z, Czarny J, Cheng Z, Duan J (2007) Promotion of plant growth by ACC deaminase producing soil bacteria. Eur J Plant Pathol 119:329–339. https://doi.org/10.1007/978-1-4020-6776-1_8

Goddard M-L, Belval L, Martin IR, Roth L, Laloue H, Deglène-Benbrahim L, Valat L, Bertsch C, Chong J (2021) Arbuscular mycorrhizal Symbiosis triggers major changes in primary metabolism together with modification of defense responses and signaling in both roots and leaves of *Vitis vinifera*. Front Plant Sci 12:1675. https://doi.org/10.3389/fpls.2021.721614

Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016) Alleviation of mercury toxicity in wheat by the interaction of mercury-tolerant plant growth-promoting rhizobacteria. J Plant Growth Regul 35:1000–1012. https://doi.org/10.1007/s00344-016-9598-x

González-García MP, Vilarrasa-Blasi J, Zhiponova M, Divol F, Mora-Garcia S, Russinova E, Cano-Delgado AI (2011) Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development 138:849–859. https://doi.org/10.1242/dev.057331

Gooding MJ, Addisu M, Uppal RK, Snape JW, Jones HE (2012) Effect of wheat dwarfing genes on nitrogen-use efficiency. J Agric Sci 150:3–22. https://doi.org/10.1017/S0021859611000414

Grichko VP, Glick BR (2001) Ethylene and flooding stress in plants. Plant Physiol Biochem 39:1–9. https://doi.org/10.1016/S0981-9428(00)01213-4

Gutiérrez-Mañero F, Ramos-Solano B, Probanza A, Mehouachi J, Tadeob F, Talon M (2001) The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plant 111:206–211. https://doi.org/10.1034/j.1399-3054.2001.1110211.x

Gutjahr C (2014) Phytohormone signaling in arbuscular mycorhiza development. Curr Opin Plant Biol 20:26–34. https://doi.org/10.1016/j.pbi.2014.04.003

Haagen-Smith AJ, Leech WD, Bergren WR. 1942. The estimation, isolation and, identification of auxins in plant materials. Am J Bot 29, 500-506. doi: https://doi.org/10.1126/science.93.2426.624

Hayat S, Ali B, Ahmad A (2007) Salicylic acid: biosynthesis, metabolism and physiological role in plants. In: Hayat S, Ahmad A (eds) Salicylic acid: a plant hormone. Springer, Dordrecht. https://doi.org/10.1007/1-4020-5184-0_1

He H, Ye Z, Yang D, Yan J, Xiao L, Zhong T, Yuan M, Cai X, Fang Z, Jing Y (2013) Characterization of endophytic *Rahnella* sp. JN6 from *Polygonum pubescens* and its potential in promoting growth and cd, pb, Zn uptake by *Brassica napus*. Chemosphere 90:1960–1965. https://doi.org/10.1016/j.chemosphere.2012.10.057

Hedden P, Phillips AL, Rojas MC, Carrera E, Tudzynski B (2001) Gibberellin biosynthesis in plants and fungi: a case of convergent evolution? J Plant Growth Regul 20:319–331. https://doi.org/10.1007/s003440010037

Heil M, Bostock RM (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defense. Ann Bot 89:503–512. https://doi.org/10.1093/aob/mcf076

Hibbing M, Fuqua C, Parsek M, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8:15–25. https://doi.org/10.1038/nrmicro2259

Hossain MM, Sultana F, Islam S (2017) Plant growth-promoting fungi (PGPF): phytostimulation and induced systemic resistance. Plant-microbe interactions in agro-ecological perspectives, 135–191. https://doi.org/10.1007/978-981-10-6593-4_6

Howe GA (2010) Jasmonates. In: Davies PJ (ed) Plant hormones. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-2686-7_28

Huang J, Zhu JK (2004) Plant responses to stress: abscisic acid and water stress. In: Goodman RM (ed) Encyclopedia of plant and crop science. Marcel Dekker, New York. ISBN-13: 978-0-8247-0944-0

Huang D, Wu W, Abrams SR, Cutler AJ (2008) The relationship of drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors. J Exp Bot 59:2991–3007. https://doi.org/10.1093/jxb/ern155

Hynes RK, Boyetchko SM (2006) Research initiatives in the art and science of biopesticide formulations. Soil Biol Biochem 38(4):845–849. https://doi.org/10.1016/j.soilbio.2005.07.003

Hyodo H (1991) Stress/wound ethylene. In: The plant hormone ethylene. CRC Press/Taylor and Francis Group, Boca-Raton, FL. https://doi.org/10.1201/9781351075763

Iosipenko A, Ignatov V (1995) Physiological aspects of phytohormone production by *Azospirillum brasilense* Sp 7. In: Fendrik I, del Gallo M, Vanderleyden J, de Zamaroczy M (eds) *Azospirillum* VI and Related microorganisms, NATO ASI series (series G: ecological sciences), vol 37. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-79906-8_33

Islam F, Yasmeen T, Ali Q, Ali S, Arif MS, Hussain S, Rizvi H (2014) Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. Ecotoxicol Environ Saf 104:285–293. https://doi.org/10.1016/j.ecoenv.2014.03.008

Islam F, Yasmeen T, Saleem Arif M, Riaz M, Shahzad SM, Imran Q, Ali I (2017) Combined ability of chromium (Cr) tolerant plant growth promoting bacteria (PGPB) and stress alleviator (salicylic acid) in attenuation of chromium stress in maize plants. Plant Physiol Biochem 108:456–467. https://doi.org/10.1016/j.plaphy.2016.08.014

Jalilian J, Modarres-Sanavy SAM, Saberali SF, Sadat-Asilan K (2012) Effects of the combination of beneficial microbes and nitrogen on sunflower seed yields and seed quality traits under different irrigation regimes. Field Crops Res 127:26–34. https://doi.org/10.1016/j.fcr.2011.11.001

James EK, Olivares FL (1998) Infection and colonization of sugar cane and other Graminaceous plants by endophytic diazotrophs. Crit Rev Plant Sci 17:77–119. https://doi.org/10.1080/07352689891304195

Janzen R, Rood S, Dormar J, McGill W (1992) *Azospirillum brasilense* produces gibberellins in pure culture and chemically-medium and in co-culture on straw. Soil Biol Biochem 24:1061–1064. https://doi.org/10.1016/0038-0717(92)90036-W

Jiang S, Yao J, Ma KW, Zhou H, Song J, Zhou H, Song J, He S-Y, Ma W (2013) Bacterial effector activates Jasmonate signaling by directly targeting JAZ transcriptional repressors. PLoS Pathog 9:e1003715. https://doi.org/10.1371/journal.ppat.1003715

Jian-Jun T, Wei CH, Biao M, Wan-Ke Z, Shou-Yi C, Jin-Song Z (2015) The role of ethylene in plants under salinity stress. Front Plant Sci 6:1059. https://doi.org/10.3389/fpls.2015.01059

Jorge GL, Kisiala A, Morrison E, Aoki M, Nogueira APO, Emery RJN (2019) Endosymbiotic *Methylobacterium oryzae* mitigates the impact of limited water availability in lentil (*Lens culinaris* Medik.) by increasing plant cytokinin levels. Environ Exp Bot 162:525–540. https://doi.org/10.1016/j.envexpbot.2019.03.028

Kang S-M, Khan AL, Hamayun M, Hussain J, Joo GJ, You Y-H, Kim J-G, Lee IJ (2012) Gibberellin-producing *Promicromonospora* sp. SE188 improves *Solanum lycopersicum* plant growth and influences endogenous plant hormones. J Microbiol 50:902–909. https://doi.org/10.1007/s12275-012-2273-4

Kang S-M, Khan AL, Waqas M, You Y-H, Kim J-H, Kim J-G, Hamayun M, Lee IJ (2014) Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. J Plant Interac 9:673–682. https://doi.org/10.1080/17429145.2014.894587

Karadeniz A, Topcuoğlu Ş, İnan S (2006) Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World J Microbiol Biotechnol 22:1061–1064. https://doi.org/10.1007/s11274-005-4561-1

Katsir L, Schilmiller AL, Staswick PE, He S-Y, Howe GE (2008) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc Natl Acad Sci U S A 105:7100–7105. https://doi.org/10.1073/pnas.0802332105

Kenawy A, Dailin DJ, Abo-Zaid GA, Malek RA, Ambehabati K, Zakaria K, Sayyed RZ, El Enshasy HA (2019) Biosynthesis of antibiotics by PGPR and their roles in biocontrol of plant diseases. In: Sayyed R (ed) Plant growth promoting rhizobacteria for sustainable stress management. Microorganisms for sustainability, vol *13*. Springer, Singapore. https://doi.org/10.1007/978-981-13-6986-5_1

Keswani C, Bisen K, Singh V, Sarma BK, Singh HB (2016) Formulation technology of biocontrol agents: present status and future prospects. In: Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 35–52. https://doi.org/10.1007/978-81-322-2779-3_2

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture — a review. Agron Sustain Dev 27:29–43. https://doi.org/10.1051/agro:2006011

Khan MIR, Fatma M, Per TS, Anjum NA, Khan NA (2015) Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci 6:462. https://doi.otg/10.3389/fpls.2015.00462

Khan WU, Ahmad SR, Yasin NA, Ali A, Ahmad A, Akram W (2017) Application of *bacillus megaterium* MCR-8 improved phytoextraction and stress alleviation of nickel in *Vinca rosea*. Int J Phytoremediation 19:813–824. https://doi.org/10.1080/15226514.2017.1290580

Khan N, Bano A, Ali S, Ali Babar M (2020) Crosstalk amongst phytohormones from plants and PGPR under biotic and abiotic stresses. Plant Growth Regul 90:189–203. https://doi.org/10.1007/s10725-020-00571-x

Kho ZY, Lal SK (2018) The human gut microbiome—A potential controller of wellness and disease. Front Microbiol 9:1835. https://doi.org/10.3389/fmicb.2018.01835

Kim DS, Hwang BK (2014) An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens. J Exp Bot 65:2295–2306. https://doi.org/10.1093/jxb/eru109

King RW, Evans LT (2003) Gibberellins and flowering of grasses and cereals: prising open the lid of the "florigen" black box. Annu Rev Plant Physiol Plant Mol Biol 54:307–328. https://doi.org/10.1146/annurev.arplant.54.031902.135029

Kloepper J, Leong J, Teintze M, Sichroth M (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886. https://doi.org/10.1038/286885a0

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *bacillus* spp. Phytopathology 94:1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M, Kadwell S, Launis K, Lewis K, Maddox D, PcPherson K, Meghji MR, Merlin E, Rhodes R, Warren GW, Wright M, Evola SV (1993) Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *bacillus thuringiensis*. Nat Biotechnol 11:194–200. https://doi.org/10.1038/nbt0293-194

Kurniawan A, Chuang H-W (2022) Rhizobacterial *bacillus mycoides* functions in stimulating the antioxidant defence system and multiple phytohormone signalling pathways to regulate plant growth and stress tolerance. J Appl Microbiol 132(2):1260–1274. https://doi.org/10.1111/jam.15252

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656. https://doi.org/10.1038/sj.emboj.7600121

Le Page-Degivry MT, Bidard JN, Rouvier E, Bulard C, Lazdunski M (1986) Presence of abscisic acid, a phytohormone, in the mammalian brain. Proc Natl Acad Sci U S A 83:1155–1158. https://doi.org/10.1073/pnas.83.4.1155

Leaungvutiviroj C, Ruangphisarn P, Hansanimitkul P, Shinkawa H, Ssasaki K (2010) Development of a new biofertilizer with a high capacity for N_2 fixation, phosphate and potassium solubilization and auxin production. Biosci Biotechnol Biochem 74:1098–1101. https://doi.org/10.1271/bbb.90898

Lefevere H, Bauters L, Gheysen G (2020) Salicylic acid biosynthesis in plants. Front Plant Sci 11:338. D. https://doi.org/10.3389/fpls.2020.00338

Lemke C, Potter KC, Schulte S, Peters RJ (2019) Conserved bases for the initial cyclase in gibberellin biosynthesis: from bacteria to plants. Biochem J 476:2607–2621. https://doi.org/10.1042/BCJ20190479

Letham DS, Shannon JS, McDonald IR (1964) The structure of zeatin, a factor inducing cell division. Proc Chem Soc 1984:230–231

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Biol 49:199–222. https://doi.org/10.1146/annurev.arplant.49.1.199

Li Y, Walton DC (1990) Violaxanthin is an abscisic acid precursor in water-stressed dark-grown bean leaves. Plant Physiol 92:551–559

Li Y, Xiao J, Chen L, Huang X, Cheng Z, Han B, Zhang Q, Wu C (2018) Rice functional genomics research: past decade and future. Mol Plant 11:359–380. https://doi.org/10.1016/j.molp.2018.01.007

Li X, Sun P, Zhang Y, Jin C, Guan C (2020) A novel PGPR strain *Kocuria rhizophila* Y1 enhances salt stress tolerance in maize by regulating phytohormone levels, nutrient acquisition, redox potential, ion homeostasis, photosynthetic capacity and stress-responsive genes expression. Environ Exp Bot 174:104023. https://doi.org/10.1016/j.envexpbot.2020.104023

Liao C, Yan W, Chen Z, Xie G, Deng XW, Tang X (2021) Innovation and development of the third-generation hybrid rice technology. Crop J 9:693–701. https://doi.org/10.1016/j.cj.2021.02.003

Liba CM, Ferrara FIS, Manfio GP, Fantinatti-Garboggini F, Albuquerque RC, Pavan C, Ramos PL, Moreira-Filho CA, Barbosa HR (2006) Nitrogen-fixing chemo-organotrophic bacteria isolated from cyanobacteria-deprived lichens and their ability to solubilize phosphate and to release amino acids and phytohormones. J Appl Microbiol 101:1076–1086. https://doi.org/10.1111/j.1365-2672.2006.03010.x

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Biol 50:47–65. https://doi.org/10.1146/annurev.arplant.50.1.47

Lieberman M, Kunishi AT, Mapson LW, Wardale DA (1965) Ethylene production from methionine. Biochem J 97:449–459. https://doi.org/10.1042/bj0970449

Lin Y, Sun X, Yuan Q, Yan Y (2014) Extending shikimate pathway for the production of muconic acid and its precursor salicylic acid in *Escherichia coli*. Metabol Eng 23:62–69. https://doi.org/10.1016/j.ymben.2014.02.009

Liu F, Xing S, Ma H, Du Z, Ma B (2013) Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in *Platycladus orientalis* container seedlings. Appl Microbiol Biotechnol 97:9155–9164. https://doi.org/10.1007/s00253-013-5193-2

Liu Y, Zhu A, Tan H, Cao L, Zhang R (2019) Engineering banana endosphere microbiome to improve *fusarium* wilt resistance in banana. Microbiome 7:74. https://doi.org/10.1186/s40168-019-0690-x

Lorck H (1948) Production of hydrocyanic acid by bacteria. Physiol Plantarum 1(2):142–146. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x

Lucas RE (2004) The industrial revolution: past and future. Annu Rep Federal Reserve Bank of Minneapolis 18:5–20

Ma Y, Rajkumar M, Fritas H (2008) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manag 90:831–837. https://doi.org/10.1016/j.jenvman.2008.01.014

Ma Y, Cao J, He J, Chen Q, Li X, Yang Y (2018) Molecular mechanism for the regulation of ABA homeostasis during plant development and stress responses. Int J Mol Sci 19:3643. https://doi.org/10.3390/ijms19113643

MacMillan J, Suter PJ (1958) The occurrence of gibberellin A_1 in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). Naturwissenschaften 45:46. https://doi.org/10.1007/BF00635028

Magnin-Robert M, Trotel-Aziz P, Quantinet D, Biagianti S, Aziz A (2007) Biological control of *Botrytis cinerea* by selected grapevine-associated bacteria and stimulation of chitinase and *b*-1,3 glucanase activities under field conditions. Eur J Plant Pathol 118:43–57. https://doi.org/10.1007/s10658-007-9111-2

Martínez-Hidalgo P, García JM, Pozo MJ (2015) Induced systemic resistance against *Botrytis cinerea* by *Micromonospora* strains isolated from root nodules. Front Microbiol 6:922. https://doi.org/10.3389/fmicb.2015.00922

Mattoo AK, Suttle JC (1991) The plant hormone ethylene. CRC Press, Taylor and Francis Group, Boca-Raton, FL. https://doi.org/10.1201/9781351075763

Meena M, Swapnil P, Divyanshu K, Kumar S, Harish TDN, Zehra A, Marwal A, Upadhyay RS (2020) PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: current perspectives. J Basic Microbiol 1:34. https://doi.org/10.1002/jobm.202000370

Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100. https://doi.org/10.1126/science.1203980

Mendoza-Hernández JC, Perea-Vélez YS, Arriola-Morales J, Martínez-Simón SM, Pérez-Osorio G (2016) Assessing the effects of heavy metals in ACC deaminase and IAA production on plant growth-promoting bacteria. Microbiol Res 188:53–61. https://doi.org/10.1016/j.micres.2016.05.001

Mgbemene CA, Nnaji CC, Nwozor C (2016) Industrialization and its backlash: focus on climate change and its consequences. J Environ Sci Technol 9:301–316. https://doi.org/10.3923/jest.2016.301.316

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (2018) The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front Plant Sci 9:112. https://doi.org/10.3389/fpls.2018.00112

Mishra J, Arora NK. 2016. Bioformulations for plant growth promotion and combating phytopathogens: a sustainable approach. In Bioformulations: for sustainable agriculture (pp. 3-33). Springer, New Delhi. doi: https://doi.org/10.1007/978-81-322-2779-3_1

Mishra AK, Singh VP (2011) Drought modeling—A review. J Hydrol 403:157–175. https://doi.org/10.1016/j.jhydrol.2011.03.049

Mitchell J, Mandava N, Worley J, Plimmer JR, Smith MV (1970) Brassins—A new family of plant Hormones from rape pollen. Nature 225:1065–1066. https://doi.org/10.1038/2251065a0

Mok DW, Mok MC (2001) Cytokinin metabolism and action. Annu Rev Plant Physiol Plant Mol Biol 52:89–118. https://doi.org/10.1146/annurev.arplant.52.1.89

Morel MA, Castro-Sowinski S (2013) The complex molecular signaling network in microbe–plant interaction. In: Plant microbe symbiosis: fundamentals and advances. Springer, New Delhi, pp 169–199. https://doi.org/10.1007/978-81-322-1287-4_6

Moreno D, Berli F, Piccoli P, Bottini R (2011) Gibberellins and abscisic acid promote carbon allocation in roots and berries of grape plants. J Plant Growth Regul 30:220–228. https://doi.org/10.1007/s00344-010-9186-4

Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. Physiol Plant 100:620–630. https://doi.org/10.1111/j.1399-3054.1997.tb03068.x

Morgan PW, Hall W (1964) Accelerated release of ethylene by cotton following application of Indolyl-3-acetic acid. Nature 201:99. https://doi.org/10.1038/201099a0

Müller DB, Vogel C, Bai Y, Vorholt JA (2016) The plant microbiota: systems-level insights and perspectives. Annu Rev Genetics 50:211–234. https://doi.org/10.1146/annurev-genet-120215-034952

Murcia G, Pontin M, Reinoso H, Baraldi R, Bertazza G, Gómez Talquenca S, Bottini R, Piccoli PN (2015) ABA and GA_3 increase carbon allocation in different organs of grapevine plants by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters. Physiol Plant 156:323–337. https://doi.org/10.1111/ppl.12390

Murcia G, Pontin M, Piccoli P (2018) Role of ABA and Gibberellin A_3 on gene expression pattern of sugar transporters and invertases in *Vitis vinifera* cv. Malbec during berry ripening. Plant Growth Regul 84:275–283. https://doi.org/10.1007/s10725-017-0338-4

Nagahama K, Ogawa T, Fujii T, Fukuda H (1992) Classification of ethylene-producing bacteria in terms of biosynthetic pathways to ethylene. J Ferment Bioeng 73:1–5. https://doi.org/10.1016/0922-338X(92)90221-F

Nambara E, Marion-Poll A (2005) Abscisic acid and catabolism. Annu Rev Plant Biol 56:165–185. https://doi.org/10.1146/annurev.arplant.56.032604.144046

Naz I, Bano A, Tamoor-ul-Hassan. (2009) Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. African J Biotechnol 8:5762–5766. https://doi.org/10.5897/AJB09.1176

Nazari M, Smith DL (2020) A PGPR-produced bacteriocin for sustainable agriculture: a review of thuricin 17 characteristics and applications. Front Plant Sci 11:916. https://doi.org/10.3389/fpls.2020.00916

Nazli F, Mustafa A, Ahmad M, Azhar H, Jamil M, Wang X, Shakeel Q, Imtiaz M, El-Esawi M (2020) A review on practical application and potentials of phytohormone-producing plant growth-promoting rhizobacteria for inducing heavy metal tolerance in crops. Sustainability 12:9056. https://doi.org/10.3390/su12219056

Nett R, Montanares M, Marcassa A, Lu X, Nagel R, Charles TC, Hedden P, Rojas MC, Peters RJ (2017) Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. Nat Chem Biol 13:69–74. https://doi.org/10.1038/nchembio.2232

Nouri A, Lee J, Yin X, Tyler DD, Saxton AM (2019) Thirty-four years of no-tillage and cover crops improve soil quality and increase cotton yield in Alfisols, southeastern USA. Geoderma 337: 998–1008. https://doi.org/10.1016/j.geoderma.2018.10.016

Ohkuma K, Lyon JL, Addicott FT, Smith OE (1963) Abscisin II, an abscission-accelerating substance from young cotton fruit. Science 142:1592–1593. https://doi.org/10.1126/science.142.3599.1592

Okon Y, Labandera-González C (1994) Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil Biol Biochem 26:1591–1601. https://doi.org/10.1016/0038-0717(94)90311-5

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33:197. https://doi.org/10.1007/s11274-017-2364-9

Pacurar DI, Perrone I, Bellini C (2014) Auxin is a central player in the hormone cross-talks that control adventitious rooting. Physiol Plant 151:83–96. https://doi.org/10.1111/ppl.12171

Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, Rhodes WK, Otero YI, Barry GF, Eichholtz DA, Peschke VM, Nida DL, Taylor NB, Kishore GM (1995) Development, identification, and characterization of a glyphosate-tolerant soybean line. Cell Biol Mol Genetics 35(5):1451–1461. https://doi.org/10.2135/cropsci1995.0011183X003500050032x

Park J-E, Park J-Y, Kim Y-S, Staswick PE, Jeon J, Yun J, Kim S-Y, Kim J, Lee Y-H, Park CH-M (2007) GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in Arabidopsis. J Biol Chem 282:10036–10046. https://doi.org/10.1074/jbc.M610524200

Patten C, Glick B (1996) Bacterial biosynthesis of indole 3-acetic acid. Can J Microbiol 42:207–220. https://doi.org/10.1139/m96-032

Pfeffer C, Larsen S, Song J, Dong M, Besenbacher F, Meyer RL, Kjeldsen KU, Schreiber L, Gorby YA, El-Naggar MY, Leung KM, Schramm A, Risgaard-Petersen N, Nielsen LP (2012) Filamentous bacteria transport electrons over centimetre distances. Nature 491:218–221. https://doi.org/10.1038/nature11586

Phalan B, Onial M, Balmford A, Green RE (2011) Reconciling food production and biodiversity conservation: land sharing and land sparing compared. Science 333:1289–1291. https://doi.org/10.1126/science.1208742

Pharis RP, King RW (1985) Gibberellins and reproductive development in seed plants. Annu Rev Plant Physiol 36:517–568. https://doi.org/10.1146/annurev.pp.36.060185.002505

Phinney BO (1956) Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc Natl Acad Sci U S A 42:185–189. https://doi.org/10.1073/pnas.42.4.185

Piccoli P, Travaglia C, Cohen A, Sosa CP, Masuelli R, Bottini R (2011) An endophytic bacterium isolated from roots of the halophyte *Prosopis strombulifera* produces ABA, IAA, gibberellins A_1 and A_3 and jasmonic acid in chemically defined culture medium. Plant Growth Regul 64: 207–210. https://doi.org/10.1007/s10725-010-9536-z

Pieterse CM, Leon-Reyes A, Van der Ent S, Van Wees SC (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316. https://doi.org/10.1038/nchembio.164

Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pimentel D (1996) Green revolution agriculture and chemical hazards. Sci Total Environ 188:86–98. https://doi.org/10.1016/0048-9697(96)05280-1

Pontin M, Murcia G, Fontana A, Bolcato L, Bottini R, Piccoli P (2021) Nitric oxide and abscisic acid regulate osmo-protective and anti-oxidative mechanisms related to water stress tolerance of grapevines. Aust J Grape Wine Res 27(3):392–405. https://doi.org/10.1111/ajgw.12485

Pozo MJ, López-Ráez JA, Azcón-Aguilar C, García-Garrido JM (2015) Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytol 205: 1431–1436. https://doi.org/10.1111/nph.13252

Rademacher W (1994) Gibberellin formation in microorganisms. Plant Growth Regul 15:303–314. https://doi.org/10.1007/BF00029903

Rani U, Kumar V (2019) *In: nanobiotechnology in bioformulations*. Microbial bioformulations: present and future aspects. Springer, Cham, pp 243–258. https://doi.org/10.1007/978-3-030-17061-5_10

Raskin I (1992a) Role of salicylic acid in plants. Ann Rev Plant Physiol Plant Mol Biol 43:439–463. https://doi.org/10.1146/annurev.pp.43.060192.002255

Raskin I (1992b) Salicylate, a new plant hormone. Plant Physiol 99:799–803. https://doi.org/10.1104/pp.99.3.799

Reinhardt D, Wiemken A, Boller T (1991) Induction of ethylene biosynthesis in compatible and incompatible interactions of soybean roots with *Phytophthora megasperma* f. sp. *glycinea* and its relation to phytoalexin accumulation. J Plant Physiol 138:394–399. https://doi.org/10.1016/S0176-1617(11)80512-2

Reinoso H, Luna V, Dauría C, Pharis R, Bottini R (2002) Dormancy in peach (*Prunus persica* L.) flower buds VI. Effects of gibberellins and an acylcyclohexanedione (trinexapac-ethyl) on bud morphogenesis in field experiments with orchard trees and on cuttings. Can J Bot 80:656–663. https://doi.org/10.1139/b02-051

Reynolds JF, Stafford Smith DM, Lambin EF, Turner BL, Mortimore M, Batterbury SPJ, Downing TE, Dowlatabadi H, Fernández RJ, Herrick JE, Huber-Sannwald E, Jiang H, Leemans R, Lynam T, Maestre FT, Ayarza M, Walker B (2007) Global desertification: building a science for dry land development. Science 316:847–851. https://doi.org/10.1126/science.1131634

Ribaudo CM, Krumpholz EM, Cassán FD, Bottini R, Cantore ML, Curá JA (2006) *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. J Plant Growth Regul 25:175–185. https://doi.org/10.1007/s00344-005-0128-5

Rodríguez H, González T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91:552–555. https://doi.org/10.1007/s00114-004-0566-0

Romano I, Ventorino V, Pepe O (2020) Effectiveness of plant beneficial microbes: overview of the methodological approaches for the assessment of root colonization and persistence. Front Plant Sci 11:6. https://doi.org/10.3389/fpls.2020.00006

Rood SB, Mandel R, Pharis RP (1989) Endogenous gibberellins and shoot growth and development in *Brassica napus*. Plant Physiol 89:269–273. https://doi.org/10.1104/pp.89.1.269

Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H (2013) The biology of strigolactones. Trends Plant Sci 18:72–83. https://doi.org/10.1016/j.tplants.2012.10.003

Saad MM, Eida AA, Hirt H (2020) Tailoring plant-associated microbial inoculants in agriculture: a roadmap for successful application. J Exp Bot 71:3878–3901. https://doi.org/10.1093/jxb/eraa111

Salazar-Cerezo S, Martínez-Montiel N, García-Sánchez J, Pérez-y-Terrón R, Martínez-Contreras RD (2018) Gibberellin biosynthesis and metabolism: a convergent route for plants, fungi and bacteria. Microbiol Res 208:85–98. https://doi.org/10.1016/j.micres.2018.01.010

Salomon MV, Bottini R, De Souza Filho GA, Cohen A, Moreno D, Gil M, Piccoli P (2014) Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis of defense-related terpenes in in vitro cultured grapevine. Physiol Plant 151:359–374. https://doi.org/10.1111/ppl.12117

Salomon MV, Purpora R, Bottini R, Piccoli P (2016) Rhizosphere associated bacteria trigger accumulation of terpenes in leaves of *Vitis vinifera* L. cv. Malbec that protect cells against reactive oxygen species. Plant Physiol Biochem 196:295–304. https://doi.org/10.1016/j.plaphy.2016.05.007

Salomon MV, Funes Pinter I, Piccoli P, Bottini R (2017a) Use of plant growth-promoting rhizobacteria as biocontrol agents: induced systemic resistance against biotic stress in plants. In: Kalia VC (ed) Microbial applications 2. Springer, New York, pp 133–152. https://doi.org/10.1007/978-3-319-52669-0_7

Salomon MV, Piccoli P, Funes Pinter MI, Stirk W, Kulkarni M, VanStaden J, Bottini R (2017b) Bacteria and a smoke-water extracts improve growth and induce the synthesis of volatile defense mechanisms in *Vitis vinifera* L. Plant Physiol Biochem 120:1–9. https://doi.org/10.1016/j.plaphy.2017.09.013

Sang MK, Kim JD, Kim BS, Kim KD (2011) Root treatment with rhizobacteria antagonistic to *phytophthora* blight affects anthracnose occurrence, ripening, and yield of pepper fruit in the plastic house and field. BioControl 101(6):666–678. https://doi.org/10.1094/PHYTO-08-10-0224

Sansberro P, Mroginski L, Bottini R (2004) Foliar sprays with ABA promote growth of *Ilex paraguariensis* by alleviating diurnal water stress. Plant Growth Regul 42:105–111. https://doi.org/10.1023/B:GROW.0000017476.12491.02

Santino A, Taurino M, De Domenico S, Bonsegna S, Poltronieri P, Pastor V, Flors V (2013) Jasmonate signaling in plant development and defense response to multiple (a)biotic stresses. Plant Cell Rep 32:1085–1098. https://doi.org/10.1007/s00299-013-1441-2

Santoro V, Schiavon M, Visentin I, Constán-Aguilar C, Cardinale F, Celi L (2021) Strigolactones affect phosphorus acquisition strategies in tomato plants. Plant Cell Environ 44(11):3628–3642. https://doi.org/10.1111/pce.14169

Sarma RK, Saikia R (2014) Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. Plant Soil 377:111–126. https://doi.org/10.1007/s11104-013-1981-9

Sasse JM (2003) Physiological actions of Brassinosteroids: an update. J Plant Growth Regul 22: 276–288. https://doi.org/10.1007/s00344-003-0062-3

Schisler DA, Slininger PJ, Behle RW, Jackson MA (2004) Formulation of bacillus spp. for biological control of plant diseases. Phytopathology 94:1267–1271. https://doi.org/10.1094/PHYTO.2004.94.11.1267

Schneider G, Schliemann W (1994) Gibberellin conjugates: an overview. Plant Growth Regul 15: 247–260. https://doi.org/10.1007/BF00029898

Scholz VV, Martin BC, Meyer R, Schramm A, Fraser MW, Nielsen LP, Kendrick GA, Risgaard-Petersen N, Burdorf LDW, Marshall IPG (2021) Cable bacteria at oxygen-releasing roots of aquatic plants: a widespread and diverse plant–microbe association. New Phytol 232(5): 2138–2151. https://doi.org/10.1111/nph.17415

Scopel E, Findeling A, Chavez Guerra E, Corbeels M (2005) Impact of direct sowing mulch-based cropping systems on soil carbon, soil erosion and maize yield. Agron Sustain Dev 25:425–432. https://hal.archives-ouvertes.fr/hal-00886259

Shahzad R, Khan AL, Bilal S, Waqas M, Kang SM, Lee IJ (2017) Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in *Oryza sativa*. Environ Exp Bot 136:68–77. https://doi.org/10.1016/j.envexpbot.2017.01.010

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 11(118):131

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:a001438. https://doi.org/10.1101/cshperspect.a001438

Sparks E, Wachsman G, Benfey PN (2013) Spatiotemporal signaling in plant development. Nature Rev Gen 14:631–644. https://doi.org/10.1038/nrg3541

Spolaor LT, Simões Azeredo Gonçalves L, Pais A, dos Santos OJ, Martinez de Oliveira AL, Scapim CA, Bengosi Bertagna FA, Kuki MC (2016) Plant growth-promoting bacteria associated with nitrogen fertilization at topdressing in popcorn agronomic performance. Bragantia 75:33–40. https://doi.org/10.1590/1678-4499.330

Stringlis IA, Zhang H, Pieterse CM, Bolton MD, de Jonge R (2018) Microbial small molecules–weapons of plant subversion. Nat Prod Rep 35:410–433. https://doi.org/10.1039/C7NP00062F

Strzelczyk E, Kamper M, Li C (1994) Cytocinin-like-substances and ethylene production by *Azospirillum* in media with different carbon sources. Microbiol Res 149:55–60. http://www.bashanfoundation.org/contributors/retired/Li-C_Y/cylicarbonsources.pdf

Suganuma N, Yamauchi H, Yamamoto K (1995) Enhanced production of ethylene by soybean roots after inoculation with *Bradyrhizobium japonicum*. Plant Sci 111:163–168. https://doi.org/10.1016/0168-9452(95)04239-Q

Takahashi N, Phinney BO, MacMillan J (1991) Gibberellins. Springer-Verlag, Berlin, p 426. https://doi.org/10.1007/978-1-4612-3002-1

Takino J, Kozaki T, Sato Y, Liu C, Ozaki T, Minami A, Oikawa H (2018) Unveiling biosynthesis of the phytohormone abscisic acid in fungi: unprecedented mechanism of core scaffold formation catalyzed by an unusual sesquiterpene synthase. J Amer Chem Soc 140:12392–12395. https://doi.org/10.1021/jacs.8b08925

Tamura S (1991) Historical aspects of gibberellins. In: Takahashi N, Phinney BO, MacMillan J (eds) Gibberellins. Springer, New York, NY. https://doi.org/10.1007/978-1-4612-3002-1_1

Taniguchi S, Hosokawa-Shinonaga Y, Tamaoki D, Yamada S, Akimitzu K, Gomi K (2013) Jasmonate induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. Plant Cell Environ 37:451–461. https://doi.org/10.1111/pce.12169

Tanimoto E (1991) Gibberellin requirement for the Normal growth of roots. In: Takahashi N, Phinney BO, MacMillan J (eds) Gibberellins. Springer, New York, NY. https://doi.org/10.1007/978-1-4612-3002-1_22

Tanimoto E (2005) Regulation of root growth by plant Hormones—roles for auxin and gibberellin. Critical Rev Plant Sci 24:249–265. https://doi.org/10.1080/07352680500196108

Tardieu F, Davies WJ (1992) Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol 98:540–545. https://doi.org/10.1104/pp.98.2.540

Tardieu F, Katerji N, Bethenod O, Zhang J, Davies WJ (1991) Maize stomatal conductance in the field: its relationship with soil and plant water potentials, mechanical constraints and ABA concentration in the xylem sap. Plant Cell Environ 14:121–126. https://doi.org/10.1111/j.1365-3040.1991.tb01378.x

Tauger MB (2010) Agriculture in world history. Taylor & Francis Group, New York, NY. https://doi.org/10.4324/9780203847480

Tilman D, Clark M (2015) Food, agriculture & the environment: can we feed the World & Save the earth? Daedalus 144:8–23. https://doi.org/10.1162/DAED_a_00350

Travaglia C, Cohen AC, Reinoso H, Castillo C, Bottini R (2007) Exogenous abscisic acid increases carbohydrate accumulation and redistribution to the grains in wheat grown under field conditions of soil water restriction. J Plant Growth Regul 26:285–289. https://doi.org/10.1007/s00344-007-9018-3

Travaglia C, Reinoso H, Bottini R (2009) Application of abscisic acid promotes yield in field-cultured soybean by enhancing production of carbohydrates and their allocation in seed. Crop Pasture Sci 60:1131–1136. https://doi.org/10.1071/CP08396

Travaglia C, Reinoso H, Luna C, Cohen A, Castillo C, Bottini R (2010) Exogenous ABA increases yield in field-grown wheat with a moderate water restriction. J Plant Growth Regul 29:366–374. https://doi.org/10.1007/s00344-010-9147-y

Trewavas A (2000) Signal perception and transduction. In: Buchanan B, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD. ISBN: 978-0-470-71421-8

Ullah N, Ditta A, Imtiaz M, Li X, Ullah Jan A, Mehmood S, Rizwan MS, Rizwan M (2021) Appraisal for organic amendments and plant growth-promoting rhizobacteria to enhance crop productivity under drought stress: a review. J Agron Crop Sci 207:783–802. https://doi.org/10.1111/jac.12502

Umehara M, Cao M, Akiyama K, Akatsu T, Seto Y, Hanada A, Li W, Takeda-Kamiya N, Morimoto Y, Yamaguchi S (2015) Structural requirements of Strigolactones for shoot branching inhibition in rice and arabidopsis. Plant Cell Physiol 56:1059–1072. https://doi.org/10.1093/pcp/pcv028

Van der Ent S, Van Wees SC, Pieterse CM (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70(13-14):1581–1588. https://doi.org/10.1016/j.phytochem.2009.06.009

Van Wees SCM, Van der Ent S, Pieterse CMJ (2008) Plant immune responses triggered by beneficial microbes. Curr Opinion Plant Biol 11(4):443–448. https://doi.org/10.1016/j.pbi.2008.05.005

Vega FA, Covelo EF, Cerqueira B, Andrade ML (2009) Enrichment of marsh soils with heavy metals by effect of anthropic pollution. J Hazard Mater 170:1056–1063. https://doi.org/10.1016/j.jhazmat.2009.05.074

von der Goltz J, Dar A, Fishman R, Mueller ND, Barnwal P, McCord GC (2020) Health impacts of the Green revolution: evidence from 600,000 births across the developing world. J Health Econ 74:102373. https://doi.org/10.1016/j.jhealeco.2020.102373

Wajeeha S, Saadia N, Zahid A (2017) Strigolactones biosynthesis and their role in abiotic stress resilience in plants: a critical review. Front Plant Sci 8:1487. https://doi.org/10.3389/fpls.2017.01487

Wang H, Deng XW (2018) Development of the "third-generation" hybrid Rice in China. Genomics Proteomics Bioinformatics 16:393–396. https://doi.org/10.1016/j.gpb.2018.12.001

Wang L, Ruan Y-L (2013) Regulation of cell division and expansion by sugar and auxin signaling. Front Plant Sci 4:163. https://doi.org/10.3389/fpls.2013.00163

Wang L, Duan C, Wu D, Guan Y (2014) Quantification of endogenous brassinosteroids in sub-gram plant tissues by in-line matrix solid-phase dispersion–tandem solid phase extraction coupled with high performance liquid chromatography–tandem mass spectrometry. J Chromatogr A 1359:44–51. https://doi.org/10.1016/j.chroma.2014.07.037

Went FW (1926) On growth-accelerating substances in the coleoptile of *Avena sativa*. Proc Sect Sci Koninklijke Akad Wetenschappen Amsterdam 305:10–19. https://www.dwc.knaw.nl/DL/publications/PU00015412.pdf

Woo SL, Pepe O (2018) Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. Front Plant Sci 9:1801. https://doi.org/10.3389/fpls.2018.01801

Xu ZJ, Nakajima M, Suzuki Y, Yamaguchi I (2002) Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. Plant Physiol 129:1285–1295. https://doi.org/10.1104/pp.001784

Xu Z, Ge Y, Zhang W, Zhao Y, Yang G (2018) The walnut *JrVHAG1* gene is involved in cadmium stress response through ABA-signal pathway and MYB transcription regulation. BMC Plant Biol 18:19. https://doi.org/10.1186/s12870-018-1231-7

Yakhin OI, Lubyanov AA, Yakhin IA, Brown PH (2017) Biostimulants in plant science: a global perspective. Front Plant Sci 7:2049. https://doi.org/10.3389/fpls.2016.02049

Yamada S, Kano A, Tamaoki D, Miyamoto A, Shishido H, Miyoshi S, Taniguchi S, Akimitsu K, Gomi K (2012) Involvement of OsJAZ8 in Jasmonate-induced resistance to bacterial blight in rice. Plant Cell Physiol 53:2060–2072. https://doi.org/10.1093/pcp/pcs145

Yang P, Wen Q, Yu R, Han X, Deng XW, Chen H (2020) Light modulates the gravitropic responses through organ-specific PIFs and HY5 regulation of LAZY4 expression in Arabidopsis. Proc Natl Acad Sci U S A 117:18840–18848. https://doi.org/10.1073/pnas.2005871117

Ye Q, Zhu W, Li L, Zhang S, Yin Y, Ma H, Wang X (2010) Brassinosteroids control male fertility by regulating the expression of key genes involved in Arabidopsis anther and pollen development. Proc Natl Acad Sci U S A 107:6100–6105. https://doi.org/10.1073/pnas.0912333107

Yu M-H, Zhao ZZ, He J-X (2018) Brassinosteroid signaling in plant–microbe interactions. Int J Mol Sci 19:4091. https://doi.org/10.3390/ijms19124091

Zaman M, Kurepin LV, Catto W, Pharis RP (2015) Evaluating the use of plant hormones and biostimulators in forage pastures to enhance shoot dry biomass production by perennial ryegrass (*Lolium perenne* L.). J Sci Food Agric 96:715–726. https://doi.org/10.1002/jsfa.7238

Zeevaart JAD (1999) Abscisic acid metabolism and its regulation. In: Hooykaas PPJ, Hall MA, Libbenga KR (eds) Biochemistry and molecular biology of plant hormones. Elsevier, Amsterdam, pp 189–207. eBook ISBN: 9780080860947

Zhang J, Davies WJ (1989) Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant Cell Environ 12:73–81. https://doi.org/10.1111/j.1365-3040.1989.tb01918.x

Zhao N, Zhao M, Tian Y, Wang Y, Han C, Fan M, Guo H, Bai M-Y (2021) Interaction between BZR1 and EIN3 mediates signalling crosstalk between brassinosteroids and ethylene. New Phytol 232(6):2308–2323. https://doi.org/10.1111/nph.17694

Zhu Z, Bai Y, Lv M, Tian G, Zhang X, Li L, Jiang Y, Ge S (2020) Soil fertility, microbial biomass, and microbial functional diversity responses to four years fertilization in an apple orchard in North China. Hort Plant J 6:223–230. https://doi.org/10.1016/j.hpj.2020.06.003

Chapter 6
The Bacterial-Fungal Consortia: Farmer's Needs, Legal and Scientific Opportunities, and Constraints

Marco Nuti, Laura Ercoli, and Elisa Pellegrino

Abstract In the ecosystem, the plant microbiome is often in association with other microorganisms like bacteria, actinobacteria, and fungi that carry on different roles for the ecosystem. The functional microbiome of the rhizosphere has been studied widely for improvement and enhancement of crop productivity which directly supports a farmer first and later the economy of any country. The integrated functions of bacteria, in the development of synergies with fungal partners, have several advantages. In most of these, many are required to be revised and targeting them according to farmers' needs to propagate conventional agriculture to less environment-impacting types of agriculture. Thus, focusing on it is important to re-look at the constraints of developing biofertilizers using multiple microorganisms. This review addresses the development of need-based and microbial consortia for farmers and the economy.

Keywords Microbial consortia · Rhizobacteria · Rhizosphere · Ecosystem · Farmers

6.1 Introduction

In nature, very rarely microbes live and act alone (Delosse 2019; Nuti 2021). The microbial components of the microbiota are usually found as associations (bacterial, actinobacterial, fungal) in the ecosystems (soil, water, plants, animals, and humans). The biodiversity of these associations determines by and large the functionality of the various microbiomes. Starting from the notion that new properties arise in complex systems as a result of the interactions at a lower level, animals and plants are no longer heralded as autonomous entities, but rather as biomolecular networks

---

M. Nuti (✉)
Sant'Anna School of Advanced Studies, Research Centre of Crop Sciences, Pisa, Italy

Department of Agriculture, Food and Environment (DAFE), University of Pisa, Pisa, Italy

L. Ercoli · E. Pellegrino
Sant'Anna School of Advanced Studies, Research Centre of Crop Sciences, Pisa, Italy
e-mail: laura.ercoli@santannapisa.it; e.pellegrino@santannapisa.it

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_6

composed of the host plus its associated microbes, i.e., "holobionts," super-organisms formed by two components, the macrobiont and the microbiont (Bordenstein and Theis 2015). The cell density of the latter expressed per gram of weight or ml of gastric fluids is often greater than or close to that of the cells forming the macrobiont. Consequently, the biodiversity of the microsymbiont greatly affects the macrosymbiont (Sánchez-Cañizares et al. 2017). This is the case of the human intestinal microbiome (Thursby and Juge 2017), the gut microbiome of the termites (Mathew et al. 2021; Rosengaus et al. 2011), the rumen of ruminants (Cholewińska et al. 2021; Mizrahi et al. 2021), and the microbiome of the plant rhizosphere (Xun et al. 2021; Berendsen et al. 2012). Inside the common truffles (*Tuber* spp.), there are up to 10^8 culturable bacterial cells per gram of truffle "tissue" (Sbrana et al. 2002). In each of these ecosystems, the microbiomes carry on different, integrated functions which allow the macrobiont to survive, to feed on the nutrients metabolized by the microbiont (e.g., the nitrogen from nitrogen-fixing bacteria in the nodules of forage or grain legumes, the phosphorus taken up by mycorrhizal plant root symbionts), to grow better due to the microbially produced biostimulatory substances, to be resilient to abiotic stresses such as water stress, and to be resilient to biotic stresses such as the endophytic bacteria for plants just like the lactic acid bacteria do in the human intestine.

In the transition from conventional agriculture to less environment-impacting types of agriculture, e.g., organic, conservative, regenerative, and symbiotic, the use of microorganisms either as single strains or as microbial consortia has increased remarkably. "Microbials," i.e., products based on living microorganisms, are currently used as (a) biopesticides, namely, pest management agents to control several noxious pests and pathogens infesting crops, gardens, plant nurseries, and forests and causing severe yield and quality losses; (b) biostimulants, i.e., microorganisms which can stimulate natural processes to improve nutrient uptake, nutrient efficiency, and crop quality; (c) soil improvers, i.e., products to be added to the soil to maintain or improve its properties, e.g., green composted amendments; and (d) biofertilizers, i.e., microorganisms living alone or in consortia which provide direct nutrients to plants, such as the symbiotic bacteria living in the nitrogen-fixing nodules of leguminous crops or the symbiotic arbuscular mycorrhizal fungi living in the roots of crops, transferring soluble phosphates, zinc, and iron directly to the plant.

The aim of this review is to highlight the various facets of the above categories and in particular the farmer's needs, the legal frameworks, and the scientific opportunities and constraints. The latter include the discussion on how to create appropriate microbial consortia.

6.2 The Farmer's Need

Many factors usually affect the choice of a product by the farmer: availability of the product, namely, its efficacy in the field, the cost on the market, and the ease of transport and delivery. When a new category of products appears on the market, it is often publicized in advance and reaches the farmers without prior appropriate training. The "microbials" (as biopesticides, biostimulants, soil improvers, and biofertilizers) have encountered some difficulty in the market at the beginning just because they were "new" products. It is therefore imperative to provide the farmers with all the information which comes from the scientific arena and from an independent, critical evaluation of the reliability and field efficacy of commercially available products, leading to optimizing the use of these new products, possibly through a participatory approach (Kiss 2019).

6.2.1 Biopesticides

This category of microbials includes products acting as targeted fungicides, herbicides, insecticides, and nematicides. They may present several advantages over their chemical counterparts and are expected to occupy a large share of the market in the coming years. Without denying the role played in the conventional agriculture by synthetic pesticides during the last six decades, the need for more sustainable control strategies of pests has emerged due to secondary adverse effects of synthetic pesticides in terms of environmental contamination, insect pest resistance, and presence of residues (Fenibo et al. 2021). Biopesticides are a densely populated category of innovative tools for farmers. In 2020, the microbials (including viruses) registered by EPA in the USA as active ingredient insecticides were 136. In the European Union (EU 2022), there are 65 registered active microorganisms, and considering that each of them can give rise to different formulations, the overall number of formulated products, registered at national level in each of the 27 European member states, is much higher. In India, the microbial pesticides were included in the schedule to the Insecticides Act, 1968, while *Beauveria bassiana* for commercial production and distribution was included in the Gazette of India on March 26, 1999 (Keswani et al. 2013). In other countries of the Organisation for Economic Co-operation and Development (OECD) such as Australia, Canada, and Japan, there are similar legislative provisions. In the EU, the authorization procedure, for both active microorganism and formulated product, includes the risk assessment for human toxicological aspects, for ecotoxicology, environmental fate, residues, and operator/workers/bystanders' exposure. Before entering the procedure of risk assessment, the efficacy of the products is evaluated, and only if found positive, other assessments are carried out. Fungi represent more than 50% of registered biopesticides, followed by bacteria and viruses. The bio-insecticidal (microbial) products are represented by microfungi (one or more different strains of *Beauveria bassiana*, *Metarhizium anisopliae*, *Hirsutella*, *Isaria*, *Lecanicillium*, *Paecilomyces*, and *Verticillium*), along with bacteria (one or more strains of *Bacillus*, *Burkholderia*, *Chromobacterium*, *Pasteuria*, *Pseudomonas*, *Serratia*, and *Yersinia*), actinobacteria (*Streptomyces*), yeasts (*Candida*, *Saccharopolyspora*), and baculoviruses (species-specific viruses active against chewing and biting insects). Possibly *Bacillus thuringiensis (B.t.)* as a bio-insecticide still holds the majority of the global biopesticide market, valued at USD 1457 million in 2018 and expected to reach USD 2820 million by 2024. The different biovars of *B.t.* are effective on different target insect pests (*B.t. israelensis* against dipterans such as blackflies and mosquitoes, *B.t. tenebrionis* against coleopterans such as *Leptinotarsa decemlineata*, *B.t. aizawai* against lepidopteran larvae such as *Cydalima perspectalis*, *B.t. kurstaki* against lepidopteran larvae, e.g., *Tuta absoluta*). The microbial biofungicides are represented by one or more strains of bacteria (i.e., *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Pseudomonas syringae*), actinobacteria (i.e., *Streptomyces lydicus*, *Streptomyces griseoviridis*), fungi (i.e., *Aureobasidium pullulans*, *Coniothyrium minitans*, *Duddingtonia flagrans*, *Gliocladium catenulatum*, *Myrothecium verrucaria*, *Trichoderma asperellum*, *T. gamsii*, *T. harzianum*, *Ulocladium oudemansii*), and yeasts (i.e., *Candida oleophila*). The target phytopathogens include the most common fungal agents of plant disease such as *Alternaria*, *Botrytis*, *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Sclerotinia*. Then there are the microbial herbicides (EU 2022; Value-Market-Research 2022). Examples include *Cercosporella riparia* to control the growth of *Ageratina riparia* and *Puccinia chondrillina* to control *Chondrilla juncea* (skeleton weed). Several studies have also supported the efficacy of microbial herbicides such as *Phragmidium violaceum* to control *Rubus* spp. (wild blackberry), *Cercospora rodmanii* to control the water hyacinth, and *Colletotrichum gloeosporioides* to control *Aeschynomene virginica*. The modalities of use in the field, along with the warnings for handling, and the target pest to be controlled are obligatory in the label and accompanying leaflets, considering also that many of the biopesticides are classified as “low risk” substances. However, they are somehow “the new” products, and the farmers need to be better trained. Teachers need to be trained too, since their preparation dates to the older time when these products were not yet on the market. Therefore, governmental agencies and the private sector should fill this gap, through appropriate “hands-on courses” and field demonstrations. In the scientific and technical literature, there are several reviews dealing with biopesticides (Cornelius et al. 2019; Idris et al. 2020; Samada and Tambunan 2020; Ukoroije and Otayor 2020; Fenibo et al. 2021; Hernández-Fernández et al. 2021; Kumar et al. 2021). The efficacy of the main microbials as biopesticides and biostimulants is summarized in Table 6.1.

6.2.2 *Biostimulants*

The microbial biostimulants are a relatively new category of products available for the farmers. The physiology of several individual microbial species was mostly described in the second half of the last century, and it clearly indicated that hormones, vitamins, and other plant stimulatory substances are produced *ex planta*, but only from the onset of this century, it has been possible to measure the *in planta* effects and their impact on crop production and on soil health. In the EU, the Reg. 2019/1009 provides the regulatory framework for biostimulants which can be

Table 6.1 Effects of microbial consortia of various sources on the plant growth promotion

Microbial (active substance)	Crop/source of isolation	Efficacy/effect	References
Azospirillum spp., *Pseudomonas* spp.	Rice (*Oryza sativa*)	Increase the growth and the yield	Braga et al. (2018)
Bacillus spp., *Pseudomonas* spp., and *Azospirillum* spp.	Seedlings and cuttings	Increase the germination and the rooting of cuttings; biocontrol of bacterial wilt and the survival of plants after transplanting	Angulo et al. (2014)
Pseudomonas frederiksbergensis	Red pepper (*Capsicum annuum* L.)	Biostimulant under water and salt stress conditions	Chatterjee et al. (2017)
Paenibacillus illinoisensis, *Bacillus* spp.	Peanut (*Arachis hypogaea* L.)	Increase growth and yield	Liu et al. (2017)
Bacillus spp., *Pseudomonas* spp.	Soil	Control potential of *Meloidogyne javanica* and *Ditylenchus* nematodes	Turatto et al. (2017)
Micromonospora spp.	Alfalfa (*Medicago sativa* L.)	Plant probiotic bacteria	Martínez-Hidalgo et al. (2014)
Streptomyces lydicus WYEC 108	Grass, ornamentals, vegetables, and forest species	Biocontrol by soil-borne plant pathogens and foliar diseases	Sousa and Olivares (2016)
Streptomyces avermitilis	Ornamentals, vegetables, and forest species	Biocontrol of nematodes and insects	Sousa and Olivares (2016)
Actinomadura spp.	Vegetable grain	Bioherbicide/biopesticide producers 2,4-dihydro-4-(β-D-ribofuranosyl)-1,2,4 (3H)-triazol-3-one (herbicide)	Barka et al. (2016)
Streptomyces violaceusniger YCED-9	Soil	Antifungal agent	Barka et al. (2016)
Corynebacterium spp., *Pseudonocardia dioxanivorans*, *Streptomyces* spp., *Micromonospora* spp., *Streptomyces* sp. MBCN152-1, *S. lydicus* WYEC 108	Vegetables, fruits, and grains	Plant growth-promoting bacterium. Biocontrol agent and biofungicide	Salwan and Sharma (2020)
AMF native consortia *Glomus fasciculatum*, *G. etunicatum*, *G. intraradices*, *G. mosseae*, *Scutellospora* sp.	Root carrot (*Daucus carota* L.), tomato (*Solanum lycopersicum* L.)	Increased plant and root carrot (*Daucus carota* L.), and tomato (*Solanum lycopersicum* L.) at greenhouse and field level	Regvar et al. (2003)

(continued)

Table 6.1 (continued)

Microbial (active substance)	Crop/source of isolation	Efficacy/effect	References
Glomus fasciculatum, *Glomus clarum*, *Glomus etunicatum*, *Glomus versiforme*	Long pepper (*Piper longum* L.)	Improve growth at greenhouse and field level	Singh and Gogoi (2012)
Glomus intraradices	Tomato (*Solanum lycopersicum* L.)	Improve yield at field level	Makus (2004)
Rhizophagus intraradices, *Glomus aggregatum*, *G. viscosum*, *G. etunicatum*, *G. claroideum*	Corn (*Zea mays* L.)	Improves crop growth, yield, and grain quality	Berta et al. (2013)
Rhizophagus irregularis	Bread wheat (*Triticum aestivum* L.)	Increases grain Fe concentration, no effect on grain yield	Pellegrino et al. (2020)
Rhizophagus irregularis	Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.)	Increases grain Fe and Zn concentration, no effect on grain yield	Ercoli et al. (2017)
Multiple consortia/single isolates	Alfalfa (*Medicago sativa* L.)	Improves forage yield and nutrient and fatty acid content in forage	Pellegrino et al. (2022)
Funneliformis mosseae and *Rhizophagus irregularis*	Chickpea (*Cicer arietinum* L.)	Increases grain yield and grain Fe and Zn concentration	Pellegrino and Bedini (2014)

microbial and non-microbial, as we discuss in paragraph 3 of this review. Microbial biostimulants include both fungi and bacteria, and their target crops practically embrace all horticultural, cereal, legume, and several tree crops either in nurseries or in the field. The effects of a treatment with a microbial biostimulant span from stimulation of plant growth, increase of plant tolerance to salt and water stress, increase of germination and growth of root system, and delayed senescence up to improvements of the quality of crops in terms of content of plant antioxidants and other secondary metabolites relevant for human health. Some of the well-known microbial biofertilizers such as *Rhizobia* and *Azospirillum* can exhibit, depending on the agronomic conditions or management, also the stimulatory functions typical of the biostimulants. Examples of microbials as biostimulants, including the references, are reported in Table 6.1. The biostimulants are found sometimes in the market as biopesticides as remarked recently by Pirttilä et al. (2021), and this confusion might require more stringent control on the content of the label or even clearer terminology in the legislative provisions. Biopesticides cannot be marketed, at least in the EU, as biostimulants and vice versa. Literature reviews on microbial biostimulants are available (Aguilar et al. 2020; Fadiji et al. 2022; Ganugi et al. 2021), also containing a meta-analysis of a restricted number of data (Castiglione et al. 2021).

6.2.3 Soil Conditioners

Soil is a limited, irreplaceable, and non-renewable environmental asset. Any soil degradation represents a loss for present and future generations. In a recent position paper prepared by a number of non-profit organizations and academic institutions (Soil Health: Civil Society Calls For European Leadership In "The Challenge To Combat Land Degradation Consultation about the proposal of a Soil Health Law" Position Paper, March 2022), it was stressed that "combatting soil degradation is crucial to overcome global challenges, framed by the 2030 Agenda of the United Nations, starting from those related to food security, sustainability of cities, protection of biodiversity, mitigation and adaptation to climate change, prevention of desertification. It is essential for the establishment of a bioeconomy capable of developing safe alternatives to dependence on fossil resources, pursuing circularity in the use of materials. Healthy soils are also the result and, at the same time, prerequisite of the agroecological transition in the food systems. These are some of the reasons why healthy soils are the seedbed in which the European Green Deal should take root." Soil deterioration includes both natural and man-made events, the latter usually known as "soil degradation." This process causes a lowering of the actual or potential capacity of soil to give rise to products or services. Soil is a complex ecosystem formed by four phases: solid, gases, water, and biophase. The latter gives the soil the trait of a living organism, as it is formed by a variety of microbes, micro- and meso-fauna. The microbial community represents a relevant component by weight: 1 hectare of land of 25 cm depth, weighing 3000 tons and having 3.5% organic matter (the majority of European agricultural soils are below 2%), contains up to 3 tons of microbes or 10^9 culturable microbial cells per gram d.w. of soil, belonging to up 2000 different taxa, which are functionally interconnected within aggregates (small macro-aggregates, 250–2000 μm, and micro-aggregates, 53–250 μm) (Piazza et al. 2019; Pellegrino et al. 2022a, b). The microbiome in bulk soil and around the roots (i.e., the rhizosphere) lives almost never alone (Delosse 2019). It grows, multiplies, or reproduces within the aggregates, never as mono-specific (except in rare extremophilic conditions), but rather in multi-specific microbial consortia, providing various ecosystem services. Soil degradation therefore consists in a progressive de-structurization of the aggregates which are the major players in the maintenance of the biogeochemical cycles and the mobilization of nutrients for the plant nutrition and health and in the maintenance of soil functional biodiversity. The limiting factor of the soil functional biodiversity remains the content of organic matter, namely, 1.75% organic carbon corresponding to ca. 3.5% organic matter (Lynch et al. 2004). The fundamental role of biodiversity for the maintenance of our quality of life on the Earth is highlighted by the United Nations: "Biodiversity, including the number, abundance, and composition of genotypes, populations, species, functional types, communities, and landscape units, strongly influences the provision of ecosystem services and therefore human well-being." Anthropogenic soil losses have been recognized for more than almost five decades (Nearing et al. 2017), and early in the 1970s, the OECD and the European

Economic Community (EEC) affiliated member states already warned that “Loss of productive soil is one of the most pressing and difficult problems facing the future of mankind.” Annual losses through erosion were 0.3% of total areas in the emerging countries, and 30% of plowing layer was affected by degradation in the USA in the last 200 years, along with crop yield decrease and subsequent need for higher energy inputs in agriculture. The European Commission officially has enlisted the following causes of soil losses (Nuti 2016): erosion, pollution (localized and diffuse), salinization/alkalization, decrease of organic matter content (today 84% of agricultural soils in EU are below the threshold of 3.5%), cementation and overbuilding, flooding, compaction, and loss of soil biodiversity. The latter is mainly due to inappropriate agronomic management practices. The Pan-European Soil Erosion Risk Assessment (PESERA) map (Kirby et al. 2008) has estimated annual soil losses between 1 and 50 t per ha: Italy is in pole position (often losses are 20–50 t ha^{-1}), along with the Pyrenees region and Greece, although a lower rate of soil degradation is a diffuse event in EU. The process is irreversible in a 50–100-year span in the soils where the losses are higher than 1 t ha¯1 and there is a low soil formation speed. In that sense, any mitigation measure such as the stimulation of agronomic practices leading to carbon sequestration and carbon sink formation should be put in place. It is highly possible that in the next 30 years, only soil will be able to immobilize significant amounts of carbon and therefore reduce the actual levels of carbon dioxide. Alternative strategies require more than 30 years to capture amounts of carbon dioxide relevant to counteract climatic changes. Maybe this is the reason why we have begun talking about “regenerative agriculture” and “regenerative soils” as an approach of true eco-sustainable agriculture at the global level. If an active soil organic matter is brought back to more than 3.5%, the soil micro- and macro-aggregates will allow eventually to maintain the functional biodiversity. The use of soil improvers, such as green composted amendments, possibly rich in an active microbiota relevant for the organic matter turnover, might play a pivotal role in this strategy, to reconstitute the structure of the soil and increase at the same time its organic matter content. Aggregates protect soil organic matter and stabilize it. In a recent study, Pellegrino et al. (2021) found that fungi along with protists such as *Cercozoa* play major roles in soil structuring and carbon cycling. Reviews are available in recent literature for soil improvers (Bambdad et al. 2022; Feldmann et al. 2022).

6.2.4 Biofertilizers

The biofertilizers, according to Vessey (2003), can be defined as biological products containing living microorganisms that, when applied to seed, plant surfaces, or soil, promote plant growth by several mechanisms such as increasing the supply of nutrients, increasing root biomass or root area, and increasing nutrient uptake capacity of the plant. This term is not legally binding, and the prefix bio- merely indicates its living matter component. Often in the scientific and technical literature,

Table 6.2 Brockwell's scale for visual inspection of nodule formation (Brockwell 1982)

Nodule score	Distribution and number of effective nodules	
	Crown	Elsewhere
0	0	0
0.5	0	1–4
1.0	0	5–9
1.5	0	≥10
2.0	Few	0
2.5	Few	Few
3.0	Many	0
4.0	Many	Few
5.0	Many	Many

biofertilizers and biostimulants are considered synonyms or even equivalent terms. However, according to the EU Reg. 2019/1009, the function of a fertilizer is to provide nutrients for the plant growth, and there are microorganisms enlisted fulfilling this task (*Azotobacter* spp., *Rhizobium* spp., and *Azospirillum* spp. and mycorrhizal fungi, i.e., dinitrogen-fixing bacteria and arbuscular mycorrhizal fungi providing soluble phosphorus to the plant), while biostimulants are products which stimulate plants' nutrition processes without providing nutrients directly. Biofertilizers in general are known for their cost-effectiveness, environment-friendly nature, and composition. These are effective alternatives or integrative tools to mineral fertilizers.

The dinitrogen-fixing microorganisms are probably the oldest known biofertilizer, dating back to the end of the nineteenth century. The symbionts of legume crops are collectively called rhizobia although they belong to different taxonomic genera (*Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium*, *Ensifer*, *Burkholderia*) and are able to induce the formation of dinitrogen-fixing nodules on compatible hosts of Leguminosae (Peix et al. 2015), i.e., *Rhizobium leguminosarum* for pea, faba bean, vetchling, and lentil; *Rhizobium phaseoli* for common bean; *Rhizobium ciceri* for chickpea; *Sinorhizobium meliloti* for alfalfa and other medics, yellow melilot, and fenugreek; *Rhizobium trifolii* for clover; *Bradyrhizobium lupini* for lupins; *Mesorhizobium loti* for trefoil; *Rhizobium sullae* for sulla; *Rhizobium vigna* for cowpea and other *Vigna* species for peanut; *Rhizobium simplex* for sainfoin; and *Bradyrhizobium japonicum* for soybean. The amount of nitrogen annually fixed (60 to more than 250 kg ha^{-1}) varies among legume crops and may depend on agronomic management practices and environmental conditions (soil and climate). In Europe, inoculation is essential for soybean because European soils do not contain the required species, and this concept of inoculation can be extended to all agronomic condition in which the nodules of the forage or grain legume crop are absent or malfunctioning. One simple field test is the visual inspection of the nodules and the assignment to a score scale such as Brockwell's scale (Table 6.2): crops scoring less than 3.0 would need to be inoculated with the appropriate rhizobia. Other field tests are also available (Pommeresche and Hansen 2017). Detached nodules from root canopy brought to the lab or in field with portable gas chromatograph can be assessed for nitrogen fixation by using the acetylene reduction assays (Soper et al. 2021). Mineral nitrogen in soil such as nitrate at 6–7 mM and at 12 mM can have serious deleterious effect on biological dinitrogen fixation and on the formation of nodules (Pampana et al. 2018). The literature on rhizobia and rhizobial bioformulations is vast (Arora et al. 2017) and could total for more than 20,000 papers. In the last few years, approximately 13,247 peer-reviewed journal papers only on soybean production have been produced, of which 731 focused on soil management (Pagano and Miransari 2016).

Mycorrhizal fungi are among the most used microbial fertilizers globally, showing a remarkable biostimulatory effect on crops, improving their yields and quality. The arbuscular mycorrhizal fungi (AMFs) belong to the phylum Glomeromycota, which includes 5 orders (Archaeosporales, Diversisporales, Gigasporales, Glomerales, and Paraglomerales), 29 genera, and more than 200 species. They are reportedly present as symbionts of more than 80% of the land plants present in the biosphere (Castiglione et al. 2021). Hundreds of scientific papers report the beneficial effects of mycorrhizal inoculation, mainly with species of *Rhizophagus* and *Funnelliformis*, at the greenhouse and field level, recently reviewed by Aguilar et al. (2020) and Castiglione et al. (2021), which can be considered effective biostimulants. In addition to the clear effects on crops, AMFs improve also the physical properties of the soil by modifying its structure, e.g., by inducing the entanglement of the soil particles with each other through the synthesis of glomalin, a glycoprotein, thus maintaining or creating the soil structure (Bedini et al. 2009). In this sense, AMFs can be considered soil improvers. Finally, AMFs can provide directly to the plants macro-nutrients such as phosphorus and micro-nutrients such as Zn and Mg. In this sense, they act as true (bio)fertilizers. This triple efficacy will be exerted in different ratios, depending on the biomass density of the inoculant, its formulation, and its composition in terms of genera and species present in the inoculant and its interaction with soil AMF community. Sporadically, it has been observed that AMF protects the plant against nematodes or soil-borne fungi. To help farmers to predict the efficacy of a mycorrhizal inoculation, a database on plant productivity is available (Chaudhari et al. 2016), although it would be desirable to update it since most field data has been obtained during the last 7–8 years.

6.3 Legal Framework

Biopesticides are a densely populated category of innovative tools for farmers. In 2020, the microbials (including viruses) registered by EPA in the USA as active ingredient insecticides were 136. In the European Union (EU 2022), there are 65 registered active microorganisms, and considering that each of them can give rise to different formulations, the overall number of formulated products, registered at national level in each of the 27 European member states, is much higher. In India, the microbial pesticides were included in the schedule to the Insecticides Act, 1968, while *Beauveria bassiana* for commercial production and distribution was included in the Gazette of India on March 26, 1999 (Keswani et al. 2013). In other countries of the OECD such as Australia, Canada, and Japan, there are similar legislative provisions. In the EU, the authorization procedure, for both active microorganism and formulated product, includes the risk assessment for human toxicological aspects, for ecotoxicology, environmental fate, residues, and operator/workers/bystanders' exposure. Before entering the procedure of risk assessment, the efficacy of the products is evaluated, and only if found positive, other assessments are carried out.

For biofertilizers, in the EU, the Reg. 2019/1009 (EU 2019), of which the full implementation deadline was on July 16, 2022, sets the rules for marketing the inorganic and organic fertilizers, intended as substances or mixtures, microorganisms, or any other material applied or intended to be applied to plants or their rhizosphere or to mushrooms and their mycosphere with the purpose of providing nutrients or improving their nutrition efficiency. Among the 11 families of constituent materials, there are microbials, and among the 7 product functional categories, there are the fertilizers, the amendments, and the biostimulants. The positive short list reported in the provision could be expanded to include new microbials, if there is scientific evidence supporting their efficacy on specific crops and safety for both the environment and consumers.

The "soil improvers or soil conditioners," according to Regulation (EU) 2019/1009 (product functional category 3), are "fertilizing products whose function is to maintain, improve or protect the physical or chemical properties, the structure or the biological activity of the soil to which it is added." The concept of maintenance and improvement of physical chemical properties of the soil, jointly with its biological activity, is a new science-based provision in a legislative context.

The biostimulants (microbial and non-microbial) are defined by the same EU Regulation as "products stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, availability of confined nutrients in soil or rhizosphere." After July 16, 2022, there will be the obligation to present a conformity declaration in order to deliver the product on the EU market, and the 27 member states need to adapt their national legislation on fertilizer to the rules set by the new Regulament. This implies defining methods of analysis, particularly for those products which are newly introduced, i.e., the microbials. The existence of only a few accredited organizations might slow down the implementation process at the national level. Furthermore, out of the 200 harmonized provisions which would guarantee the conformity declaration, in some member states, less than 100 are ready, and the deadline is within 2024/2025. The adoption of the QR code on the label should facilitate the marketing of the newly introduced products.

The pesticides fall under a different EU provision, i.e., the Reg. 1107/2009, which lays down the rules for the authorization of plant protection products in commercial form and for their placing on the market and use and control within the community (EU 2009). However, setting the rules for the microbial plant protection products (the so-called biopesticides) has taken more time than for the chemical pesticides. The reasons for that have been discussed by Sundh and Eilenberg (2021). Similarly, in other countries, the chemical plant protection products are regulated (e.g., the Public Law 106–224 of June 20, 2000, also known as the Plant Protection Act in the USA), but the microbial pesticides have been regulated by later provisions (e.g., by EPA in the USA, https://www.epa.gov/pesticides/biopesticides); an overview of representative legislations has been published by Kabaluk et al. (2010).

6.4 Scientific Opportunities and Constraints

Designing a microbial consortium is not an easy task. Ideally, the farmers would need a custom-tailored product, but normally, this is not available yet, just as the custom-tailored medicaments for humans. The available consortia on the market cover a bunch of crops, agronomic situations, and pedo-climatic areas. The single-strain inoculants and microbial consortia can be developed to produce all categories of products, i.e., biopesticides, biostimulants, soil improvers, or biofertilizers. Rhizobia have been successfully used for more than a century as single-strain biofertilizer of legume crops, and *Bacillus thuringiensis* has been successfully used for almost a century as a single-strain bio-insecticide. In the first case, the success (i.e., formation of dinitrogen-fixing nodules on the legume crop) was mainly due to the specificity of the microbial inoculant and to the use of an adequate number of cells to each seed (i.e., more than 1000 for small-seeded legumes up to 10,000 for large-seeded legumes) during the seed coating. The second generation of rhizobial inoculants was developed, allowing less erratic, more constant results in the field. In the second case, the selection of appropriate mono-specific strains and the possibility of large-scale production of microbial cells in pure culture played a major role for the success of the biopesticide. However, the more recent development of dual-strain biopesticides and of microbial consortia for the production of biostimulants and biofertilizers has encountered interest and favorable acceptance by the farmers. In these cases, the rationale is simple. When complex substrates are used, it would be difficult for a single microorganism to accomplish all the biochemical steps required to colonize, and partially degrade, the substrate. We can take advantage of the cooperative nature of microbes in nature and let each strain or species do what they normally would do naturally. Typical examples are the production of soil improvers or amendments based on lignocellulosic materials, such as the solid waste generated during the extraction of olive oil from olives (eight million tons is available each year in the Mediterranean countries, i.e., Spain, Italy, and Greece) or the solid waste generated during the coffee grain processing around the world. In these cases, there are additional difficulties to prepare the amendment because of the presence of polyphenols and fats which are inhibitory of the microbial activities. Thus, only an accurate strain selection and the formation of compatible inter-specific or inter-strain microbial consortia can ensure the degradation of the recalcitrant molecules of the substrate and the contemporary formation of humic substances to ameliorate the soil physical chemical properties, the formation of biostimulatory substances for the plants, and the formation of carbon sinks, along the detoxification of the initial substrate (Echeverria et al. 2012; Agnolucci et al. 2013; Echeverria et al. 2017, 2022). At field level, microbial consortia prepared with mycorrhizal fungi have proven to be effective in enhancing the forage yield and the fatty acid content of alfalfa (*Medicago sativa*) (Pellegrino et al. 2022a, b). The opportunity of enhancing both yields at field level and quality of crops opens new horizons and challenging perspectives. However, many "unknowns" are waiting to be unraveled, and the entangled life of fungi (Sheldrake 2020) is far from being disentangled and profitably used by all farmers. There are more than 10 products of biostimulants and biofertilizers containing microbial consortia of up to 30 strains, whose precise biochemical mechanisms are still to be clarified (Macik et al. 2020). There are other constraints that we should circumvent by stimulating R & D activities: the mass scale production of some mycorrhizal fungi which for the moment are used as "crude inoculum" to prepare biofertilizers, the coupling of fungal inoculants with the appropriate cultivars of different crops, and the selection of a wider range of epiphytic bacteria to be used as biopesticides. Having at hand the molecular tools to identify "new" microorganisms or to monitor the strains in the field, it appears that this availability has paved the way to an eco-friendly and safe use of microbials in agriculture.

Acknowledgment Not applicable.

Conflict of Interest Authors declare no conflict of interest.

References

Agnolucci M, Cristani C, Battini F, Palla M, Cardelli R, Saviozzi A, Nuti M (2013) Microbially-enhanced composting of olive mill solid waste (wet husk): bacterial and fungal community dynamics at industrial pilot and farm level. Bioresour Technol 134:10–16. https://doi.org/10.1016/j.biortech.2013.02.022

Aguilar A, Valdez G, Nuti M (2020, 1902) Ecosystem functions of microbial consortia in sustainable agriculture. Agronomy 10. https://doi.org/10.3390/agronomy10121902

Angulo VC, Sanfuentes EA, Rodríguez F, Sossa KE (2014) Caracterización de rizobacterias promotoras de crecimiento en plántulas de Eucalyptus nitens. Rev Argent Microbiol 46:338–347

Arora NK, Verma M, Mishra J (2017) Rhizobial bioformulations: past, present and future. In: Mehnaz S (ed) Rhizotrophs: plant growth promotion to bioremediation. Microorganisms for sustainability, vol 2. Springer, Singapore. https://doi.org/10.1007/978-981-10-4862-3_4

Bambdad H, Papari S, Lazarovits G, Berruti F (2022) Soil amendments for sustainable agriculture: microbial organic fertilizers. Soil Use Manag 38:94–120

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, Van Wezel GP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1–43

Bedini S, Pellegrino E, Avio L, Pellegrini S, Bazzoffi P, Argese E, Giovannetti M (2009) Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. Soil Biol Biochem 41(7):1491–1496. https://doi.org/10.1016/j.soilbio.2009.04.005

Berendsen RL, Corné MJP, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trend Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berta G, Copetta A, Gamalero E, Bona E, Cesaro P, Scarafoni A, D'Agostino G (2013) Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. Mycorrhiza 24:161–170

Bordenstein SR, Theis KR (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol 13(8):e1002226

Braga LF, De Oliveira FA, Couto EAPD, Santos KFDN, Ferreira EPDB, Martin-Didonet CCG (2018) Polyphasic characterization of bacteria obtained from upland rice cultivated in Cerrado soil. Braz J Microbiol 49:20–28

Brockwell J (1982) Selection of rhizobia for inoculants. In: Nitrogen fixation in legumes. Academic Press, Australia, p 173

Castiglione AM, Mannino G, Contartese V, Bertea CM, Ertani A (2021) Microbial biostimulants as response to modern agriculture needs: composition, role and application of these innovative products. Plan Theory 10:1533. https://doi.org/10.3390/plants10081533

Chatterjee P, Samaddar S, Anandham R, Kang Y, Kim K, Selvakumar G, Sa T (2017) Beneficial soil bacterium *Pseudomonas frederiksbergensis* OS261 augments salt tolerance and promotes red pepper plant growth. Front Plant Sci 8:705

Chaudhari VB, Rúa MA, Antoninka A, Bever JD, Cannon J, Craig A, Duchicela J, Frame A, Gardes M, Gehring C et al (2016) MycoDB, a global database of plant response to mycorrhizal fungi. Sci Data 3:160028

Cholewińska P, Górniak W, Wojnarowski K (2021) Impact of selected environmental factors on microbiome of the digestive tract of ruminants. BMC Vet Res 17(1):25. https://doi.org/10.1186/s12917-021-02742-y

Cornelius G, Lohiya G, Sharma R (2019) Biopesticides: an alternative approach for agricultural output (food) and environmental safety. Int J Eng Res Technol 8(11):578–590. ISSN: 2278-0181

Delosse M-A (2019) Jamais seul: ces microbes qui construisent les plantes, les animaux et les civilisations. Ed. Actes Sud, Les Mèjan, Pl. Barberova, 13200 Arles, France

Echeverria C, Sania EM, Obando S, Nuti M (2022) Scientific, technical, and social challenges of coffee rural production in Ecuador. In: Alem H, Jena PR (eds) Sustainable agricultural value chain. IntechOpen. https://doi.org/10.5772/intechopen.104747. ISBN 978-1-83969-756-2. http://www.intechopen.com/books/sustainable-agricultural-value-chain

Echeverria MC, Cardelli R, Bedini S, Colombini A, Incrocci L, Castagna A, Agnolucci M, Cristani C, Ranieri A, Saviozzi A, Nuti M (2012) Microbially-enhanced composting of wet olive husks. Bioresour Technol 104:509–517. https://doi.org/10.1016/j.biortech.2011.11.042

Echeverria MC, Pellegrino E, Nuti M (2017) The solid wastes of coffee production and of olive oil extraction: management perspectives in rural areas (Chapter 9). In: Mihai FC (ed) Solid waste management in rural areas. IntechOpen, pp 165–189. ISBN 978-953-51-3486-2. https://doi.org/10.5772/intechopen.69427

Ercoli L, Schüßler A, Arduini I, Pellegrino E (2017) Strong increase of durum wheat iron and zinc content by field-inoculation with arbuscular mycorrhizal fungi at different soil nitrogen availabilities. Plant Soil 419(1):153–167

EU (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

EU (2019) Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulation (EC) No. 1069/2009 and (EC) No. 1107/2009 and repealing Regulation (EC) No. 2003/2003. Off. J. Eur. Union 2019, 1–114. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1009&from=EN. Accessed 25 Aug 2022

EU (2022) EU Pesticide Database. Search Active substances, safeners and synergists (europa.eu); accessed January 14th, 2022. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as. Accessed 25 Aug 2022

Fadiji AE, Babalola OO, Santoyo G, Perazzolli M (2022) The potential role of microbial biostimulants in the amelioration of climate change-associated abiotic stresses on crops. Front Microbiol 12:829099. https://doi.org/10.3389/fmicb.2021.829099

Feldmann F, Jehle J, Bradáčová K et al (2022) Biostimulants, soil improvers, bioprotectants: promoters of bio-intensification in plant production. J Plant Dis Prot. https://doi.org/10.1007/s41348-022-00567-x

Fenibo EO, Ijoma GN, Matambo T (2021) Biopesticides in sustainable agriculture: a critical sustainable development driver governed by green chemistry principles. Front Sustain Food Syst 5:619058. https://doi.org/10.3389/fsufs.2021.619058

Ganugi P, Martinelli E, Lucini L (2021) Microbial biostimulants as a sustainable approach to improve the functional quality in plant-based foods: a review. Curr Opin Food Sci 41:217–223. ISSN 2214-7993. https://doi.org/10.1016/j.cofs.2021.05.001

Hernández-Fernández M, Cordero-Bueso G, Ruiz-Muñoz M, Cantoral JM (2021) Culturable yeasts as biofertilizers and biopesticides for a sustainable agriculture: a comprehensive review. Plan Theory 10:822. https://doi.org/10.3390/plants10050822

Idris AL, Fan X, Muhammad MH et al (2020) Ecologically controlling insect and mite pests of tea plants with microbial pesticides: a review. Arch Microbiol 202:1275–1284. https://doi.org/10.1007/s00203-020-01862-7

Kabaluk JT, Svircev AM, Goettel MS, Woo SG, Eds. (2010) The use and regulation of microbial pesticides in representative jurisdictions worldwide. IOBC Global 99. https://www.iobc-global.org/

Keswani C, Singh SP, Singh HB (2013) *Beauveria bassiana*: status, mode of action, applications and safety issues. Biotech Today 3(1):16–20. https://doi.org/10.5958/j.2322-0996.3.1.002

Kirby MJ, Irvine BJ, Jones RJA, Govers G, PESERA team (2008) The PESERA coarse scale erosion model for Europe. I – Model rationale and implementation. Eur J Soil Sci 59(6): 1293–1306. https://doi.org/10.1111/j.1365-2389.2008.01072.x

Kiss J (2019) Pesticides in agriculture: are we sustainable yet? J Consumer Protect Food Safety 14: 205–207

Kumar J, Ramlal A, Mallick D et al (2021) An overview of some biopesticides and their importance in plant protection for commercial acceptance. Plan Theory 10:1185. https://doi.org/10.3390/plants10061185

Liu D, Yang Q, Ge K, Hu X, Qi G, Du B, Liu K, Ding Y (2017) Promotion of iron nutrition and growth on peanut by *Paenibacillus illinoisensis* and Bacillus sp. strains in calcareous soil. Braz J Microbiol 48:656–670

Lynch JM, Benedetti A, Insam H, Nuti MP, Smalla K, Torsvik V, Nannipieri P (2004) Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol Fertil Soil 40:363–385

Macik M, Gryta A, Frac M (2020) Biofertilizers in agriculture: an overview on concepts, strategies and effects on soil microorganisms. Adv Agron 162:31–87

Makus D (2004) Mycorrhizal inoculation of tomato and onion transplants improves earliness. Acta Hortic 631:275–281

Martínez-Hidalgo P, Galindo-Villardón P, Trujillo ME, Igual JM, Martínez-Molina E (2014) Micromonospora from nitrogen fixing nodules of alfalfa (*Medicago sativa* L.). A new promising plant probiotic bacteria. Sci Rep 4:6389

Mathew G, Raveendran S, Huang C-C, Pandey A, Parameswaran B (2021) Microbial diversity in termite gut ecosystem and their role in lignocelluloses degradation. In: Microbial diversity and ecology in hotspots. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-323-90148-2.00002-X

Mizrahi I, Wallace RJ, Moraïs S (2021) The rumen microbiome: balancing food security and environmental impacts. Nat Rev Microbiol 19:553–566. https://doi.org/10.1038/s41579-021-00543-6

Nearing MA, Xie Y, Liu B, Ye Y (2017) Natural and anthropogenic rates of soil erosion. Int Soil Water Cons Res 5(2):77–84. ISSN 2095-6339. https://doi.org/10.1016/j.iswcr.2017.04.001

Nuti M (2016) Quantification of soil losses due to erosion, pollution and urbanization. El Diario FICAYA Emprende 7:23–27. Numero Especial “Tecnología y medio ambiente” ISSN 1390-9290. www.utn.edu.ec/ficayaemprende

Nuti M (2021) Una società invisibile: l’importanza dei microbi in agricoltura. Carocci editore, Roma, pp.1-71. ISBN 978-88-430-7429-7

Pagano MC, Miransari M (2016) In: Miransari M (ed) Abiotic and biotic stresses in soybean production in the importance of soybean production worldwide. Academic Press, Elsevier, Amsterdam, pp 1–26. ISBN 9780128015360. https://doi.org/10.1016/B978-0-12-801536-0.00001-3

Pampana S, Masoni A, Mariotti M, Ercoli L, Arduini I (2018) Nitrogen fixation of grain legumes differs in response to nitrogen fertilisation. Exp Agric 54(1):66–82. https://doi.org/10.1017/S0014479716000685

Peix A, Ramírez-Bahena MH, Velázquez E, Bedmar EJ (2015) Bacterial associations with legumes. Crit Rev Plant Sci 34(1-3):17–42. https://doi.org/10.1080/07352689.2014.897899

Pellegrino E, Bedini S (2014) Enhancing ecosystem services in sustainable agriculture: biofertilization and biofortification of chickpea (*Cicer arietinum* L.) by arbuscular mycorrhizal fungi. Soil Biol Biochem 68:429–439

Pellegrino E, Piazza G, Arduini I, Ercoli L (2020) Field inoculation of bread wheat with Rhizophagus irregularis under organic farming: variability in growth response and nutritional uptake of eleven old genotypes and a modern variety. Agronomy 10(3):333

Pellegrino E, Piazza G, Helgason T, Ercoli E (2021) Eukaryotes in soil aggregates across conservation management: major roles of protists, fungi and taxa linkages in soil structuring and C stock. Soil Biol Biochem 163:108463. https://doi.org/10.1016/j.soilbio.2021.108463

Pellegrino E, Nuti M, Ercoli L (2022a) Multiple arbuscular mycorrhizal fungal consortia enhance yield and fatty acids of Medicago sativa: a two-year field study on agronomic traits and tracing of fungal persistence. Front Plant Sci 13:814401. https://doi.org/10.3389/fpls.2022.814401; https://www.frontiersin.org/article/10.3389/fpls.2022.814401

Pellegrino E, Piazza G, Helgason T, Ercoli L (2022b) Microbiome structure and interconnection in soil aggregates across conservation and conventional agriculture allow to identify main prokaryotic and fungal taxa related to soil functioning. Soil Biol Biochem 175:108833

Piazza G, Ercoli L, Nuti M, Pellegrino E (2019) Interaction between conservation tillage and nitrogen fertilization shapes prokaryotic and fungal diversity at different soil depths: evidence from a 23-year field experiment in the mediterranean area. Front Microbiol 10:Article 2047. https://doi.org/10.3389/fmicb.2019.02047

Pirttilä AM, Mohammad Parast Tabas H, Baruah N, Koskimäki JJ (2021) Biofertilizers and biocontrol agents for agriculture: how to identify and develop new potent microbial strains and traits. Microorganisms 9:817. https://doi.org/10.3390/microorganisms9040817

Pommeresche R, Hansen S (2017) Examining root nodule activity on legumes. FertilCrop Technical Note. Research Institute of Organic Agriculture (FiBL) and Norwegian Centre for Organic Agriculture (NORSØK), Frick and Tingvoll. https://orgprints.org/31344/

Regvar M, Vogel-Mikuš K, Ševerkar T (2003) Effect of AMF inoculum from field isolates on the yield of green pepper, parsley, carrot, and tomato. Folia Geobot 38:223–234

Rosengaus RB, Zecher CN, Schultheis KF, Brucker RM, Bordenstein SR (2011) Disruption of the termite gut microbiota and its prolonged consequences for fitness. Appl Environ Microbiol 77(13):4303–4312. https://doi.org/10.1128/AEM.01886-10

Salwan R, Sharma V (2020) Molecular and biotechnological aspects of secondary metabolites in actinobacteria. Microbiol Res 231:126374

Samada LH, Tambunan USF (2020) Biopesticides as promising alternatives to chemical pesticides: a review of their current and future status. OnLine J Biol Sci 20:66–76. https://doi.org/10.3844/ojbsci.2020.66.76

Sánchez-Cañizares C, Jorrín B, Poole PS, Tkacz A (2017) Understanding the holobiont: the interdependence of plants and their microbiome. Curr Opin Microbiol 38:188–196., ISSN 1369-5274. https://doi.org/10.1016/j.mib.2017.07.001

Sbrana C, Agnolucci M, Bedini S, Lepera A, Toffanin A, Giovannetti M, Nuti MP (2002) Diversity of culturable bacterial populations associated to *Tuber borchii* ectomycorrhizas and their activity on *Tuber borchii* mycelial growth. FEMS Microbiol Lett 211:195–201

Sheldrake M (2020) Entangled life: how fungi make our worlds, change our minds and shape our futures. Random House Publ., New York, p 366. ISBN 0525510311

Singh RK, Gogoi P (2012) Augmented growth of long pepper in response to arbuscular mycorrhizal inoculation. J For Res 23:339–344

Soper FM, Simon C, Jauss V (2021) Measuring nitrogen fixation by the acetylene reduction assay (ARA): is 3 the magic ratio? Biogeochemistry 152:345–351. https://doi.org/10.1007/s10533-021-00761-3

Sousa JADJ, Olivares FL (2016) Plant growth promotion by streptomycetes: ecophysiology, mechanisms and applications. Chem Biol Technol Agric 3:1–12

Sundh I, Eilenberg J (2021) Why has the authorization of microbial biological control agents been slower in the EU than in comparable jurisdictions? Pest Manag Sci 77(5):2170–2178. https://doi.org/10.1002/ps.6177

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474(11): 1823–1836. https://doi.org/10.1042/BCJ20160510

Turatto MF, Dourado FDS, Zilli JE, Botelho GR (2017) Control potential of Meloidogyne javanica and Ditylenchus spp. using fluorescent Pseudomonas and Bacillus spp. Braz J Microbiol 49:54–58

Ukoroije RB, Otayor RA (2020) Review on the bio-insecticidal properties of some plant secondary metabolites: types, formulations, modes of action, advantages and limitations. Asian J Res in Zoology 3(4):27–60

Value-Market-Research (2022) Global Biopesticides Market Overview, Forecast, Analysis | Global Report, 2027 (valuemarketresearch.com); https://www.valuemarketresearch.com/report/biopesticides-market. Accessed 24 June 2022

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586. https://doi.org/10.1023/A:1026037216893

Xun W, Shao J, Shen Q, Zhang R (2021) Rhizosphere microbiome: functional compensatory assembly for plant fitness. Comput Struct Biotechnol J 19:5487–5493. https://doi.org/10.1016/j.csbj.2021.09.035. PMID: 34712394; PMCID: PMC8515068

Part II
Contribution to Agriculture and Sustainability

Chapter 7
Sustainable Improvement of Productivity and Quality of Agricultural Crops Using a Microbial Consortium

Somayeh Emami, Hossein Ali Alikhani, and Vida Kardgar

Abstract Excessive consumption of chemical fertilizer for improving the yield and health of agricultural crops eliminates the ecosystem balance in the water and soil environment. For the environmental concerns and high cost of chemical fertilizer, the production of biological fertilizers has been considered. In sustainable agricultural systems, microbial inoculants have special importance in increasing productivity and sustainable soil fertility management. The use of microbial inoculants leads to stability of soil resources, maintains long-term production capacity, and prevents environmental pollution. Decades of research in greenhouse and field conditions have shown that these inoculants are more effective on plants' growth when used as a consortium. In addition to the individual effects of microorganisms, productivity and quality of agricultural crops can be improved by inoculation with other microorganisms due to their synergistic effects. The association of different organisms in microbial consortium enhances fertility and health status of soil for the growth of legume and non-legume plants. This chapter presents an overview of recent researches on the soil microbial consortia, mechanisms, and their impact on improving the productivity and quality of agricultural crops.

Keywords Microbial consortia · Nitrogen fixer · Phosphate solubilizer · Phytostimulation · Biocontrol

---

S. Emami (✉) · H. A. Alikhani
Department of Soil Science, College of Agriculture and Natural Resource, University of Tehran, Tehran, Iran
e-mail: emamisomaye@ut.ac.ir

V. Kardgar
Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Tehran, Iran

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_7

7.1 Introduction

Population growth, increasing human demand for food, and environmental crises are among the most important challenges in the world. The world's current population is 7.8 billion and is projected to reach 9.0 billion in the next 30 years (Glick 2015; Ramakrishna et al. 2019). These issues are related to agricultural production, and it is necessary to think of a solution for them. In addition, abiotic and biotic stresses are a threat to agricultural production. Soil salinization, soil erosion and destruction, drought and global warming, diseases, and pathogens are among the threats to agricultural crop production (Numan et al. 2018).

According to these conditions, it is clear that the pressure on soil and water resources has started, and they must withstand more pressure in the future. Due to the difficulty of increasing the area under cultivation, maximum effort must be focused on increasing production per unit area. Therefore, attention to soil fertility and how to improve it to achieve sustainable production, food security, and environmental protection is essential (Numan et al. 2018; Ayangbenro and Babalola 2020).

Fertilizer is critical factor in plant nutrition, and the optimal use of fertilizer is the main concern of the agricultural sector (Tilman et al. 2002; Ramakrishna et al. 2019). The Green Revolution, which was formed with the introduction and supply of chemical fertilizers, increased agricultural production by increasing plant growth and yield indices and posed risks to the environment and limited water and soil resources. Therefore, the continuation of traditional methods is a threat to food security and soil and water resources (Ramakrishna et al. 2019; Ayangbenro and Babalola 2020). Researchers are trying to find ways to increase the quantity and quality of agricultural products by emphasizing the protection of non-renewable sources of the environment, especially soil and water.

In recent years, the use of soil microbial inoculants in the form of biofertilizers or biocontrol agents in agriculture has been considered by researchers (Numan et al. 2018; Emami et al. 2020; Ayangbenro and Babalola 2020). Improving the growth of various agricultural crops by soil microbial inoculants has been proven in the greenhouse (Emami et al. 2020) and field experiments (Shirmohammadi et al. 2020). These inoculants can have positive and economic effects on legume (alfalfa and soybeans) and non-legume (wheat, rice, and maize) plants (Guiñazú et al. 2010; Korir et al. 2017; Emami et al. 2020; Shirmohammadi et al. 2020). Plant response to soil inoculation with microbial inoculants varies depending on the bacteria and fungi strains, plant species, soil properties, concentration and quality of the inoculum, and environmental conditions (Etesami and Maheshwari 2018; Kour et al. 2020; Ayangbenro and Babalola 2020). Sometimes inoculated microorganisms cannot survive in the soil because they compete with the native soil biota. An effective inoculant in terms of plant growth-promoting (PGP) traits in a specific area may not have the same effect on the plant in other soil and climatic conditions. Isolation and study of native isolates can help to produce suitable inoculants for local products (Meena et al. 2017; Etesami and Maheshwari 2018; Shirmohammadi et al. 2020). In addition, the use of microbial consortia is preferred to a single-specific organism for biofertilization (Emami et al. 2020). The efficiency of consortia is higher due to the synergistic relationship between microorganisms and the production of various metabolites. Ullah et al. (2017) stated that consortium of rhizobia (*Rhizobium leguminosarum* and *Mesorhizobium ciceri*) was more effective than single-strain inoculation for improving wheat growth in water-deficient conditions. The use of arbuscular mycorrhizal fungi (*Funneliformis mosseae*) and phosphate-solubilizing fungi (*Apophysomyces spartima*) as a consortium reduced salt stress effects on beach palm growth and enhanced phosphorus and magnesium uptake (Zai et al. 2021). The present chapter summarizes recent researches on soil microbial consortia, mechanisms, and their impact on improving the productivity and quality of agricultural crops.

7.2 Soil Microbial Consortia

A conceivable solution to reduce the adverse effects of continued use of chemical fertilizers is the application of biological fertilizer. Biofertilizer is a substrate containing microorganisms that stimulate plant growth and yield by enhancing the bio-availability and absorption of macro- and micro-nutrients for plants. Microorganisms increase plant productivity by nitrogen fixation, or increasing the availability of nutrients (e.g., phosphate solubilization), or increasing plant access to nutrients by improving plant root systems (through the production of hormones) (Saharan and Nehra 2011; Devi et al. 2018; Emami et al. 2020). In addition, the microorganisms that improve plant growth by controlling pathogens are called biopesticides (Mukhopadhyay 1996; Devi et al. 2018). In some cases, microorganisms increase plant productivity in both ways. For example, some species of *Burkholderia* have biocontrol properties on the pathogenic fungus *Fusarium* spp. and stimulate maize growth in iron deficiency conditions through siderophore production. Various studies showed that an isolate can't communicate with all soil conditions and with all host plants successfully due to the partial resistance to environmental changes, insufficient colonization, and fluctuation in metabolite production (Dowling and O'Gara 1994; Raupach and Kloepper 1998; Shirmohammadi et al. 2020). A suitable solution is the formulation of biofertilizers with several isolates. Numerous PGP traits of different microorganisms can be helpful for the development of biological inoculants. The effectiveness of microbial consortia in improving plant growth (through biofertilization and biocontrol) has been reported in previous researches (Ruíz-Sánchez et al. 2011; Emami et al. 2018; Ríos-Ruiz et al. 2020). In these reports, microbial consortia contain a combination of bacteria, bacteria and fungi, as well as bacteria and yeast (Janisiewicz and Bors 1995; Nandakumar et al. 2001). Using several microorganisms together can increase the efficiency, durability, and effectiveness of inoculants and provide a combination of different PGP traits together without the use of genetic engineering.

Belimov et al. (1995) reported a significant positive effect on the grain yield of barley by co-inoculation of *Azospirillum lipoferum* 137 + *Agrobacterium* *radiobacter* 10 and *Azospirillum lipoferum* 137 + *Arthrobacter mysorens* 7 compared to the pure culture. Adesemoye et al. (2009) stated that the combination of *Bacillus pumilus* and *Bacillus amyloliquefaciens* with 75% required nitrogen for maximum yield of plant improved the growth indices of tomato plants and the amount of nitrogen uptake at the same level (statistically) by treating 100% required nitrogen fertilizer without inoculation. Rhizospheric phosphate-solubilizing fluorescent pseudomonads stimulate plant growth, while co-inoculation of rhizosphere and endophytic fluorescent pseudomonad strains has a synergistic effect on the wheat plant growth (Emami et al. 2020).

7.3 Mechanism of Microbial Consortia in the Improvement of Productivity and Quality of Agricultural Crops

Numerous mechanisms are known to explain how soil microbial consortia affect plant growth, which can be divided into three categories: biofertilization (nitrogen fixation, phosphate solubilization, and ionophore production(, phytostimulation (auxins,' cytokinins,' and gibberellins' production), and biocontrol (antimicrobial secondary metabolites) (Saharan and Nehra 2011; Emami et al. 2018).

7.3.1 Biofertilization

7.3.1.1 Nitrogen Fixation

Nitrogen is a crucial nutrient for plant growth and is one of the main components of the synthesis of proteins, nucleic acids, and other cellular compounds. Although nitrogen makes up 78% of the air, it is still one of the most limiting factors for plant growth, and nitrogen fertilizers are often used to address its deficiency, which increases production costs (Rashid et al. 2016). Under such conditions, the utilization of atmospheric nitrogen through the process of biological nitrogen fixation is a suitable option (Franche et al. 2009; Pérez-Montaño et al. 2014). Benefits of biological nitrogen fixation include reducing production costs, reducing groundwater pollution, increasing plant protein production, increasing soil nitrogen residues for future crops, and increasing soil fertility (Pérez-Montaño et al. 2014; Rashid et al. 2016). Different bacterial genera such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Enterobacter*, and *Pseudomonas* are capable of nitrogen fixation (Demba Diallo et al. 2004; Franche et al. 2009; Duc et al. 2009). Increased yields of rice (Malik et al. 1997; Zhou et al. 2020; Panneerselvam et al. 2021), alfalfa (Zhu et al. 2016; Pourbabaee et al. 2021), soybeans (Afzal et al. 2010), maize (de Salamone et al. 1996; Di Salvo et al. 2018; Ke et al. 2019), and wheat (Boddey et al. 1986; da Silveira et al. 2016; de Souza Gênero et al. 2020) were obtained by application of nitrogen-fixing bacteria in many experiments. Various estimates have determined the contribution of symbiotic nitrogen-fixing bacteria in soil nitrogen supply to about 44–200 kg N/ha per year (Soderlund and Roswall 1982). Nitrogen fixation contributes to nitrogen balance in the plant and increases nitrogenase activity in inoculated roots. Associative and free-living nitrogen-fixing bacteria are important inoculators of non-legume plants, especially cereals (Kader et al. 2002; Rodrigues et al. 2015; Kour et al. 2020). Free-living bacteria can stabilize up to about 15–20 kg N/ha per year and associative bacteria up to 20–40 kg N/ha per year (Jaga and Singh 2010; Kour et al. 2020). The amount of nitrogen stabilized by these bacteria can be effective in maintaining long-term soil fertility. Although native populations of these bacteria are present in the soil, they may not be effective in nitrogen fixation. Therefore, efficient and effective strains of these bacteria are usually used as biofertilizers. Research has shown that the process of nitrogen fixation by microorganisms requires energy derived from available organic carbon. For this reason, the application of green, organic, and some chemical fertilizers is effective in the nitrogen fixation process.

7.3.1.2 Improvement of the Nutrient Bio-availability

A variety of microorganisms, including bacteria and fungi, can improve the bio-availability of macro- and micro-nutrients in the rhizosphere of the host plant (Pérez-Montaño et al. 2014). These microorganisms act through the solubilization of inaccessible forms of nutrients or the production of ionophores, including siderophores (Richardson et al. 2009; Rashid et al. 2016).

Deficiency of the phosphorus after nitrogen is the most vital element that limits plant growth. Most soils have large reserves of total phosphorus, but plant-available phosphorus is often a small portion of this total phosphorus (Pérez-Montaño et al. 2014). The plants can absorb only two absorbable forms of mono-hydrogen phosphate ($H_2PO_4^-$) and di-hydrogen phosphate (HPO_4^{2-}). Phosphate-solubilizing bacteria with the secretion of organic acids and phosphatases are the most common biological methods that facilitate the conversion of non-absorbable phosphorus into plant-absorbable forms (Rodriguez et al. 2004; Richardson et al. 2009; Rashid et al. 2016). In addition, the interaction of plants with mycorrhizal fungi reduces the adverse effects of nutrient deficiency and drought and salinity stresses (Nasto et al. 2014). In this type of relationship, the fungus in response to receiving organic matter from the host plant increases the absorption of nutrients and plant resistance to environmental stresses, diseases, and pests. Mycorrhizal fungi with a wide hyphal network increase the efficiency of plants in absorbing water and nutrients, specifically immobile elements of phosphorus, zinc, and copper (Kour et al. 2020). Studies show that the roots of mycorrhizal plants can use insoluble sources of phosphorus in soil that is not available to plant roots (Ozgonen and Erkilic 2007). The results of a study by Gyaneshwar et al. (2002) showed that mycorrhizal fungi are an inexpensive and efficient solution to increase the agronomic efficiency of phosphate rock. Also, this biological relationship improves the uptake of nitrogen, potassium, and magnesium in poor soils.

One of the important methods of supplying potassium required by plants is potassium-solubilizing microorganisms (KSM) such as bacteria and fungi. The KSM improve the solubility of K-bearing minerals in the soil and the availability of potassium to plants by producing organic and inorganic acids (e.g., gluconic, α-ketogluconic, oxalic, citric, acetic, citric, succinic, and nitric acids) (Etesami et al. 2017; Yadav 2017). Among the KSM in soil are *Pseudomonas*, *Bacillus*, and *Klebsiella* bacteria (Lian et al. 2002; Etesami et al. 2017; Yadav 2017) and *Penicillium* and *Aspergillus* fungi (Meena et al. 2016; Pandey et al. 2020).

Iron is an essential element for plant growth, and its deficiency has been reported in calcareous soils. Plant roots prefer to absorb iron in reduced form (Fe^{2+}), but ferric iron (Fe^{3+}) is more common in calcareous soils, which is easily converted to the form of precipitated iron oxides (Kraemer 2004; Gontia-Mishra et al. 2016; Scavino and Pedraza 2013). Some plants typically secrete organic compounds (phytosiderophores) that bind to iron (Fe^{3+}) and increase its absorption. Phytosiderophores transfer ferric iron to the root surface where it is reduced to Fe^{2+} and absorbed more rapidly by the plant (Scavino and Pedraza 2013). The production of chelating compounds (siderophores) by microorganisms to improve the availability of iron was demonstrated by Crowley et al. (1991) and Radzki et al. (2013), which improved the uptake of iron in plants. It has been shown that several plant species can absorb the siderophore-iron III complex. This role of microorganisms is more important in calcareous soils. Unlike phytosiderophores, produced and secreted by only a few monocot plants belonging to the Gramineae family, microbial siderophore production ability has been proven by a very wide range of fungi and bacteria (Radzki et al. 2013; Mehnaz 2013). Although siderophore production capacity is present in all aerobic and facultative anaerobic bacteria and fungi, the production potential of these substances is very different in different microbial species and even in different strains within each species. The concentration of bacterial and fungal siderophores in the soil is between 4–300 and 30–240 μmole per gram of soil, respectively (Barton and Hemming 1993).

7.3.2 Phytostimulation

7.3.2.1 Production of Plant Growth Regulators

Soil microorganisms can increase plant growth and yield by synthesizing various plant growth regulators (PGRs) (Bloemberg and Lugtenberg 2001; Pérez-Montaño et al. 2014; Enespa and Chandra 2020). The PGRs are organic substances that affect the physiological processes of plants in very low concentrations. Because the concentration of PGRs is an indicator of the regulation of plant physiological processes, slight changes in the amount of PGRs can lead to changes in plant growth characteristics. The most important PGRs are auxins, gibberellins, cytokines, abscisic acid, and ethylene (Bloemberg and Lugtenberg 2001; Glick 2012; Emami et al. 2019). Microorganisms by producing various PGRs can increase root length and area and consequently increase plant growth and yield. A wide range of bacterial (*Pseudomonas*, *Enterobacter*, *Bacillus*, *Paenibacillus*, *Rhizobium*, *Pantoea*, and *Azospirillum*) and fungal (*Trichoderma*, *Penicillium*, and *Fusarium*) genera can produce cytokines, gibberellic acid, and indole acetic acid (Bottini et al. 2004; Glick 2012; Gowtham et al. 2017; Meena et al. 2017; Emami et al. 2019; Turaeva et al. 2020).

7.3.2.2 Production of ACC Deaminase

One of the valuable mechanisms in the plant-microorganism relationship, especially in terms of environmental stress, is related to ethylene. Ethylene is a potent regulator of plant growth that affects plant metabolism (Van de Poel et al. 2015). Ethylene in the plant can stimulate or inhibit growth, depending on the concentration, type of physiological processes, and growth stage of the plant (Glick 2012; Van de Poel et al. 2015; Nath et al. 2017). Low-level ethylene synthesis increases initial growth and root development, but higher levels of ethylene inhibit longitudinal root growth. Some bacterial species can reduce ethylene levels in plants by producing the 1-aminocyclopropane-1-carboxylate (ACC) *deaminase* enzyme (Glick 2012; Nath et al. 2017; Meena et al. 2017; Bharti and Barnawal 2018). These bacteria reduce ACC levels by producing ACC *deaminase* and hydrolyzing ACC for nitrogen supply (Glick 2012; Meena et al. 2017; Bharti and Barnawal 2018; Lobo et al. 2019). The ACC is a precursor to ethylene production. With this action, the amount of ethylene around the plant roots does not exceed a certain amount, and the growth of roots and plants is improved. In addition, the production of auxin in bacteria activates the synthesis pathway of the ACC *deaminase* enzyme, so that it can degrade the ethylene precursor. The results of an experiment on the maize (*Zea mays* L.) plant showed that inoculation with some strains of *Pseudomonas* resulted in a significant increase in height, root weight, and total maize biomass compared to the control. It seems that these strains increase the root growth of the plant by reducing the inhibition of ethylene in the roots, and as a result, with the improvement of root growth, yield and stem growth also increase (Shaharoona et al. 2006).

7.3.3 Biocontrol

7.3.3.1 Production of Antibiotics

Bacteria belonging to the genera *Pseudomonas* and *Bacillus* play an active role in suppressing and reducing the population of pathogenic microorganisms (Meena et al. 2017). The production of well-known antibiotics such as subtilin, subtilosin A, sublancin (ribosomal synthesis), and bacilysin, chlorotetain, mycobacillin, rhizocticin, and difficidin, surfactin, iturin and fengycin (nonribosomal peptide synthesis and polyketide synthase) by *Bacillus* strains reduce the growth of pathogenic microorganisms (Leclère et al. 2005; Goswami et al. 2016). The most famous antibiotic-producing *Bacillus* strains are *B. subtilis* 168 and *B. amyloliquefaciens* FZB42 (Goswami et al. 2016). Among the most famous strains of the antibiotic-producing *Pseudomonas* strains are *P. fluorescens* and *P. aeruginosa*. These species can produce the antibiotics amycin A, azomycin, butyrolactone, 2,4-diacetylphloroglucinol, ecomycin, kanosamine, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, pseudomonic acid, rhamnolipids, and viscosinamide (Goswami et al. 2016).

7.3.3.2 Cell Wall-Degrading Enzymes

Microorganisms destroy pathogens by producing cell wall-degrading enzymes such as chitinase, glucanase, cellulase, and protease (Neeraja et al. 2010; Pérez-Montaño et al. 2014). The chitinase enzyme breaks down the insoluble linear polymer β-1,4-N-acetyl-glucosamine, a major component of fungal cell walls (Neeraja et al. 2010; Goswami et al. 2016). Gram-positive bacteria with chitinolytic activity include *Bacillus licheniformis*, *B. cereus*, *B. circulans*, *B. subtilis*, and *B. thuringiensis*, and gram-negative bacteria include *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter agglomerans*, and *P. fluorescens* (Shrestha et al. 2015; Goswami et al. 2016; Chalotra et al. 2019). β-1,3-Glucanase produced by *Paenibacillus* and *Streptomyces* easily degrades the cell wall of the pathogenic fungus *Phytophthora capsici*, *Rhizoctonia solani*, and *Fusarium oxysporum* (Park et al. 2012; Compant et al. 2005). Also, *Burkholderia cepacia* T1A-2B and *Pseudomonas* sp. T4B-2A strongly inhibit the growth of some pathogenic fungi *Rhizoctonia solani* and *Sclerotium rolfsii* (De Curtis et al. 2010).

7.3.3.3 Production of Siderophore

Different genera of bacteria and fungi can produce siderophore. But the well-known genus is *Pseudomonas* (Meena et al. 2017). *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* produce various siderophores such as pseudobactin, pyochelin, and pyoverdine. Rhizospheric bacteria usually release these compounds to increase their competitive potential (Hillel 2008; Gontia-Mishra et al. 2016; Lamont et al. 2017; Meena et al. 2017). In addition, these compounds also improve plant iron nutrition. In general, siderophore-producing microorganisms through (a) improving the iron nutrition, (b) restricting the growth of other microorganisms through antibiotic molecules released by these bacteria, and (c) preventing the growth of pathogenic microorganisms, especially fungi, by limiting their access to iron increase the health of plants (Lamont et al. 2017; Meena et al. 2017).

7.3.3.4 Hydrogen Cyanide Production

Hydrogen cyanide (HCN) biosynthesis has been reported in both eukaryotes and prokaryotes. Among prokaryotes, the production of hydrogen cyanide is more common in proteobacteria. Its production has been reported in fluorescent *Pseudomonas* (such as *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Pseudomonas protegens*), *Chromobacterium violaceum*, some rhizobia, and other bacteria (Blumer and Haas 2000; Rudrappa et al. 2008; Ramette et al. 2011). HCN is a toxic substance and controls the population of pathogenic microorganisms. It has a positive effect in inhibition of root-knot and black rot in tomato and tobacco roots created by *Meloidogyne javanica* and *Thielaviopsis basicola*, respectively (Siddiqui 2006).

7.3.3.5 Induced Systemic Resistance

Studies show that inoculation of plants with some bacteria, such as *Pseudomonas*, increases the resistance of host plants' immune systems and prevents the contamination of plant tissues with pathogenic microorganisms. In *Arabidopsis*, carnation, and radish plants, *Pseudomonas* spp. have the potential to induce systemic resistance. In this condition, "O antigenic side chain" of the bacterial outer membrane lipopolysaccharides plays the role of inducing determinant. The pseudobactin siderophores induce systemic resistance in *Arabidopsis* and tobacco. Also, pseudomonine siderophores induce salicylic acid production in radish, which subsequently strengthens the plant's immune system (Van Loon and Bakker 2006; Goswami et al. 2016).

7.3.4 Multiple Mechanisms of Action

According to scientific findings, multiple mechanisms are responsible for increasing the growth and yield of inoculated plants with soil microbial inoculants. In addition to improving nutrients' bio-availability, production of hormones by microorganisms in the rhizosphere, production of ACC deaminase, control of plant pathogens, and production of siderophore are among the mechanisms to improve productivity and quality of agricultural crops (Table 7.1). For example, phosphate-solubilizing microorganisms, in addition to organic and inorganic phosphate solubilization, increase plant growth via different mechanisms (i.e., the enhancement of the micronutrients' bio-availability, the production and secretion of metabolites, and the improvement of N_2 fixation). In fact, in some cases, a particular microorganism exhibits several mechanisms. Antoun et al. (1998) showed that out of 266 *Rhizobium* and *Bradyrhizobium* species, 83% produced siderophore, 58% produced IAA, 54% dissolved insoluble phosphate, and 11% had destructive effects on the plant growth.

Table 7.1 Plant growth-promoting traits of beneficial microorganisms

Isolates	Plant growth-promoting traits	References
Alcaligenes sp. B16	IAA production Siderophore production nifH gene presence	Devi et al. (2018)
Arthrobacter chlorophenolicus BHU3	IAA production Phosphate solubilization N_2 fixation HCN production	Kumar et al. (2021)
Bacillus sp. M7c	Phosphate solubilization	Guiñazú et al. (2010)
Bacillus megaterium BHU1	IAA production Siderophore production Phosphate solubilization N_2 fixation	Kumar et al. (2021)
Bacillus thuringiensis C25	β-1,3-Glucanase activity Protease activity Chitinase activity	Shrestha et al. (2015)
Bradyrhizobium japonicum TAL 377	IAA production Gibberellic acid production	Afzal et al. (2010)
Enterobacter spp. BHU5	IAA production Siderophore production N_2 fixation HCN production	Kumar et al. (2021)
Enterobacter ludwigii HG 2	IAA production Siderophore production Phosphate solubilization Potassium solubilization Zinc solubilization Mercury tolerant ACC deaminase activity	Gontia-Mishra et al. (2016)
Fusarium moniliforme UzGC-12	IAA production Gibberellic acid production	Turaeva et al. (2020)
Klebsiella pneumoniae HG 3	IAA production Siderophore production Phosphate solubilization Exopolysaccharide secretion Potassium solubilization Zinc solubilization Mercury tolerant ACC deaminase activity	Gontia-Mishra et al. (2016)
Mesorhizobium ciceri SRC8	IAA production Siderophore production Phosphate solubilization Exopolysaccharide secretion Organic acid production	Ullah et al. (2017)
Paenibacillus sp. B1	IAA production Phosphate solubilization	Li et al. (2020)
Paenibacillus polymyxa BHU2	IAA production Phosphate solubilization N_2 fixation	Kumar et al. (2021)

(continued)

Table 7.1 (continued)

Isolates	Plant growth-promoting traits	References
Penicillium canescens UzCF-54	IAA production Gibberellic acid production	Turaeva et al. (2020)
Pseudomonas sp. 54RB	IAA production Phosphate solubilization Gibberellic acid production	Afzal et al. (2010)
Pseudomonas sp. 19Fv1T	IAA production Siderophore production Phosphate solubilization	Bona et al. (2017)
Pseudomonas spp.	IAA production Siderophore production Phosphate solubilization	Emami et al. (2020)
Pseudomonas spp.	Chitinase activity Lipase activity Protease activity 2,4-Diacetylphloroglucinol production	Chalotra et al. (2019)
Pseudomonas sp. FM7d	Phosphate solubilization	Guiñazú et al. (2010)
Pseudomonas aeruginosa (strains B4, B23, B25, and B35)	IAA production Siderophore production HCN production Chitinase activity (B4, B35)	Devi et al. (2018)
Pseudomonas alkylphenolica PF9	Tricalcium phosphate (Ca3HPO4) solubilization	Farssi et al. (2021)
Pseudomonas mosselii E240	IAA production Siderophore production Phosphate solubilization	Emami et al. (2020)
Rhizobium leguminosarum SRL5	IAA production Siderophore production Phosphate solubilization Exopolysaccharide secretion Organic acid production	Ullah et al. (2017)
Serratia marcescens BHU4	IAA production Siderophore production Phosphate solubilization N_2 fixation HCN production	Kumar et al. (2021)
Serratia marcescens B8	IAA production Siderophore production Chitinase activity HCN production nifH gene presence	Devi et al. (2018)
Sinorhizobium meliloti Rm41	Phosphate solubilization	Farssi et al. (2021)
Streptomyces torulosus PCPOK-0324	1,3-β-D-Glucanase activity	Park et al. (2012)
Trichoderma harzianum UzCF-55	IAA production Gibberellic acid production	Turaeva et al. (2020)

IAA indole-3-acetic acid, *HCN* hydrogen cyanide

Also, Emami et al. (2019) reported that 88%, 59.2%, 38%, 24%, and 18% of the rhizospheric isolates had the ability of IAA and siderophore production, solubilization of organic and inorganic phosphate, and HCN production, respectively. Co-inoculation of mung bean (*Vigna radiata* L.) with *Rhizobium* (N_2 fixer) and *Bacillus* sp. (phosphate solubilizer) enhanced inoculated plant growth not only due to their phosphate solubilization and N_2 fixation abilities but also due to their efficiency in IAA production (Qureshi et al. 2011). Production of IAA by bacteria changed plant IAA level and increased root properties and root nodulation. Likewise, Figueiredo et al. (2008) showed that production of cytokinin by *Paenibacillus polymyxa* DSM 36 (phosphate solubilizer) affects nitrogen fixation in roots of common bean (*Phaseolus vulgaris* L.) plants by stimulating rhizobial growth and improving the number of infections in roots and nodule formation.

7.4 Effect of Soil Microbial Consortia on Productivity and Quality of Agricultural Crops

Various greenhouse and field studies have confirmed the effectiveness of microbial consortia on plant growth and crop yields to reduce the level of chemical fertilizer use and reduce environmental problems caused by agricultural activities. These microbial consortia show beneficial effects on productivity and quality of agricultural crops, and different types of them have been commercialized for use in agriculture. The synergistic effect between nitrogen fixer and phosphate solubilizer bacteria enhances fixed nitrogen level and efficiency of nutrients' use. This fact was confirmed by Belimov et al. (1995), Wani et al. (2007)), and Li et al. (2020). Wani et al. (2007) reported that co-inoculation of chickpea plants (*Cicer arietinum* L.) by phosphate-solubilizing bacteria (*Pseudomonas* or *Bacillus*) and two N_2-fixing bacteria (*Mesorhizobium cicero* and *Azotobacter chroococcum*) improved soil nutrient availability (N and P), raised root nodulation, and enhanced plant growth compared to single inoculation. Similarly, co-inoculation of faba bean (*Vicia faba* L.) plants with *Rhizobium* (N_2 fixer) and *Bacillus megaterium* (phosphate solubilizer) improved seed quality and yield of plant (Rugheim and Abdelgani 2012). Co-inoculation of mung bean plants by *Bacillus* strains (*B. aryabhattai* S10 and *B. subtilis* ZM63) improved plant growth, nutritional status, and nodulation of plants (Ahmad et al. 2019). In another way, PGP rhizospheric bacteria act synergistically with PGP endophytic bacteria. Emami et al. (2020) reported that wheat plants treated with *Pseudomonas* sp. R185 (rhizospheric phosphate solubilizer) and *Pseudomonas mosselii* E240 (endophytic phosphate solubilizer) exhibited an increase in plant height, shoot dry weight, root dry weight, and grain yield, compared to the control plants. Mycorrhizal fungi can interact beneficially with symbiotic, associative, and free-living N_2 fixer bacteria, phosphate solubilizer bacteria, and other beneficial microorganisms and enhance their positive effects on plants. Bacteria around the roots can develop mycorrhizal symbiosis by producing auxin and other hormones. Mycorrhizal fungi can also increase root secretion and consequently increase soil microbial population. *Glomus fasciculatum* in association with *Mesorhizobium ciceri* (N_2 fixers) and *Serratia* sp. (phosphate solubilizer) through different mechanisms enhance legume plant growth (Zaidi et al. 2010). Table 7.2 shows the effect of soil microbial consortia on the productivity and quality of agricultural crops.

7.5 Future Considerations and Conclusion

Today, fertilizers are widely used as a tool to increase the quantity of production, especially in developing countries, but it is necessary to pay attention to the quality of agricultural products in order to prevent the accumulation of nutrients and other pollutants in plant tissues and the destruction of valuable soil and water resources. Obviously, the use of biological fertilizers instead of chemical fertilizers can provide environmental health and sustainability of production resources and by improving the quality of food products can have a significant impact on promoting public health. Soil microbial consortia that contain several microorganisms with different PGP traits (such as nitrogen fixation, phosphate solubilization, siderophore production, hormone production, and their biocontrol properties against fungi and pathogens) are very effective. The use of soil microbial consortia is an effective way to overcome the problem of nutrient deficiencies and environmental stresses in plants, as well as environmental pollution. If the cost of biological fertilizers' production is reduced and the use efficiency of fertilizers is increased, the preference of biological fertilizers over chemical fertilizers is undoubtedly an expert and correct decision. In the first stage, achieving a microorganism with PGP ability and then achieving the responsible genes allows the researcher to effectively use from these biological capabilities in the farm. The effectiveness of soil microbial consortia obtained from researches should be evaluated in agricultural and horticultural fields. Different tests in different climatic conditions and in the presence of different host plants and monitoring the positive effects of these inoculants need to be performed before commercialization and mass production. In a general conclusion, it can be said that soil microbial consortia can increase the speed and the rate of plant growth and thus increase crop yield through synergistic effects for growth promoters and adverse impact for growth inhibitors.

Table 7.2 Effect of soil microbial consortia on the productivity and quality of agricultural crops

Plant	Microbial consortium	Effect	References
Alfalfa (*Medicago sativa* L.)	*Bacillus cereus* *Bacillus altitudinis* *Comamonas* *Stenotrophomonas maltophilia*	Increased plant growth and plant antioxidant enzyme activity	Shahzad et al. (2020)
	Pseudomonas alkylphenolica *Sinorhizobium meliloti*	Increased plant P content, plant height, plant fresh and dry biomass	Farssi et al. (2021)
Chickpea (*Cicer arietinum* L.)	*Mesorhizobium ciceri* *Azotobacter chroococuum* *Bacillus* sp.	Increased plant biomass and N and P uptake	Wani et al. (2007)
	Pseudomonas fluorescens *Mesorhizobium* sp.	Increased plant biomass, nodule number, and nutrient content	Verma et al. (2012)
	Pseudomonas aeruginosa *Trichoderma harzianum* *Mesorhizobium* sp.	Increased plant growth under the stress of the pathogen *S. rolfsii*	Singh et al. (2014)
Maize (*Zea mays* L.)	*Rhizoglomus irregulare* *Pseudomonas putida*	Increased maize productivity and P use efficiency	Pacheco et al. (2021)
	Azospirillum brasilense *Pseudomonas putida* *Acinetobacter* sp. *Sphingomonas* sp.	Increased plant growth and decreased the application of chemical fertilizer	Molina-Romero et al. (2021)
Potato (*Solanum tuberosum* L.)	*Pseudomonas aeruginosa* *Serratia marcescens* *Alcaligenes faecalis*	Increased plant growth and antifungal activities	Devi et al. (2018)
Rice (*Oryza sativa* L.)	*Glomus intraradices* *Azospirillum brasilense*	Increased rice growth and physiological traits under well-watered and drought conditions	Ruíz-Sánchez et al. (2011)
	Pseudomonas putida *Chlorella vulgaris*	Decreased the accumulation of arsenic and improved the level of nutrient elements in rice	Awasthi et al. (2018)

(continued)

Table 7.2 (continued)

Plant	Microbial consortium	Effect	References
	Burkholderia ubonensis *Burkholderia vietnamiensis* *Citrobacter bitternis*	Increased the productivity of rice and decreased the use of nitrogen fertilizer by up to 25%	Ríos-Ruiz et al. (2020)
Sunflower (*Helianthus annuus* L.)	*Rhizophagus irregularis* *Chryseobacterium humi* *Ochrobactrum haematophilum*	Increased biomass production and accumulation of nutrient (N, P, K, Ca, Mg) and decreased Na content in plant tissue in saline condition	Pereira et al. (2016)
Tomato (*Solanum lycopersicum* L.)	*Rhizophagus intraradices* *Glomus aggregatum* *Glomus viscosum* *Claroideoglomus etunicatum* *Claroideoglomus claroideum* *Pseudomonas fluorescens* *Pseudomonas* sp.	Increased yield and quality (the level of sugars and vitamins) of tomato	Bona et al. (2017)
Wheat (*Triticum aestivum* L.)	Photosynthetic microbial consortium	Decreased about 50% of the nitrogen chemical fertilization when combined with the biofertilizer	Ramírez-López et al. (2019)
	Pseudomonas sp. R185 *Pseudomonas mosselii* E240	Increased plant height, shoot dry weight, root dry weight, and grain yield, in wheat cultivars	Emami et al. (2020)
	Bacillus megaterium *Arthrobacter chlorophenolicus* *Enterobacter* sp. *Pseudomonas aeruginosa*	Increased plant health, yield, and nutrient content	Kumar et al. (2021)

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Afzal A, Bano A, Fatima M (2010) Higher soybean yield by inoculation with N-fixing and P-solubilizing bacteria. Agron Sustain Dev 30(2):487–495

Ahmad M, Adil Z, Hussain A, Mumtaz MZ, Nafees M, Ahmad I, Jamil M (2019) Potential of phosphate solubilizing bacillus strains for improving growth and nutrient uptake in mung bean and maize crops. Pak J Agri Sci 56:283–289

Antoun H, Beauchamp J, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth-promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67

Awasthi S, Chauhan R, Dwivedi S, Srivastava S, Srivastava S, Tripathi RD (2018) A consortium of alga (*Chlorella vulgaris*) and bacterium (*Pseudomonas putida*) for amelioration of arsenic toxicity in rice: a promising and feasible approach. Environ Exp Bot 150:115–126

Ayangbenro AS, Babalola OO (2020) Reclamation of arid and semi-arid soils: the role of plant growth-promoting archaea and bacteria. Curr Plant Biol 25:100173

Barton LL, Hemming BC (1993) Iron chelating in plants and soil microorganisms. Academic Press, San Diego, CA

Belimov AA, Kojemiakov AP, Chuvarliyeva CN (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate solubilizing bacteria. Plant Soil 173:29–37

Bharti N, Barnawal D (2018) Amelioration of salinity stress by PGPR: ACC deaminase and ROS scavenging enzymes activity. In: Singh AK, Kumar A, Singh PK (eds) PGPR amelioration in sustainable agriculture: food security and environmental management. Woodhead Publishing, Duxford/Kidlington, pp 85–106

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Blumer C, Haas D (2000) Iron regulation of the hcnABC genes encoding hydrogen cyanide synthase depends on the anaerobic regulator ANR rather than on the global activator GacA in *Pseudomonas fluorescens* CHA0. Microbiology 146(10):2417–2424

Boddey RM, Baldani VL, Baldani JI, Döbereiner J (1986) Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field grown wheat. Plant Soil 95:109–121

Bona E, Cantamessa S, Massa N, Manassero P, Marsano F, Copetta A, Lingua G, D'Agostino G, Gamalero E, Berta G (2017) Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza 27(1):1–1

Bottini R, Cassán F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503

Chalotra RA, Mallick SA, Gupta MO, Sharma D, Gupta SA (2019) Production of cell wall degrading enzymes and antibiotic by Pseudomonads for assessing their biocontrol potential. Indian J Agric Sci 89(6):994–997

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Crowley DE, Wang YC, Reid CP, Szaniszlo PJ (1991) Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant Soil 130:179–198

da Silveira AP, Sala VM, Cardoso EJ, Labanca EG, Cipriano MA (2016) Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation. Appl Soil Ecol 107: 313–319

De Curtis F, Lima G, Vitullo D, De Cicco V (2010) Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. Crop Prot 29(7):663–670

de Salamone IG, Döbereiner J, Urquiaga S, Boddey RM (1996) Biological nitrogen fixation in *Azospirillum* strain-maize genotype associations as evaluated by the 15N isotope dilution technique. Biol Fertil Soils 23:249–256

de Souza Gênero JF, Messa VR, Beladeli MN, da Costa AC, Júnior JB (2020) Rhizosphere nitrogen-fixing bacteria (free-living) contribute to nitrogen absorption in wheat. Rhizosphere 16:100245

Demba Diallo M, Willems A, Vloemans N, Cousin S, Vandekerckhove TT, De Lajudie P, Neyra M, Vyverman W, Gillis M, Van der Gucht K (2004) Polymerase chain reaction denaturing gradient gel electrophoresis analysis of the N_2-fixing bacterial diversity in soil under *Acacia tortilis* ssp. *raddiana* and *Balanites aegyptiaca* in the dryland part of Senegal. Environ Microbiol 6(4): 400–415

Devi AR, Sharma GD, Majumdar PB, Pandey P (2018) A multispecies consortium of bacteria having plant growth promotion and antifungal activities, for the management of Fusarium wilt complex disease in potato (*Solanum tuberosum* L.). Biocatal Agric Biotechnol 16:614–624

Di Salvo LP, Cellucci GC, Carlino ME, de Salamone IE (2018) Plant growth-promoting rhizobacteria inoculation and nitrogen fertilization increase maize (*Zea mays* L.) grain yield and modified rhizosphere microbial communities. Appl Soil Ecol 126:113–120

Dowling DN, O'Gara F (1994) Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends Biotechnol 12:133–141

Duc L, Noll M, Meier BE, Bürgmann H, Zeyer J (2009) High diversity of diazotrophs in the fore field of a receding alpine glacier. Microb Ecol 57(1):179–190

Emami S, Alikhani HA, Pourbabaei AA, Etesami H, Motashare Zadeh B, Sarmadian F (2018) Improved growth and nutrient acquisition of wheat genotypes in phosphorus deficient soils by plant growth-promoting rhizospheric and endophytic bacteria. Soil Sci Plant Nutr 64(6): 719–727

Emami S, Alikhani HA, Pourbabaei AA, Etesami H, Sarmadian F, Motessharezadeh B (2019) Effect of rhizospheric and endophytic bacteria with multiple plant growth promoting traits on wheat growth. Environ Sci Pollut Res 26(19):19804–19813

Emami S, Alikhani HA, Pourbabaee AA, Etesami H, Motasharezadeh B, Sarmadian F (2020) Consortium of endophyte and rhizosphere phosphate solubilizing bacteria improves phosphorous use efficiency in wheat cultivars in phosphorus deficient soils. Rhizosphere 14:100196

Enespa JP, Chandra P (2020) Halophilic microbes from plant growing under the hypersaline habitats. Plant Microb Sustain Agric 25:317

Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Saf 156:225–246

Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects a review. J Soil Sci Plant Nutr 17(4):897–911

Farssi O, Saih R, El Moukhtari A, Oubenali A, Mouradi M, Lazali M, Ghoulam C, Bouizgaren A, Berrougui H, Farissi M (2021) Synergistic effect of *Pseudomonas alkylphenolica* PF9 and *Sinorhizobium meliloti* Rm41 on Moroccan alfalfa population grown under limited phosphorus availability. Saudi J Biol Sci 28:3870–3879

Figueiredo MV, Martinez CR, Burity HA, Chanway CP (2008) Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World J Microbiol Biotechnol 24:1187–1193

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012: 963401

Glick BR (2015) Introduction to plant growth-promoting bacteria. Beneficial plant-bacterial interactions. Springer, Cham, pp 1–28

Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016) Alleviation of mercury toxicity in wheat by the interaction of mercury-tolerant plant growth-promoting rhizobacteria. J Plant Growth Regul 35:1000–1012

Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food Agric 2:1–19

Gowtham HG, Duraivadivel P, Hariprasad P, Niranjana SR (2017) A novel split-pot bioassay to screen indole acetic acid producing rhizobacteria for the improvement of plant growth in tomato (*Solanum lycopersicum* L.). Sci Hortic 224:351–357

Guiñazú LB, Andrés JA, Del Papa MF, Pistorio M, Rosas SB (2010) Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*. Biol Fertil Soils 46(2):185–190

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. In: Food security in nutrient-stressed environments: exploiting plants' genetic capabilities. Springer, Netherlands, pp 133–143

Hillel M (2008) Balanced crop nutrition: fertilizing for crop and food quality. Turk J Agric For 32: 183–193

Jaga P, Singh V (2010) Effect of biofertilizer, nitrogen and sulphur on sorghum-mustard cropping system. In: Proceedings of National Seminar on Soil Security for Sustainable Agriculture held at College of Agriculture, Nagpur

Janisiewicz WJ, Bors B (1995) Development of a microbial community of bacterial and yeast antagonists to control wound-invading postharvest pathogens of fruits. Appl Environ Microbiol 61:3261–3267

Kader MA, Mian MH, Hoque MS (2002) Effects of *Azotobacter* inoculant on the yield and nitrogen uptake by wheat. J Biol Sci 2:259–261

Ke X, Feng S, Wang J, Lu W, Zhang W, Chen M, Lin M (2019) Effect of inoculation with nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the microbiome indigenous to the rhizosphere. Syst Appl Microbiol 42(2):248–260

Korir H, Mungai NW, Thuita M, Hamba Y, Masso C (2017) Coinoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front Plant Sci 8:141

Kour D, Rana KL, Yadav AN, Yadav N, Kumar M, Kumar V, Vyas P, Dhaliwal HS, Saxena AK (2020) Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal Agric Biotechnol 23:101487

Kraemer SM (2004) Iron oxide dissolution and solubility in the presence of siderophores. Aquat Sci 66:3–18

Kumar A, Maurya BR, Raghuwanshi R (2021) The microbial consortium of indigenous rhizobacteria improving plant health, yield and nutrient content in wheat (*Triticum aestivum*). J Plant Nutr 44:1942–1956

Lamont JR, Wilkins O, Bywater-Ekegärd M, Smith DL (2017) From yogurt to yield: potential applications of lactic acid bacteria in plant production. Soil Biol Biochem 111:1–9

Leclère V, Béchet M, Adam A, Guez JS, Wathelet B, Ongena M, Thonart P, Gancel F, Chollet-Imbert M, Jacques P (2005) Mycosubtilin overproduction by *Bacillus subtilis* BBG100 enhances the organism's antagonistic and biocontrol activities. Appl Environ Microbiol 71: 4577–4584

Li Y, Li Q, Guan G, Chen S (2020) Phosphate solubilizing bacteria stimulate wheat rhizosphere and endosphere biological nitrogen fixation by improving phosphorus content. Peer J 8:9062

Lian B, Fu P, Mo D, Liu C (2002) A comprehensive review of the mechanism of potassium releasing by silicate bacteria. Acta Mineral Sin 22:179–183

Lobo CB, Tomás MS, Viruel E, Ferrero MA, Lucca ME (2019) Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. Microbiol Res 219:12–25

Malik KA, Bilal R, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing plant-promoting rhizobacteria (PGPR) with kallar grass and rice. Plant Soil 194:37–44

Meena VS, Maurya BR, Verma JP, Meena RS (2016) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi, pp 1–20

Meena VS, Meena SK, Verma JP, Kumar A, Aeron A, Mishra PK, Bisht JK, Pattanayak A, Naveed M, Dotaniya ML (2017) Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. Ecol Eng 107:8–32

Mehnaz S (2013) Secondary metabolites of Pseudomonas aurantiaca and their role in plant growth promotion. In: Arora NK (ed) Plant microbe symbiosis: fundamentals and advances. Springer, New Delhi, pp 373–393

Molina-Romero D, Juárez-Sánchez S, Venegas B, Ortíz-González CS, Baez A, Morales-García YE, Muñoz-Rojas J (2021) A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. Front Sustain Food Syst 4:293

Mukhopadhyay AN (1996) Recent Innovations in Plant Disease Control by Eco Friendly Biopesticides, Presidential Address, (Agricultural Sciences), 83rd Annual Meeting Indian Science Congres Patiala India

Nandakumar S, Babu R, Viswanathan J, Sheela J, Raguchander R, Samiyappan R (2001) A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. Biol Control 46:493–510

Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC (2014) Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. Ecol Lett 17(10):1282–1289

Nath D, Maurya BR, Meena VS (2017) Documentation of five potassium- and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. Biocatal Agric Biotechnol 10:174–181

Neeraja C, Anil K, Purushotham P, Suma K, Sarma PV, Moerschbacher BM, Podile AR (2010) Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. Crit Rev Biotechnol 30:231–241

Numan M, Bashir S, Khan Y, Mumtaz R, Shinwari ZK, Khan AL, Khan A, Ahmed AH (2018) Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. Microbiol Res 209:21–32

Ozgonen H, Erkilic A (2007) Growth enhancement and Phytophthora blight (*Phytophthora capsici* Leonian) control by arbuscular mycorrhizal fungal inoculation in pepper. Crop Protect 26:1682–1688

Pacheco I, Ferreira R, Correia P, Carvalho L, Dias T, Cruz C (2021) Microbial consortium increases maize productivity and reduces grain phosphorus concentration under field conditions. Saudi J Biol Sci Jan 28:232–237

Pandey D, Kehri HK, Zoomi I, Singh U, Chaudhri KL, Akhtar O (2020) Potassium solubilizing microbes: diversity, ecological significances. Plant Microb Sustain Agric 25:263

Panneerselvam P, Senapati A, Sharma L, Nayak AK, Kumar A, Kumar U, Prabhukarthikeyan SR, Mitra D, Sagarika MS (2021) Understanding rice growth-promoting potential of *Enterobacter* spp. isolated from long-term organic farming soil in India through a supervised learning approach. Curr Res Microb Sci 2:100035

Park JK, Kim JD, Park YI, Kim SK (2012) Purification and characterization of a 1, 3-β-d-glucanase from *Streptomyces torulosus* PCPOK-0324. Carbohydr Polym 87(2):1641–1648

Pereira SI, Moreira H, Argyras K, Castro PM, Marques AP (2016) Promotion of sunflower growth under saline water irrigation by the inoculation of beneficial microorganisms. Appl Soil Ecol 105:36–47

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny MR, Jiménez-Guerrero I, López-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169(5–6):325–336

Pourbabaee AA, Khazaei M, Alikhani HA, Emami S (2021) Root nodulation of alfalfa by *Ensifer meliloti* in petroleum contaminated soil. Rhizosphere 17:100305

Qureshi MA, Shakir MA, Iqbal A, Akhtar N, Khan A (2011) Coinoculation of phosphate solubilizing bacteria and rhizobia for improving growth and yield of mungbean (*Vigna radiata* L.). J Anim Plant Sci 21:491–497

Radzki W, Mañero FG, Algar E, García JL, García-Villaraco A, Solano BR (2013) Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. Antonie Van Leeuwenhoek 104:321–330

Ramakrishna W, Yadav R, Li K (2019) Plant growth promoting bacteria in agriculture: two sides of a coin. Appl Soil Ecol 138:10–18

Ramette A, Frapolli M, Fischer-Le Saux M, Gruffaz C, Meyer JM, Défago G, Sutra L, Moënne-Loccoz Y (2011) *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. Syst Appl Microbiol 34(3):180–188

Ramírez-López C, Esparza-García FJ, Ferrera-Cerrato R, Alarcón A, Cañizares-Villanueva RO (2019) Short-term effects of a photosynthetic microbial consortium and nitrogen fertilization on soil chemical properties, growth, and yield of wheat under greenhouse conditions. J Appl Phycol 31:3617–3624

Rashid MI, Mujawar LH, Shahzad T, Almeelbi T, Ismail IM, Oves M (2016) Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiol Res 183:26–41

Raupach GS, Kloepper JW (1998) Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158–1164

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321: 305–339

Ríos-Ruiz WF, Torres-Chávez EE, Torres-Delgado J, Rojas-García JC, Bedmar EJ, Valdez-Nuñez RA (2020) Inoculation of bacterial consortium increases rice yield (*Oryza sativa* L.) reducing applications of nitrogen fertilizer in San Martin region, Peru. Rhizosphere 14:100200

Rodrigues AC, Bonifacio A, de Araujo FF, Junior MA, Figueiredo MD (2015) *Azospirillum* sp. as a challenge for agriculture. In: Maheshwari DK (ed) Bacterial metabolites in sustainable agroecosystem. Springer, Cham, pp 29–51

Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91(11):552–555

Rudrappa T, Splaine RE, Biedrzycki ML, Bais HP (2008) Cyanogenic pseudomonads influence multitrophic interactions in the rhizosphere. PLoS One 3(4):2073

Rugheim AM, Abdelgani ME (2012) Effects of microbial and chemical fertilization on yield and seed quality of faba bean (*Vicia faba*). Int Food Res J 19:417–422

Ruíz-Sánchez M, Armada E, Muñoz Y, de Salamone IE, Aroca R, Ruíz-Lozano JM, Azcón R (2011) *Azospirillum* and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. J Plant Physiol 168:1031–1037

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res:1–30

Scavino AF, Pedraza RO (2013) The role of siderophores in plant growth-promoting bacteria. In: Bacteria in agrobiology: crop productivity. Springer, Berlin, Heidelberg, pp 265–285

Shaharoona B, Arshad M, Zahir ZA, Khalid A (2006) Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38:2971–2975

Shahzad A, Bano A, Siddiqui S (2020) Effect of bacterial consortium on alfalfa (*Medicago sativa* L.) plant nutrient uptake and antioxidant enzymes at different levels of oily sludge. Isr J Plant Sci 67:158–170

Shirmohammadi E, Alikhani HA, Pourbabaei AA, Etesami H (2020) Improved phosphorus (P) uptake and yield of wheat fed with P fertilizer by drought-tolerant phosphate-solubilizing fluorescent pseudomonads strains: a field study in drylands. J Soil Sci Plant Nutr 20(4): 2195–2211

Shrestha A, Sultana R, Chae JC, Kim K, Lee KJ (2015) *Bacillus thuringiensis* C25 which is rich in cell wall degrading enzymes efficiently controls lettuce drop caused by *Sclerotinia minor*. Eur J Plant Pathol 142(3):577–589

Siddiqui ZA (2006) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui ZA (ed) PGPR: biocontrol and biocontrol. Springer, Netherlands, pp 112–142

Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB (2014) Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during *Sclerotium rolfsii* infection. Microbiol Res 169:353–360

Soderlund R, Roswall T (1982) The nitrogen cycle. In: Hutzinger J (ed) The handbook of environmental chemistry. The natural environment and the biogeochemical cycles, vol 1B. Springer Verlag, Berlin, pp 60–81

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677

Turaeva B, Soliev A, Eshboev F, Kamolov L, Azimova N, Karimov H, Zukhritdinova N, Khamidova K (2020) The use of three fungal strains in producing of indole-3-acetic acid and gibberellic acid. Plant Cell Biotechnol Mol Biol 21:32–43

Ullah S, Khan MY, Asghar HN, Akhtar MJ, Zahir ZA (2017) Differential response of single and co-inoculation of *Rhizobium leguminosarum* and *Mesorhizobium ciceri* for inducing water deficit stress tolerance in wheat. Ann Microbiol 67(11):739–749

Van de Poel B, Smet D, Van Der Straeten D (2015) Ethylene and hormonal cross talk in vegetative growth and development. Plant Physiol 169:61–72

Van Loon LC, Bakker PA (2006) Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 39–66

Verma JP, Yadav J, Tiwari KN (2012) Enhancement of nodulation and yield of chickpea by co-inoculation of indigenous *Mesorhizobium* spp. and Plant Growth–Promoting Rhizobacteria in Eastern Uttar Pradesh. Commun Soil Sci Plant Anal 43(3):605–621

Wani PA, Khan MS, Zaidi A (2007) Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. J Plant Nutr Soil Sci 170:283–287

Yadav AN (2017) Agriculturally important microbiomes: biodiversity and multifarious PGP attributes for amelioration of diverse abiotic stresses in crops for sustainable agriculture. Biomed J Sci Tech Res 1:1–4

Zai XM, Fan JJ, Hao ZP, Liu XM, Zhang WX (2021) Effect of co-inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing fungi on nutrient uptake and photosynthesis of beach palm under salt stress environment. Sci Rep 11(1):1–11

Zaidi A, Khan MS, Rizvi A, Saif S, Ahmad B, Shahid M (2010) Role of phosphate-solubilizing bacteria in legume improvement, in microbes for legume improvement. Springer, Cham, pp 175–197

Zhou Y, Bao J, Zhang D, Li Y, Li H, He H (2020) Effect of heterocystous nitrogen-fixing cyanobacteria against rice sheath blight and the underlying mechanism. Appl Soil Ecol 153: 103580

Zhu RF, Tang FL, Liu JL, Liu FQ, Deng XY, Chen JS (2016) Co-inoculation of arbuscular mycorrhizae and nitrogen fixing bacteria enhance alfalfa yield under saline conditions. Pak J Bot 48(2):763–769

Chapter 8
Consortia of Probiotic Bacteria and Their Potentials for Sustainable Rice Production

M. A. Baset Mia, Afrina Momotaj, and Tofazzal Islam

Abstract The application of plant probiotic bacteria for a sustainable environment-friendly agriculture system has created new avenues with integration of modern technology and eco-friendly management approaches. With a goal of using minimum chemical fertilizer and getting sustainable yield in cereal grains, especially of the rice crop, biological alternatives, such as biofertilizers, are inevitable for achieving sustainable development goal 2 (SDG-2). Rice, as the prime and staple food of Asian countries, also requires careful fertilization which is expensive and hazardous to the environment. The root and rhizosphere of rice harbor a plethora of probiotic bacteria belonging to diverse taxonomic groups. The use of alternative approaches especially the formulation and application of biofertilizer using the identified elite strains of probiotic bacteria is a blooming technology that can create a sustainable and environment-friendly rice production system. And consequently, significant developments have been achieved in using biofertilizers for rice cultivation throughout the world. Among the biofertilizer technologies, the majority of them are occupied by plant-associative or free-living bacteria under the genera of *Rhizobium*, *Bacillus*, *Paraburkholderia*, *Delftia*, *Pseudomonas*, *Lysobacter*, *Azotobacter*, *Azospirillum*, *Enterobacter*, *Brevibacterium*, etc. A large body of literature is available on the development of biofertilizers that are capable of steady supply of N through fixation of atmospheric nitrogen and solubilization of essential nutrient elements in the rhizosphere, and provide other benefits to partially supplement the use of chemical fertilizers. It appears that the application of probiotic bacterial

---

M. A. B. Mia
Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

A. Momotaj
Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

T. Islam (✉)
Institute of Biotechnology and Genetic Engineering (IBGE), Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh
e-mail: tofazzalislam@bsmrau.edu.bd

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_8

consortium as biofertilizer could be a sustainable approach for rice production. This chapter reviews and updates the isolation and identification of probiotic bacteria, and the effects of their consortium as well as their mode of beneficial effects for sustainable rice production systems.

Keywords Biofertilizers · Plant growth-promoting rhizobacteria · Plant probiotics · N_2 fixation · Sustainable development

8.1 Introduction

Rice is the principal food crop as well as the staple diet of more than three billion people throughout the world. It is interesting to note that more than 100 countries are growing rice across diversified climatic conditions (Abdullah et al. 2006; Skamnioti and Gurr 2009; Hosseyni and Soltani 2013). Among the rice-consuming peoples in the world, around half of Asia's people rely upon rice as their daily principal meal source (Hosseyni and Soltani 2013; Muthayya et al. 2014; Soltani et al. 2013). The major Asian rice-producing countries are China, India, Bangladesh, Indonesia, Thailand, the Philippines, Vietnam, and Myanmar accounting for approximately 90% of the world's total rice production (Khush 2005; Abdullah et al. 2006; Muthayya et al. 2014; WEF 2022). It is also the maximum crucial meal grain withinside the diets of loads of millions of Asians, Africans, and Latin Americans living in the tropics and subtropics (Yoshida 1981). Rice production is a high input requiring system that requires a handsome amount of money which is a burden for ordinary and marginal farmers.

Generally, production of rice requires three major inputs, namely, water, fertilizer, and pesticides. Growth and development of rice plants are very crucial and often limit the process due to a lack of an adequate supply of water and fertilizers. Among the fertilizers applied for rice cultivation, the N fertilizer is the most important, which frequently limits the growth and development of the rice plant. To increase rice yield in both upland and lowland rice culture systems, a large amount of N fertilizer is required. Keeping this process continuous, of course, using chemical fertilizer is expensive and hazardous to the environment as well (Hirt 2020). As a result, farmers are facing a serious economic problem, and consequently, they are moving their focus from rice production to another enterprise. Hence, an alternative approach is urgently required for boosting the rice production system in the world, especially the resource-poor marginal farming system. In this regard, the use of biofertilizer prepared from the elite strains of plant probiotic bacteria is considered one of the alternative and renewable natural resources from both nutrition and protection of rice plants (Khan et al. 2017; Rahman et al. 2018; Hirt 2020). Application of a suitable plant growth-promoting bacterium or a consortium of compatible living bacteria increases the growth and yield of rice plants with suppression of disease and pests (Trân Van et al. 2000; Islam et al. 2005; Rahman et al. 2018). These beneficial probiotic bacteria can be either rhizospheric or endophytic that have been co-evolved with rice (Islam and Hossain 2012; Khan et al. 2017).

Moreover, these probiotic bacteria either alone or their consortium can accumulate nutrients, increase plant growth by alleviating stress tolerance as a biocontrol agent, and improve soil health by making available nutrients from complex forms (Islam et al. 2019a; Hirt 2020). Microbial consortia (MC) are symbiotic communities of two or more microbial species or strains. A consortium of microbes typically outperforms an inoculum of a single microbial species (Behera et al. 2021). The term "consortium" was coined by Johannes Reinke in 1872 to describe the interdependence of microorganisms that unites them. They are the beneficial microbes performing as biofertilizers, bioprotectants, controllers, and biostimulants that can increase crop growth and mitigate health and environmental problems. Probiotic bacteria can live as endophytic, symbiotic, or free-living organisms and benefit inoculated plants (de Souza Vandenberghe et al. 2017).

Additionally, probiotics are live bacteria recognized as beneficial for the functioning of the plant body. It is interesting to note that many plant probiotics are like bacteria that are naturally living in an animal body. Animal beings, especially humans, may already be taking some of these good bacteria if they eat fermented products, namely, yogurt and cheese. Generally, probiotics are lactic acid-producing bacteria that are useful to the host plant or animal. In the case of a plant, the probiotics are referred to as endophytic or rhizospheric bacteria. Endophytes arise as imperative additives of plant microbiomes; some of them play pivotal roles in plant improvement and plant responses to pathogens and abiotic stresses, while others produce beneficial and/or exciting secondary metabolites. The recognition of their abilities to influence plant phenotypes and convey beneficial compounds via genetic and molecular interactions has paved the way for those abilities to be exploited for the fitness and welfare of plants, people, and ecosystems (Burragoni and Jeon 2021). Biofertilizer, especially plant probiotics, can be used as a crop growth enhancer by reducing the use of chemical fertilizer and even pesticides and ensuring a higher pleasant product (García-Fraile et al. 2012). A large body of literature suggests that application of the probiotic bacteria not only improves the growth and yield of crop plants but also significantly improves the quality of the plant products. Beneficial effects of probiotic bacteria in rice and other crops are well established, and some of them are already commercialized as biofertilizers and biopesticides (Sarker et al. 2012; Khan et al. 2017; Rahman et al. 2018). Consortia of beneficial bacteria or probiotic bacterial inoculation perform better as compared to the single inoculation (Menéndez and Paço 2020). In this chapter, we summarized various types of probiotic bacteria in rice, their diversity, technique of isolation, and their mode of beneficial effects. Finally, we discussed the future projection of consortia of plant probiotic bacterial technology in the upcoming rice production system in the world.

8.2 Consortia of Probiotic Bacteria for Rice

8.2.1 Probiotic Bacteria

The term plant probiotic bacteria was first coined by Haas and Keel to designate a consortium of microorganisms that perform beneficial activities to the host plant especially fulfilling three essential criteria, namely, (i) efficient root colonizer, (ii) ability to induce systemic resistance to their host, and (iii) presence of direct antagonistic traits on pathogens (Haas and Keel 2003). A vast array of probiotic bacteria inhabit the niche of the rice ecosystem, where more than 400 have been documented by Pang et al. (2020). They are found in association with the tissue of roots as rhizospheric, endophytic, and phyllosphere in the stem, leaves, flowers, and seeds (Naher et al. 2009). Most of them are root-associated bacteria that differed from irrigated to non-irrigated ecosystems (Toju et al. 2019). A list of rice plant probiotic bacteria which are beneficial for growth, development, and yield enhancement has been summarized in Table 8.1.

8.2.2 Consortia of Probiotic Bacteria

A microbial consortium is typically defined as a collection of different microorganisms with the capacity to cooperate in a community. Probiotic consortia are more than one microbial interacting population, which can be found in diversified environmental niches. They consist of more than one member and combine strains from the same or different genera to acquire phytostimulatory properties. However, it is challenging to simulate natural environmental circumstances because a significant portion of soil flora is still not cultivable in the lab. The consortia are beneficial for managing soil and nutrient mobilization, disease control, stress tolerance, and ecosystem management (Kumar et al. 2017; Padmaperuma 2020). In consortia, bacterial cultures must mix with one another and not be antagonistic to one another. Saha et al. (2016) found significant beneficial effects on the growth and development of rice through enhanced nitrogen metabolism when the probiotic inocula are applied in the form of consortia. Recently, new consortia are gaining importance such as *Trichoderma* plus *Azotobacter* that can be developed as agricultural probiotics to support the agro-ecosystem while enhancing crop quantity and quality as reported by several researchers (Woo and Pepe 2018). Ray et al. (2017) reported that foods that have undergone fermentation are the natural habitats of a variety of food-grade microbes, which not only provide bioactive compounds to the food material but may also directly benefit human health. Similarly, Arif et al. (2020) found that plant growth and development can be enhanced, and pathogens and abiotic stresses can be mitigated by altering the microbiome through inoculating with a consortium of plant growth-promoting rhizobacteria (PGPR). Plant growth and disease resistance capacity against soil-borne pathogens can be improved by manipulating the rhizosphere

Table 8.1 List of some probiotic bacteria and their beneficial effects on rice

Species of probiotic bacteria	Host plant	Beneficial effect	References
Azospirillum brasilense	Rice crop	Increase plant growth and yield	Salamone et al. (2012)
Azospirillum lipoferum	Rice crop	Increase plant growth and yield	Chandran et al. (2020)
Azotobacter sp. strain Avi2 (MCC 3432)	Rice crop	Increase growth of plant and yield	Banik et al. (2018)
Azoarcus spp.	Rice crop	N_2 fixation	Reinhold-Hurek and Hurek (1998)
Bacillus megaterium	Rice crop	Enhance plant growth	Shakeel et al. (2015)
Bacillus sphaericus	Rice crop	Increase seed germination, seedling vigor, and plant growth	Mia and Shamsuddin (2009)
Bacillus spp. PSB9 and PSB16	Aerobic rice	Enhance P uptake and increase root and shoot growth	Panhwar et al. (2011)
Burkholderia sp. (BRRh-2), *Burkholderia* sp. (BRRh-3), *Burkholderia* sp. (BRRh-4)	Rice crop	Increase plant growth and grain yield	Khan et al. (2017)
Herbaspirillum frisingense, *H. rubrisubalbicans*, and *H. seropedicae*	Rice crop	Increase plant growth	Alves et al. (2014)
Klebsiella pneumoniae, *K. oxytoca*, and *K. planticola*	Rice crop	Capable of fixing N_2 and are classified as associative N_2 fixers	Cooney et al. (2014)
Pseudomonas aeruginosa	Rice crop	Increase phosphate-solubilizing activity	Khan et al. (2017)
Pseudochrobactrum sp.	Rice crop	Increase plant growth	Khan et al. (2017)
Lactobacillus acidophilus	Rice crop	Increase plant growth and yield	
Lactobacillus sp.	Rice crop	Minimize the post-harvest loss	Zubaidah et al. (2012)
Pseudomonas, *Bacillus*, *Klebsiella*, *Streptomyces*, *Burkholderia*, *Pantoea*, *Enterobacter*	Rice crop	Solubilize soil insoluble P and increase growth and yield of crops	Islam et al. (2019b)
Paenibacillus spp.	Rice crop	Increased nitrogen fixation and incorporation of N to the plant	Seldin (2011)
Rhizobium and *Bradyrhizobium* spp.	Rice crop	Nitrogen fixation and nutrient accumulation	Yanni et al. (2001, 2016) and Mia and Shamsuddin (2010)

microbiome. One of the most complicated and functionally active ecosystems on earth is the rhizosphere, which is a small, dynamic zone of plant root-soil interfaces that promotes plant health and reduces the effects of biotic and abiotic stress in different crop plants (Santoyo et al. 2021). In the microbial consortia system, chemical and physical connections between the two groups of organisms are made, and crop plants allow endophytic microorganisms to grow and inhabit internal portions of plants, such as roots, stems, and leaves, where beneficial interactions are secured between bacteria and the host plants (Santoyo 2022). Application of rhizobacterial consortium of *Enterobacter hormaechei* (AM122) and *Lysinibacillus xylanilyticus* (DB25) to Basmati aromatic rice resulted in improved vegetative growth, yield, and 2AP content compared to monoculture. Moreover, consortia inoculation process also increased the aroma content in rice (Harshal et al. 2021).

8.3 Type of Rice Probiotic Bacteria

Several types of probiotic bacterial consortia can improve the growth and development of rice plants upon inoculation, which are described here (Fig. 8.1).

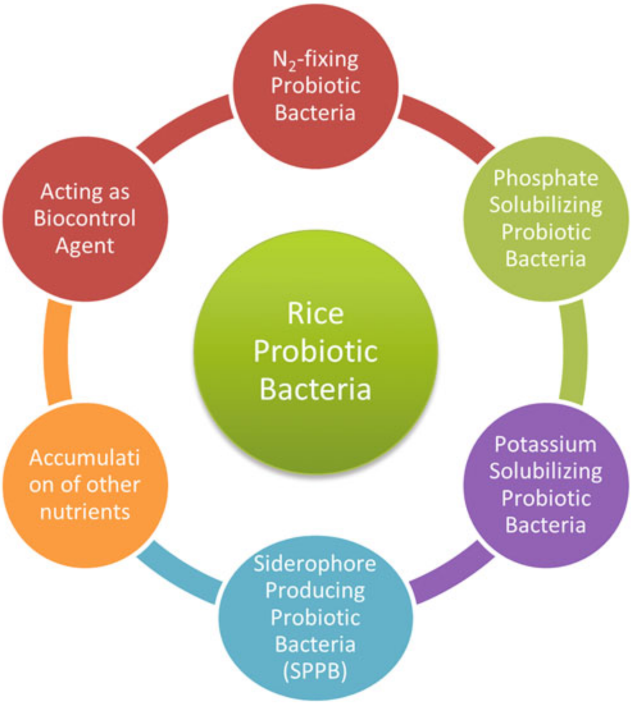


Fig. 8.1 Schematic representation of types of probiotic consortia

8.3.1 Nitrogen-Fixing Probiotic Bacteria

Nitrogen fixation and transportation of fixed N_2 by probiotic bacteria to host plants is the principal mechanism of beneficial activities (Fig. 8.2) (Hu et al. 2021b). Many research studies have been performed on N_2-fixing probiotic bacteria in rice, and a good number of probiotic species have been isolated from rice and other plant species (Table 8.1). The fixed N_2 is utilized by the host plants after the decomposition of probiotic bacteria or through transportation and excretion of NH_4^+ from probiotic bacteria to the host plants, and the latter one is more common in endophytic bacteria (Mia and Shamsuddin 2010). Generally, ammonium-excreting probiotic bacteria can release a significant amount of fixed N_2 to the host plant. Nitrogen fixation by rhizobia in association with rice roots is a new dimension where inoculated plants increase growth due to fixation of N_2 without formation of any nodule. *Bradyrhizobium* may also contribute similarly as it can fix N_2 in a free-living state (Yanni et al. 1997).

8.3.2 Phosphate-Solubilizing Probiotic Bacteria (PSPB)

Phosphorus is the primary element and second most important macronutrient after nitrogen. It is often unavailable under acidic and alkaline conditions. The P-solubilizing plant probiotic bacteria have a profound effect on P solubilization under diversified soil conditions (Molla and Chowdhury 1984; Islam et al. 2007; Islam and Hossain 2012). The most common P-solubilizing plant probiotic bacteria are *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Kushneria*, *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Serratia*, *Ralstonia*, and *Rhodococcus* genera, which do have beneficial effects on the availability of complex, unavailable P

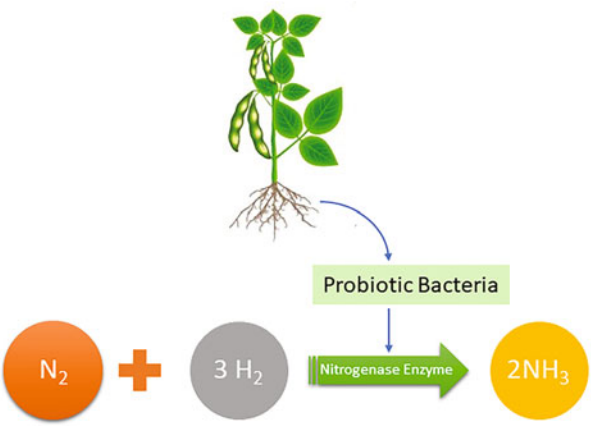


Fig. 8.2 Mechanism of atmospheric nitrogen fixation by diazotrophic plant probiotic bacteria

compounds (Alor et al. 2017). These types of bacteria produce a synergistic effect with the host plant alone or in consortium, consequently enhancing the growth and development of crop plants. Stephen et al. (2015) found two PSPB strains, namely, *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369), which recorded higher P absorption in rice under pot culture conditions. Despite phosphate solubilization, the PSPB also has a greater potential for producing phytohormones, namely, indole acetic acid, and enzymes, namely, phosphatase and phytase. Five promising strains, namely, *Pseudochrobactrum* sp., *Burkholderia* sp., *Burkholderia* sp., *Burkholderia* sp., and *Pseudomonas aeruginosa*, which were isolated from rice roots and identified through 16S rRNA gene phylogeny exhibited significant phosphate-solubilizing activity (Islam et al. 2007; Islam and Hossain 2012; Othman and Panhwar 2014). As reported by Alor et al. (2017), a great array of organic acids is produced by P-solubilizing microorganisms (Fig. 8.3). It is interesting to note that

Fig. 8.3 Different types of organic acids produced by the phosphate-solubilizing probiotic bacteria (PSPB)

the combined inoculation of two or more microbial species of PSPB and N_2 fixers has a positive impact on the growth and yield of various crops (Saxena et al. 2015).

Consortium of PSPB and diazotrophic bacterial inoculation process increased nodulation and N_2 fixation by alfalfa plants, concurrent with an increase in the P content of plant tissues. It was concluded that this was due to an improvement in P nutrition of the plant, as nodulation and N_2 fixation are P-dependent processes (Barea et al. 2005).

8.3.3 Potassium-Solubilizing Probiotic Bacteria (KSPB)

Potassium is an essential macronutrient element that has a great impact on the growth and development of crop plants. It has nonspecific functions like acting as an osmoticum, regulatory functions, and modulation of enzyme activity. This essential element is present in the soil in various forms like mineral K, non-exchangeable K, exchangeable K, and solution K. Despite this, the majority of K is present in the fixed form where probiotic bacteria, namely, *Acidithiobacillus ferrooxidans*, *Paenibacillus* spp., *Bacillus mucilaginosus*, and *B. edaphicus*, have great impact and can make available form from unavailable form through various mechanisms, namely, dissolving K-bearing minerals like biotite, feldspar, illite, muscovite, orthoclase, and mica compound. A significant number of KSPB populations, both aerobic and anaerobic bacteria, are found in the rice rhizosphere. The KSPB reduces the pH of the media and enhances the release of K from the minerals. It has been observed that consortia of KSPB performed better results compared to single inoculation in various crops (Gore and Navale 2017).

8.3.4 Siderophore-Producing Probiotic Bacteria (SPPB)

These metabolites are produced by microbes in response to an iron deficiency in the environment; thus, a few organic manipulators produce those low molecular weight (400–1500 Da) receptor protein systems with a high affinity for iron. Siderophores are secondary metabolites that act as sequestrants of iron because of their excessive dissociation rate with the aid of using this element. It produces organic manipulators that can use iron by two mechanisms: (i) directly through the Fe^{3+}-siderophore complicated via the molecular membrane, and (ii) decreased extracellular Fe^{2+} complexes (Hider and Kong 2010). This enables those plants to alter the availability of iron in their habitats by sequestering that element (Fe^{3+}-siderophore), rendering iron unavailable to phytopathogenic microbes and thereby inhibiting their growth (Kannojia et al. 2019). Currently, numerous bacterial traces have been suggested for their capacity to govern plant illnesses through siderophores, restricting the boom and colonization of iron-established phytopathogenic microorganisms (Fgaier and Eberl 2011). Yu et al. (2011) suggested that *B. subtilis* CAS15 antagonized the activity (nearly 19–94%) of 15 fungal phytopathogens belonging to the genera *Fusarium*, *Colletotrichum*, *Pythium*, *Magnaporthe*, and *Phytophthora*, by producing catecholate-kind siderophores (*bacillibactin*). On the other hand, the siderophore-generating capability of *Burkholderia* can inhibit the anthracnose in mango, *Colletotrichum gloeosporioides*, with minimal inhibitory activity. It has been reported that probiotic bacteria *Paenibacillus polymyxa* BFKC01 can significantly contribute to the absorption of iron through an activated iron acquisition mechanism and enhance the root system. These beneficial effects of producing siderophores are more pronounced when probiotics are applied as consortia.

8.3.5 Accumulation of Nutrients

When probiotic bacterial consortia (PBC) are combined with chemical fertilizers, there is a significant opportunity to improve nutrient usage efficiency while also improving crop output and soil health. In non-legume cereal crops, the PBC as part of integrated nutrient management would significantly reduce the need for the consumption of mineral fertilizers as well as the cost of cultivation, freeing up nutrients for the use of other economically significant crops. The inoculation process of plant growth-promoting rhizobacteria that could accumulate more nutrients, namely, N, K, Ca, and Mg, in tissue-cultured banana plantlets under hydroponic conditions has been documented by Mia et al. (2010a, b). Chandra and Sharma (2021) reported that under field condition, inoculation of plant growth-promoting rhizobacteria (PGPR) consortia of strain DPC9 (*Ochrobactrum anthropi*) + DPB13 (*Pseudomonas palleroniana*) + DPB15 (*P. fluorescens*) + DPB16 (*P. palleroniana*) to rice and wheat plants showed an enhanced nutrient accumulation besides fixation of N_2.

8.3.6 Act as Biocontrol Agent

The application of rice probiotic bacterial inoculation that significantly influences the control of various diseases has been documented by various researchers. The main mechanisms of controlling the pathogen are early colonization of probiotic consortia which prevent the successful colonization by the pathogenic microorganism and producing antibiotics for the control of pathogenic microorganism. A beneficial bio-inoculant for sustainable rice-wheat production in a variety of agro-ecosystems can be found in the consortium of DPC9 + DPB13 + DPB15 + DPB16-producing ACC deaminase (Chandra and Sharma 2021). The successful biocontrol activity can be achieved by the judicious application of dual or multiple mixtures of probiotic bacteria on rice (Felici et al. 2008).

8.4 Isolation and Identification of Rice Probiotic Bacteria

Plant probiotic bacteria may be isolated from rhizospheric or endophytic regions of rice roots, stems, leaves, or even seeds. The isolation of these probiotic bacteria needs growth media, and repeated subculture needs to be performed to get a pure single colony. Figure 8.4 shows a schematic representation of the isolation techniques as modified by Mia et al. (2013). The identification of isolates is mediated by various techniques, namely, biochemical, immunogold, enzyme-linked immunosorbent assay (ELISA), and the molecular technique. The most accurate and commonest technique is 16S rRNA gene sequencing (Mia et al. 2013).

8.5 Mode of Beneficial Effects of Probiotic

Several mechanisms encompass augmenting surface area accessed by plant roots, fixation of N_2, releasing of fixed N_2 and unavailable P, and production of siderophore for absorbing iron (Pii et al. 2015). Moreover, they also assist in the enhancement of nutrient accumulation in rice plants, act as biocontrol agents, and increase the resistance power of inoculated plants. Probiotic bacteria that live in close association or endophytically provide benefits by fixing N_2, producing phytohormones, siderophores, and exopolysaccharides, and producing enzymes that are more active when applied to more species in a consortium state (Hussain et al. 2022).

8.5.1 Root Colonization by Probiotic Bacteria

Successful and efficient root colonization is the precondition of beneficial effects on the host plant. The secure and established colonization ensures a good environment for interacting with the beneficial effects. Numerous bacterial developments are critical for plant colonization and invasion of the internal structure of the plant body via endophytic capacity (Mia et al. 1999). The plant roots are uncovered to soil microorganisms throughout their development and growth, permitting microorganisms to go into the plant and clearing the path for colonization of the seeds (Truyens et al. 2015; Schiltz et al. 2015). The extremely critical mechanism for finding out the endophytic capacity of the soil or rhizospheric microorganisms is the chemotaxis-caused motility to root colonization (Bacilio-Jiménez et al. 2003). The endophytic probiotic bacteria can penetrate into the root cortex that might be in the direction of the seeds, calling for microorganisms to go into the basis and become endophytes via passive penetration. Moreover, the probiotics may also penetrate through the emergence point of lateral roots or via energetic penetration using molecular wall-degrading enzymes, such as cellulase and pectinase (Fig. 8.4) (Hurek et al. 1994; Elbeltagy et al. 2000; James et al. 2002). The benefits of bacterial

Fig. 8.4 Schematic flowchart of isolation, identification, and commercialization of rice probiotic bacteria (modified from Mia and Shamsuddin 2013).

colonization within the plant exceed the risks because the host exudates may be used effectively without being hindered by an overabundance of bacteria colonizing the roots (Liu et al. 2017). In addition, endophytic microorganisms are more susceptible

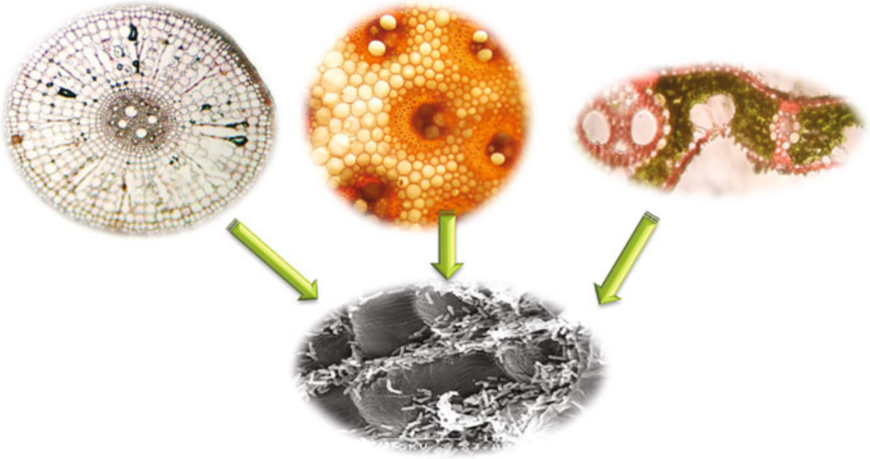


Fig. 8.5 Root colonization of probiotic bacteria inoculated with rice root; figures also show the internal structures of stem and leaf rice plant

to being blanketed by abiotic strains than rhizospheric microorganisms (Hardoim et al. 2015). Once inside the plant, positive endophytes may be able to disperse systemically and eventually reach flowers, climax, and seeds. Some endophytes use the pathway of xylem vessels in their hosts to reach the meristems. This may be via means of motion in their flagella and via means of the plant transpiration current (James et al. 2002; Compant et al. 2005), otherwise nutrient-wealthy intracellular spaces. The colonization process might be enhanced by the synthesis of extracellular polysaccharides and cell aggregation. It has been reported that probiotic bacteria like *Bacillus* sp. can synthesize extracellular polysaccharides and form a cluster of cell aggregation roots, which might have a synergistic effect on the colonization process. Similarly, larger cell aggregation has also been found in *B. megaterium* strain C4 and *Azospirillum brasilense* as reported by Bahat-Samet et al. (2004). Priming of rice seeds with probiotic bacteria resulted in high root colonization of rice plants (Figs. 8.5 and 8.6).

8.5.2 Nitrogen Fixation

The rhizospheric probiotic microbes can improve the growth and yield of rice by N_2 fixation in both upland and lowland ecosystems (Mia et al. 2007; Tang et al. 2019; Hu et al. 2021b). This is the principal mechanism of beneficial effects of probiotic microbes where substantial amounts of N_2 are fixed and transported to the tissue of the host plant, either by excretion of NH_4 from the bacteria or mineralization of decomposed bacterial dead bodies (Mia et al. 2009). The mode of transfer of fixed N_2 to host tissue depends on the nature of colonization. Bentley and Carpenter

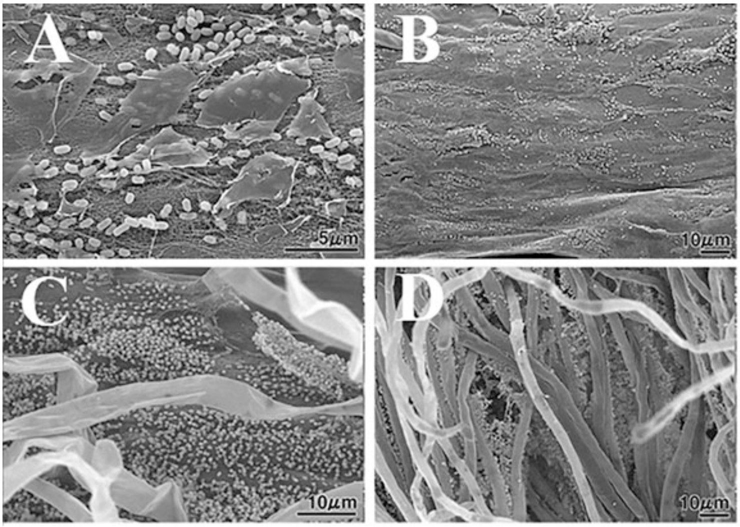


Fig. 8.6 Dense colonization of *Acinetobacter* sp. BR-25 (A–C) and *Klebsiella* sp. BR-15 (D) on the surface of roots (cv. BR29) of rice seedlings from seeds previously inoculated with bacteria (Islam et al. 2007)

(1984) reported that the epiphyllous microorganism can transfer 10–25% of their N requirement in *Welfia georgii*. The capacity to convert fixed N_2 into NH_4^+ depends on nitrogenase enzyme complex. The diazotrophic associative bacteria offer greater N for the host plant by the BNF process, which may be inhibited via assimilation of NH_4^+ and/or interference with the bad law of the nitrogenase enzymatic complex through ammonia. In vitro growth-promoting traits revealed that probiotic bacteria fixed a substantial amount of N_2, solubilized tricalcium phosphate, and produced IAA with or without the addition of L-tryptophan (Habib et al. 2016). Malik et al. (1997) found that nearly 70% N incorporation was achieved in rice by the inoculation of *Azospirillum lipoferum*, *Azospirillum brasilense*, *Azoarcus*, *Pseudomonas* as estimated by the ^{15}N isotopic dilution nuclear method. Recently, it has been shown that co-inoculation of several diazotrophic bacteria performed better in respect to N_2 fixation and utilization than monoculture. The consortium inoculation processes resulted in more synergistic effect in creating a conducive environment for making beneficial interaction between probiotic and the host plant (Maheshwari 2012).

8.5.3 Enhanced Nutrient Accumulation

Inoculation of probiotic bacterial consortia can increase the solubility of P, thereby enhancing its accumulation in rice plant in addition to N_2 fixation process. Other nutrients, namely, Ca and Mg, can also be enhanced by the uptake through the process of inoculation. Several probiotic bacterial consortia can enhance the uptake and accumulation process. Khan et al. (2017) found phosphate-solubilizing activity by the inoculation of PSPB on the seedling of BRRh-4 and BRRh-5 by root dipping (colonization) of the seedling and spraying at the flowering stage significantly enhanced the growth and grain yield of rice variety BRRI dhan-29. Interestingly, application of both strains with 50% of recommended nitrogen, phosphorus, and potassium fertilizers produced an equivalent or higher grain yield of rice compared to the control grown with fully recommended fertilizer doses (Mutalib et al. 2012).

Additionally, better grain production was achieved by combining *Pseudomonas putida*, *P. fluorescens*, or *Azotobacter chroococcum* with various inorganic nitrogen concentrations than rice plants modified with higher conventional nitrogen dosages. Tan et al. (2015) demonstrated that rice plants inoculated with the N_2-fixing species *Lysinibacillus xylanilyticus* and *Bradyrhizobium japonicum* resulted in N content of up to 63 kg ha^{-1}, which evidences the advantageous synergistic activities of the used strains over a 65-day period in greenhouse conditions.

8.5.4 Increased Plant Growth and Development

Inoculation of probiotic bacteria increases the shoot and root growth and consequently enhances the total dry matter of rice plant. These bacterial consortia encompass the formulation with *Bacillus cereus*, *Stenotrophomonas maltophilia*, or *Klebsiella pneumoniae*. They are applied in the rice rhizosphere where they significantly ($P \leq 0.05$) enhanced the growth of plants (Nevita et al. 2018). Similarly, inoculation of probiotic bacterial biofertilizer increases the growth and grain of rice by 4.83–9.16% under greenhouse conditions (Aw et al. 2020). Moreover, the inoculation system enhances the yield, grain size, and grain quality of rice plants, as reported by Lucas et al. (2009). On the other hand, *Pseudomonas* inoculation to the rice significantly increased plant morphological characteristics, namely, plant height, root length, and dry matter of shoot and root under glycophytic and even saline conditions (Sen and Chandrasekhar 2014). On the other hand, consortium application of probiotic bacteria can provide enhanced plant growth, production, and crop quality. However, selecting the right bacteria for consortia from a wide range of individually tested potent bacteria is critical.

8.5.5 Increased Root Growth

A great number of probiotic bacteria reside on the surface and endosphere and have beneficial effects on the host plants. Enhanced root stimulation is one of the important mechanisms of the beneficial effects of probiotics on rice plants. Various root parameters like length, mass, volume, and surface areas are increased by the inoculation of those bacteria. These bacteria infect the rice roots both endophytically and ectophytically, thereby changing the modulation of host plant physiology and consequently changing the morphology of the roots. The main mechanism of enhanced root morphology is the activity of phytohormones, which are produced by the probiotic bacteria associated with the rice roots. Khan et al. (2017) isolated six strains of probiotic that can produce phytohormone auxin, and they are identified as *Pseudochrobactrum* sp. (BRRh-1), *Burkholderia* sp., and *Pseudomonas aeruginosa* based on their 16S rRNA gene phylogeny. Ruiza et al. (2011) isolated two rice probiotic bacteria which can enter the internal structure via roots and lenticel, living as endophytes, producing auxin, and consequently increasing the root growth. It is assumed that the loosening of the cell wall might cause the larger roots mediated by the root colonization. Several researchers found that these bacteria performed better when inoculated in combination than when inoculated singly (Molina-Romero et al. 2021).

8.5.6 Production of Phytohormone

Phytohormones are essential biochemical compounds that act as messengers for the growth and development processes in crop plants. Production of phytohormones, namely, auxin, gibberellin, and zeatin, is performed by different types of probiotic bacteria associated with rice plants and has been documented by several authors (Maheshwari et al. 2015). They can cause crucial alterations in the physiological attributes, resulting in the changes in the morphology of rice plants. These changes may be affected either by synthesis or metabolism of phytohormones in a plant's body. Probiotic bacteria can produce significant amounts of phytohormone under controlled condition when inoculated to rice (Habib et al. 2016; Khan et al. 2017). The hormones produced are mostly related to growth promotion as well as nutritional aspects of the rice plant. Cell enlargement and an increased number of cell divisions result in enhanced root and shoot growth. The enhanced root growth is the result of root length, mass, and number, which alleviate water stress, mainly drought, and the absorption of more nutrients (Hu et al. 2021a, b). Several phytohormones, namely, auxins, gibberellins, cytokines, ethylene, and ACC deaminase, are produced by probiotic bacteria (Menendez and Garcia-Fraile 2017).

8.5.7 Production of Antibiotic

Probiotic bacterial inoculation significantly produced different types of antibiotics and protected the host plant against various types of diseases (Islam et al. 2005). These phenomena happened positively when inocula are applied as consortia. The main potential to synthesize diverse antimicrobial compounds has been significantly studied to inhibit, restrict, or remove the growth of phytopathogenic organisms (Liu et al. 2017). *Pseudomonas* and *Bacillus* are the two major bacterial genera known for their potential to provide antibiotics, together with 2,4-diacetylphloroglucinol acid and other several compounds. A big quantity of traces of the genus *Pseudomonas* are identified for generating an extensive type of antibiotics that contribute to the suppression of crop diseases, as an instance. Finally, pioluteorin and pyrrolnitrin have been proven to correctly suppress watercress disease due to *Pythium ultimum* and *Rhizoctonia solani*, respectively (Milner et al. 2019). Based on 16S rDNA gene sequencing, *Bacillus velezensis* strain DU14 was selected as a potential probiotic bacterium, and these strains showed tolerance to a pH range of 2–9 and survived in presence of 1% (w/v) of bile salt. It showed sensitivity against both broad- and narrow-spectrum antibiotics with antagonistic activities against both Gram-positive (*Bacillus cereus* ATCC-11778) and Gram-negative (*Escherichia coli* ATCC-25922) pathogens (Nevita et al. 2018; Islam et al. 2019a). Protection mechanisms of plants from diseases and other stresses by the application of probiotic bacteria are shown in Fig. 8.7.

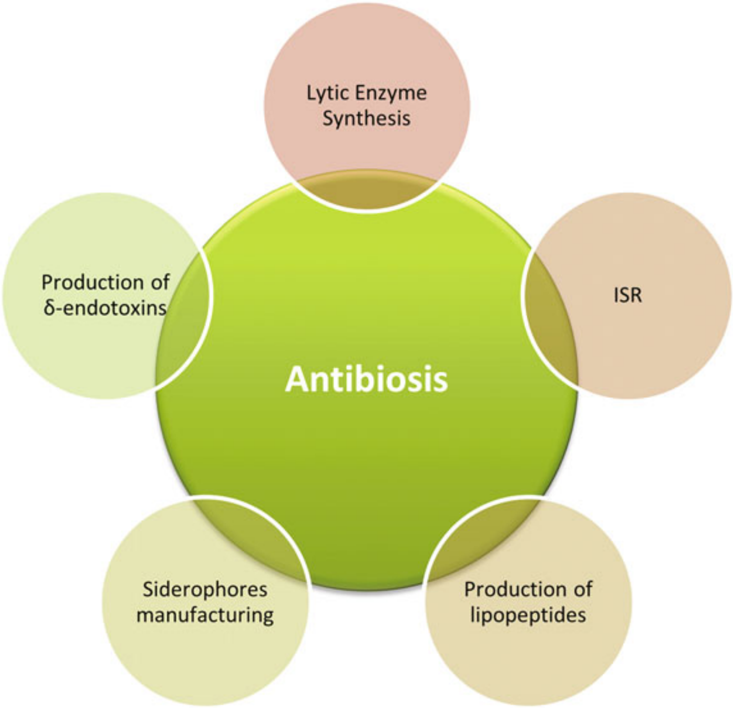


Fig. 8.7 Schematic presentation of antibiosis development of rice plant after inoculation by probiotic bacteria. ISR, induction of systemic resistance

8.5.8 Induction of Systemic Resistance (ISR) in Plant Life

Induction of systemic resistance is one of the key mechanisms for controlling various fungal and pathogenic diseases. This mechanism may be precipitated by chemical signals produced by useful microorganisms (Pérez-Montaño et al. 2014). ISR signaling depends on jasmonic acid and ethylene (Kannojia et al. 2019). So far, not all the molecular mechanisms that alter plant-useful microbe interactions have been described; however, the primary routes through which those retailers alter ISR in plant life have been identified as (i) phytohormones, (ii) pathogen-related molecular patterns (PAMPs) or microbe-related molecular patterns (MAMPs), and (iii) numerous elicitors (unstable natural compounds, siderophores, phytases, miRNAs) (Abdul Malik et al. 2020). The ISR has been evidenced in tobacco plant life, wherein PR2 (encodes β-1,3-glucanases) and PR3 (encodes a chitinase) have been activated in reaction to unstable compounds produced by *Bacillus*, conferring resistance to *Rhizoctonia solani* and *Phytophthora nicotianae* (Kim et al. 2015; Saber et al. 2015). In addition to PR genes, *Bacillus* turns on different safety mechanisms in plant life, which consist of structural modifications within the molecular wall through the buildup of lignin (Singh et al. 2016) or the manufacturing of secondary metabolites together with flavonoids, phytoalexins, auxins, and/or glucosinolates (Pretali et al. 2016). Thus, ISR has been shown in several crops (beans, carnations, cucumbers, radishes, tobacco, and tomatoes), notably decreasing the pathogenicity of numerous plant pathogens, which include fungi, bacteria, and viruses (Kannojia et al. 2019). Gopalakrishnan et al. (2012) isolated seven strains that are characterized by their physiological traits, viz., tolerance to salinity, pH, temperature, and resistance to antibiotics and fungicides on the rice plant. It is also well recognized that PGPR consortia enhance host plants' induced systemic resistance. Application of probiotic consortia inocula on rice significantly induces ISR in various crop plants, especially the rice plant, as documented by various researchers (Shabanamol et al. 2020). Consortium and coaggregate application of *Paenibacillus polymyxa* (PF-3) and *Paenibacillus faecalis* (B-19) improved ISR in rice-*Pyricularia oryzae* (Pandey et al. 2012). Additionally, Umashankari and Sekar (2011) reported that rice-*Pyricularia oryzae* was significantly controlled by consortium and co-aggregate application of *Pseudomonas fluorescens* (PF-3) and *Paenibacillus polymyxa* (B-19).

8.5.9 Production of Lipopeptides

A lipopeptide is a molecule made up of a lipid and a peptide that can self-assemble into diverse structures. The lipopeptide molecules contain a cyclic peptide related to a β-hydroxy or β-amino fatty acid chain, which are labeled into three exceptional families, primarily based totally on their amino acid series and fatty acid length (Falardeau et al. 2013; Valenzuela-Ruiz et al. 2019). Lipopeptides are synthesized with the aid of using multi-enzyme complexes known as non-ribosomal peptide synthetase (NRPS), which can be unbiased of messenger RNA (Chowdhury et al. 2015; Valenzuela-Ruiz et al. 2019); moreover, this may be low-molecular-weight compounds with amphiphilic traits that shield plant life at some point of numerous phenological degrees with the aid of using without delay suppressing the boom of pathogens or inducing systemic resistance (Hashem et al. 2019). Various bacteria, especially those belonging to the genera *Bacillus*, *Pseudomonas*, and *Streptomyces*, create these molecules as part of their *metabolism.* The plant probiotic can potentially synthesize lipopeptides upon inoculation of rice plants. Recently, Coutte et al. (2017) suggested 263 exceptional lipopeptides synthesized with the aid of 11 microbial genera, among which the *Bacillus* genus became the most considerable manufacturer with 98 exceptional lipopeptides. One has been concerned with the organic manipulation of a huge variety of phytopathogens (bacteria, fungi, and oomycetes) inflicting illnesses on important crops (Ongena and Jacques, 2008). For example, a couple of isoforms of fengycins and iturins were suggested in molecularly unfastened extracts of liquid cultures of *B. subtilis* GA1 with the capacity to inhibit *Botrytis cinerea* in apple fruits (Toure et al. 2004).

8.6 Conclusions and Future Perspective

Inoculation with probiotic bacteria promotes plant nutrition and health and increases crop yield and quality. Some of the elite strains of probiotic bacteria belonging to the genera *Rhizobium*, *Bacillus*, *Pseudomonas, Paraburkholderia*, *Enterobacter*, *Delftia*, etc. are proven to increase rice yield under nutrient-deficient conditions. Application of a consortium of the beneficial bacteria has shown better plant growth promotion than their single application to the same crop plant. The mechanisms of the beneficial effects of the probiotic bacteria consortium include but are not limited to fixation of atmospheric nitrogen, solubilization of insoluble essential nutrient elements in soils, production of various metabolites, and regulation of the expression of host genes of beneficial traits. Some of the individual bacterium or a consortium of probiotic bacterium are commercially formulated as biofertilizer and biopesticides. The application of the appropriate combination of beneficial probiotic bacteria may decrease the dependency of chemical inputs and improve the health of soils. Although the literature of the beneficial effects of the application of probiotic bacteria on rice is huge, the inconsistency of their performances in varying environmental conditions and varieties of rice is a big problem. To overcome this problem, it is important to know the impact of applied probiotics on microbiome of rice and rhizosphere. Metagenomic approaches could be used to get better understanding of the cross-kingdom cross-talks in the rhizosphere of rice after the application of certain consortium of probiotic bacteria. Obviously, a better understanding of the underlying molecular interactions between plants, probiotic bacteria, and modulated microbiome in plants and rhizosphere would help us to design an effective strategy for sustainable nutrition, protection, and yield of rice. The putative beneficial mode

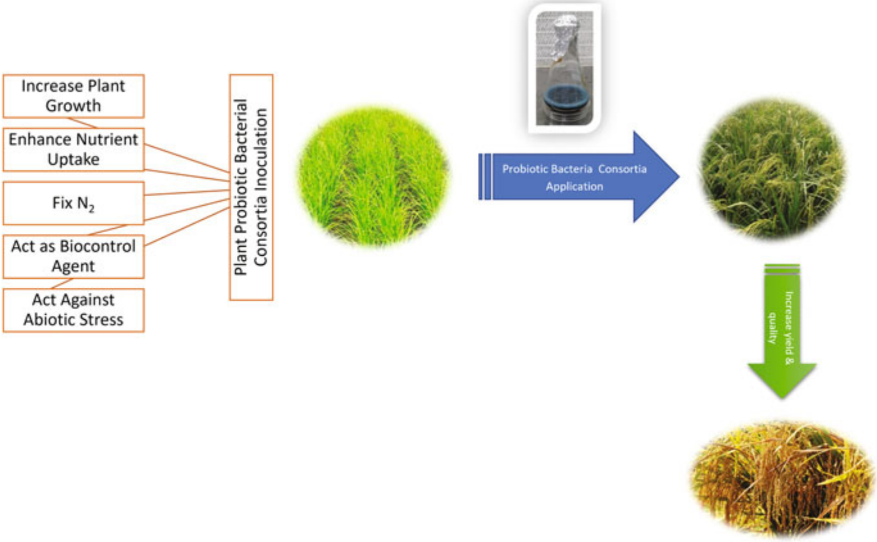


Fig. 8.8 Schematic representation of summary of mode of beneficial activities of applied probiotic bacteria

of actions of the applied probiotic bacteria on growth, yield, and health of rice plants is summarized in Fig. 8.8.

References

Abdul Malik NA, Kumar IS, Nadarajah K (2020) Elicitor and receptor molecules: orchestrators of plant defense and immunity. Int J Mol Sci:1–34

Abdullah AB, Ito S, Adhana K (2006) Estimate of rice consumption in Asian countries and the world towards 2050. In Proceedings for Workshop and Conference on Rice in the World at Stake, 2, 28–43

Alor ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971. https://doi.org/10.3389/fmicb.2017.00971

Alves GC, Videira SS, Urquiaga S, Reis VM (2014) Differential plant growth promotion and nitrogen fixation in two genotypes of maize by several Herbaspirillum inoculants. Plant Soil 387(1-2):307–321

Arif I, Batool M, Schenk PM (2020) Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trends Biotechnol 38(12):1385–1396. https://doi.org/10.1016/j.tibtech.2020.04.01

Aw XZL, Li WC, Ye ZH (2020) The effect of plant growth-promoting rhizobacteria (PGPR) on arsenic accumulation and the growth of rice plants (Oryza sativa L.). Chemosphere 242:125136. https://doi.org/10.1016/j.chemosphere.2019.125136

Bacilio-Jiménez M, Aguilar-Flores S, Ventura-Zapata E, Pérez-Campos E, Bouquelet S, Zenteno E (2003) Chemical characterization of root exudates from rice (Oryza sativa) and their effects on the chemotactic response of endophytic bacteria. Plant Soil 249(2):271–277

Bahat-Samet E, Castro-Sowinski S, Okon Y (2004) Arabinose content of extracellular Polysaccharide plays a role in cell aggregation of Azospirillum brasilense. FEMS Microbiol Lett 237:195–203

Banik A, Kumar DG, Swain P, Kumar U, Mukhopadhyay SK, Dangar TK (2018) Application of rice (Oryza sativa L.) root endophytic diazotrophic Azotobacter sp. strain Avi2 (MCC 3432) can increase rice yield under green house and field condition. Microbiol Res:S0944501318306633–. https://doi.org/10.1016/j.micres.2018.11.004

Barea JM, Werner D, Azcón-Aguilar C, Azco'n R (2005) Interactions of arbuscular mycorrhiza and nitrogen fixing symbiosis in sustainable agriculture. In: Werner D, Newton WE (eds) Agriculture, forestry, ecology and the environment. Kluwer, The Netherlands

Bentley BL, Carpenter EJ (1984) Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. Oecologia 63(1):52–56

Burragoni SG, Jeon J (2021) Applications of endophytic microbes in agriculture, biotechnology, medicine, and beyond. Microbiol Res 245:126691

Chandra D, Sharma AK (2021) Field evaluation of consortium of bacterial inoculants producing ACC deaminase on growth, nutrients and yield components of rice and wheat. J Crop Sci Biotechnol 24:293–305. https://doi.org/10.1007/s12892-020-00077-y

Chandran V, Shaji H, Mathew L (2020) Endophytic microbial influence on plant stress responses. Microbial endophytes, 161–193

Chowdhury SP, Uhl J, Grosch R, Alquéres S, Pittroff S, Dietel K, Schmitt-Kopplin P, Borriss R, Hartmann A (2015) Cyclic Lipopeptides of Bacillus amyloliquefaciens subsp. plantarum Colonizing the Lettuce Rhizosphere enhance plant defense responses toward the bottom rot pathogen Rhizoctonia solani. Mol Plant Microbe Interact. https://doi.org/10.1094/MPMI-03-15-0066-R

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Ait Barka E (2005) Endophytic colonization of Vitis vinifera L. by plant growth-promoting bacterium Burkholderia sp. strain PsJN. Appl Environ Microbiol 71(4):1685–1693

Cooney S, O'Brien S, Iversen C, Fanning S (2014) Bacteria: other pathogenic Enterobacteriaceae – Enterobacter and other genera. Encyclopedia of Food Safety, 433–441

Coutte F, Lecouturier D, Dimitrov K, Guez JS, Delvigne F, Dhulster P, Jacques P (2017) Microbial lipopeptide production and purification bioprocesses, current progress and future challenges. Biotechnol J 12(7):1600566

De Souza Vandenberghe LP, Garcia LMB, Rodrigues C, Camara MC, de Melo Pereira GV, de Oliveira J, Soccol CR (2017) Potential applications of plant probiotic microorganisms in agriculture and forestry. AIMS Microbiol 3(3):629

Elbeltagy A, Nishioka K, Suzuki H, Sato T, Sato YI, Morisaki H, Minamisawa K (2000) Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Sci Plant Nutr 46(3):617–629

Falardeau J, Wise C, Novitsky L, Avis TJ (2013) Ecological and mechanistic insights into the direct and indirect antimicrobial properties of Bacillus subtilis lipopeptides on plant pathogens. J Chem Ecol 39(7):869–878

Felici C, Vettori L, Giraldi E, Forino LMC, Toffanin A, Tagliasacchi AM, Nuti M (2008) Single and coinoculation of Bacillus subtilis and Azospirillum brasilense on Lycopersicon esculentum: effects on plant growth and rhizosphere microbial community. Appl Soil Ecol 40(2):260–270

Fgaier H, Eberl HJ (2011) Antagonistic control of microbial pathogens under iron limitations by siderophore producing bacteria in a chemostat setup. J Theor Biol 273(1):103–114

García-Fraile P, Carro L, Robledo M, Ramírez-Bahena MH, Flores-Félix JD, Fernández MT, Velázquez E (2012) Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7(5): 38122

Gopalakrishnan S, Upadhyaya HD, Vadlamudi S, Humayun P, Vidya MS, Alekhya G, Rupela O (2012) Plant growth-promoting traits of biocontrol potential bacteria isolated from rice rhizosphere. Springer Plus 1(1):1–7

Gore NS, Navale AM (2017) Effect of consortia of potassium solubilizing bacteria and fungi on growth, nutrient uptake and yield of banana. Indian J Hortic 74(2):189–197

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41:117

Habib SH, Kausar H, Saud HM, Ismail MR, Othman R (2016) Molecular characterization of stress tolerant plant growth promoting rhizobacteria (PGPR) for growth enhancement of rice. Int J Agric Biol 18:184–191

Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Harshal VD, Anupama AP, Vitthal TB, Syed GD, Altafhusain BN (2021) Rhizobacterial consortium mediated aroma and yield enhancement in basmati and non-basmati rice (*Oryza sativa* L.). J Biotechnol 328:47–58. https://doi.org/10.1016/j.jbiotec.2021.01.012

Hashem A, Tabassum B, Fathi Abd Allah E (2019) Bacillus subtilis: a plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi J Biol Sci 26:1291–1297. https://doi.org/10.1016/j.sjbs.2019.05.004H

Hider RC, Kong X (2010) Chemistry and biology of siderophores. Natural Product Report

Hirt H (2020) Healthy soils for healthy plants for healthy humans: how beneficial microbes in the soil, food and gut are interconnected and how agriculture can contribute to human health. EMBO Rep 21:e51069

Hosseyni M, Soltani J (2013) An investigation on the effects of photoperiod, aging and culture media on vegetative growth and sporulation of rice blast pathogen Pyricularia oryzae. Prog Biol Sci 3:135–143

Hu J, Yang T., Friman, V, Kowalchuk GA, Hautier Y., Li M., Wei Z. Xu Y. and Shen Q. (2021a) Introduction of probiotic bacterial consortia promotes plant growth via impacts on the resident rhizosphere microbiome. Proc R Soc B 288: 20211396. https://doi.org/10.1098/rspb.2021.1396

Hu HY, Li H, Hao MM, Ren YN, Zhang MK, Liu RY, Zhang Y, Li G, Chen JS, Ning TY (2021b) Nitrogen fixation and crop productivity enhancements co-driven by intercrop root exudates and key rhizosphere bacteria. J Appl Ecol 58:2243–2255

Hurek T, Reinhold-Hurek B, Van Montagu M, Kellenberger E (1994) Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. J Bacteriol 176(7):1913–1923

Hussain N, Bilal M, Iqbal HM (2022) Carbon-based nanomaterials with multipurpose attributes for water treatment: greening the 21st-century nanostructure materials deployment. Biomater Polym Horiz 1:1–11

Islam M, Hossain M (2012) Plant probiotics in phosphorus nutrition in crops, with special reference to rice. In: Islam MT et al (eds) Bacteria in agrobiology: plant probiotics. Springer, Switzerland, pp 325–363

Islam MT, Hashidoko Y, Deora A, Ito T, Tahara S (2005) (2005) Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne Peronosporomycetes. Appl Environ Microbiol 71(7):3786–3796. https://doi.org/10.1128/AEM.71.7.3786-3796.2005

Islam MT, Deora A, Hashidoko Y, Rahman A, Ito T, Tahara T (2007) Isolation and identification of potential phosphate solubilizing bacteria from the rhizoplane of *Oryza sativa* L. cv. BR29 of Bangladesh. Z Naturforsch 62 c:103–110

Islam T, Rahman M, Pandey P, Boehme MH, Haesaert G (2019a) Bacilli and agrobiotechnology: phytostimulation and biocontrol, vol 2. Springer Nature, Bremgarten

Islam M, Sano A, Majumder M, Hossain M, Sakagami J (2019b) Isolation and molecular characterization of phosphate solubilizing filamentous fungi from subtropical soils in Okinawa. Appl Ecol Environ Res 17:9145–9157

James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PP, Ladha JK (2002) Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Mol Plant-Microbe Interact 15(9):894–906

Kannojia P, Choudhary KK, Srivastava AK, Singh AK (2019) PGPR bioelicitors: induced systemic resistance (ISR) and proteomic perspective on biocontrol. In PGPR amelioration in sustainable agriculture. Wood head Publishing, Sawston, pp 67–84

Khan MMA, Haque E, Paul NC, Khaleque MA, Al-Garni SM, Rahman M, Islam MT (2017) Enhancement of growth and grain yield of rice in nutrient deficient soils by rice probiotic bacteria. Rice Sci 24(5):264–273

Khush GS (2005) What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol 59:1–6. https://doi.org/10.1007/s11103-005-2159-5

Kim JS, Lee J, Lee CH, Woo SY, Kang H, Seo SG, Kim SH (2015) Activation of pathogenesis-related genes by the rhizobacterium, bacillus sp. JS, which induces systemic resistance in tobacco plants. The. Plant Pathol J 31(2):195

Kumar N, Suyal DC, Sharma IP, Verma A, Singh H (2017) Elucidating stress proteins in rice (Oryza sativa L.) genotype under elevated temperature: a proteomic approach to understand heat stress response. 3 Biotech 7(3):1–8

Liu H, Carvalhais LC, Crawford M, Singh E, Dennis PG, Pieterse CM, Schenk PM (2017) Inner plant values: diversity, colonization and benefits from endophytic bacteria. Front Microbiol 8: 2552

Lucas JA, Ramos Solano B, Montes F, Ojeda J, Megias M, Gutierrez Mañero FJ (2009) Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa*) in southern Spain. Field Crop Res 114(3):404–410

Maheshwari DK (2012) Bacteria in agrobiology: plant probiotics || Consortium of Plant-Growth-Promoting Bacteria: Future Perspective in Agriculture. (Chapter 10) 185–200. doi:https://doi.org/10.1007/978-3-642-27515-9_10

Maheshwari DK, Dheeman S, Agarwal M (2015) Phytohormone-producing PGPR for sustainable agriculture. Sustainable Development and Biodiversity, 159–182

Malik KA, Bilal R, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. In Opportunities for biological nitrogen fixation in rice and other non-legumes. Springer, Dordrecht, pp 37–44

Menendez E, Garcia-Fraile P (2017) Plant probiotic bacteria: solutions to feed the world. AIMS Microbiol 3(3):502–524. https://doi.org/10.3934/microbiol.2017.3.502. PMID: 31294173; PMCID: PMC6604988

Menéndez E, Paço A (2020) Is the application of plant probiotic bacterial consortia always beneficial for plants? Exploring synergies between rhizobial and non-rhizobial bacteria and their effects on agro-economically valuable crops. Life 10:24

Mia MAB, Shamsuddin ZH (2009) Enhanced emergence and vigour seedling production of rice through plant growth promoting bacterial inoculation. Res J Seed Sci 2:96–104

Mia MAB, Shamsuddin ZH (2010) Nitrogen fixation and transportation by rhizobacteria : a scenario of rice and bananas. Int J Bot 6(3):235–242

Mia MB, Shamsuddin ZH (2013) Biofertilizer for banana production. LAP LAMBERT Academic Publishing, London

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (1999) External and internal root colonization of Azospirillum brasilense on tissue-cultured banana plantlets. In proc. eighth scientific conference of electron microscopy society, Malaysia, pp. 173–174

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2007) Associative nitrogen fixation by Azospirillum and bacillus spp. in bananas. Infomusa 16(1 & 2):11–15

Mia MB, Shamsuddin ZH, Wahab Z, Marziah M (2009) The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. Afr J Biotechnol 8(21)

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2010a) Rhizobacterial inoculation on growth and nitrogen incorporation in tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Aust J Crop Sci 4(2):85–90

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2010b) Rhizobacteria as bioenhancer for growth and yield of banana (Musa spp. cv "Berangan"). Sci Hortic 126(2):80–87

Mia M, Baset A, Hossain M, Shamsuddin ZH, Islam MT (2013) Plant-associated bacteria in nitrogen nutrition in crops, with special reference to rice and banana. Bacteria in agrobiology: crop productivity, 97–126

Milner J, Silo-Suh L, Goodman RM, Handelsman J (2019) Antibiosis and beyond: genetic diversity, microbial communities, and biological control. In ecological interactions and biological control. CRC Press, Boca Raton, FL, pp 107–127

Molina-Romero D, Juárez-Sánchez S, Venegas B, Ortíz-González CS, Baez A, Morales-García YE, Muñoz-Rojas J (2021) Bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. Front Sustain Food Syst 4. https://doi.org/10.3389/fsufs.2020.616757

Molla M, Chowdhury AA (1984) Microbial mineralization of organic phosphate in soil. Plant Soil 78:393–399

Mutalib AA, Radziah O, Shukor Y, Naher UA (2012) Effect of nitrogen fertilizer on hydrolytic enzyme production, root colonization, N metabolism, leaf physiology and growth of rice inoculated with Bacillus sp. (Sb42). Aust. J Crop Sci 6(9):1383–1389

Muthayya S, Sugimoto JD, Scott M, Maberly GF (2014) An overview of global rice production, supply, trade, and consumption. Ann N Y Acad Sci 1324(1):7–14. https://doi.org/10.1111/nyas.12540

Naher UA, Othman R, Shamsuddin ZH, Saud HM, Ismail MR (2009) Growth enhancement and root colonization of rice seedlings by Rhizobium and Corynebacterium spp. Int J Agric Biol 11:586–590

Nevita T, Sharma GD, Pandey P (2018) Differences in rice rhizosphere bacterial community structure by application of lignocellulolytic plant-probiotic bacteria with rapid composting traits. Ecol Eng 120:209–221

Ongena M, Jacques P (2008) Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 16:115–125. https://doi.org/10.1016/j.tim.2007.12.009

Othman R, Panhwar QA (2014) Phosphate-solubilizing bacteria improves nutrient uptake in aerobic Rice. In: Khan M, Zaidi A, Musarrat J (eds) Phosphate solubilizing microorganisms. Springer, Cham

Padmaperuma G (2020) Microbial consortia: Concept and application in fruit crop management. Fruit Crops:353–366. https://doi.org/10.1016/B978-0-12-818732-6.00025-3

Pandey P, Bisht S, Sood A, Aeron A, Sharma GD, Maheshwari DK (2012) Consortium of plant-growth-promoting bacteria: future perspective in agriculture. In: Maheshwari D (ed) Bacteria in agrobiology: plant probiotics. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-27515-9_10

Pang Z, Zhao Y, Xu P, Yu D (2020) Microbial diversity of upland rice roots and their influence on rice growth and drought tolerance. Microorganisms 8(9):1329

Panhwar QA, Radziah O, Zaharah AR, Sariah M, Razi IM (2011) Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice. J Environ Biol 32(5):607

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny MR, Jiménez-Guerrero I, López-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169(5-6):325–336

Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C (2015) Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process: a review. Biol Fertil Soils 51(4):403–415

Pretali L, Bernardo L, Butterfield TS, Trevisan M, Lucini L (2016) Botanical and biological pesticides elicit a similar induced systemic response in tomato (Solanum lycopersicum) secondary metabolism. Phytochemistry 130:56–63

Rahman M, Sabir AA, Mukta JA, Khan M, Alam M, Mohi-Ud-Din M, Miah M, Rahman M, Islam MT (2018) Plant probiotic bacteria *Bacillus* and *Paraburkholderia* improve growth, yield and content of antioxidants in strawberry fruit. Sci Rep 8:1–11

Ray M, Hor PK, Singh SN, Mondal KC (2017) Screening of health beneficial microbes with potential probiotic characteristics from the traditional rice-based alcoholic beverage, haria. Acta Biol Szegediensis 61(1):51–58

Reinhold-Hurek B, Hurek T (1998) Life in grasses: diazotrophic endophytes. Trends Microbiol 6 (4):139–144

Ruiza D, Agaras B, de Werrab P, Wall LG, Valverde C (2011) Characterization and screening of plant probiotic traits of bacteria isolated from rice seeds cultivated in Argentina. J Microbiol 49(6):902–912

Saber WI, Ghoneem KM, Al-Askar AA, Rashad YM, Ali AA, Rashad EM (2015) Chitinase production by Bacillus subtilis ATCC 11774 and its effect on biocontrol of rhizoctonia diseases of potato. Acta Biol Hung 66(4):436–448

Saha C, Mukherjee G, Agarwal BP, Seal A (2016) A consortium of non-rhizobial endophytic microbes from *Typha angustifolia* functions as probiotics in rice and improves nitrogen metabolism. Plant Biol 18(6):938–946. https://doi.org/10.1111/plb.1248

Salamone GIE, Funes JM, Di Salvo LP, Escobar-Ortega JS, D'Auria F, Ferrando L, Fernandez-Scavino A (2012) Inoculation of paddy rice with Azospirillum brasilense and Pseudomonas fluorescens: impact of plant genotypes on rhizosphere microbial communities and field crop production. Appl Soil Ecol 61:196–204. https://doi.org/10.1016/j.apsoil.2011.12.012

Santoyo G (2022) How plants recruit their microbiome? New insights into beneficial interactions. J Adv Res 40:45–58

Santoyo G, Urtis-Flores CA, Loeza-Lara PD, Orozco-Mosqueda MDC, Glick BR (2021) Rhizosphere colonization determinants by plant growth-promoting rhizobacteria (PGPR). Biology 10(6):475

Sarker A, Islam MT, Biswas GC, Alam MS, Hossain M, Talukder NM (2012) Screening for phosphate solubilizing bacteria inhibiting the rhizoplane of rice grown in acidic soil of Bangladesh. Acta Microbiol Immunol Hung 59:199–213

Saxena J, Saini A, Ravi I, Chandra S, Garg V (2015) Consortium of phosphate-solubilizing bacteria and fungi for promotion of growth and yield of chickpea (*Cicer arietinum*). J Crop Improv 29(3):353–369. https://doi.org/10.1080/15427528.2015.1027979

Schiltz S, Gaillard I, Pawlicki-Jullian N, Thiombiano B, Mesnard F, Gontier E (2015) A review: what is the spermosphere and how can it be studied? J Appl Microbiol 119(6):1467–1481

Seldin L (2011) Paenibacillus, nitrogen fixation and soil fertility. In Endospore-forming soil bacteria. Springer, Berlin, Heidelberg, pp 287–307

Sen S, Chandrasekhar CN (2014) Effect of PGPR on growth promotion of rice (Oryza sativa L.) under salt stress. Asian J Plant Sci Res 4(5):62–67

Shabanamol S, Edna MV, Meenu T, Karthika S, Sreekumar J, Jisha MS (2020) Enhancement of growth and yield of Rice (*Oryza sativa*) by plant probiotic endophyte, Lysinibacillus sphaericus under greenhouse conditions. Commun Soil Sci Plant Anal 51(9):1268–1282. https://doi.org/10.1080/00103624.2020.1751190

Shakeel M, Rais A, Hassan MN, Hafeez FY (2015) Root associated Bacillus sp. improves growth, yield and zinc translocation for basmati rice (Oryza sativa) varieties. Front Microbiol 6:1286

Singh JS, Koushal S, Kumar A, Vimal SR and Gupta VK (2016) Book review: microbial inoculants in sustainable agricultural productivity-vol II: functional application

Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease. Trends Biotechnol 27(3):141–150

Soltani SM, Hanafi MM, Karbalaei MT, Khayambashi B (2013) Qualitative land suitability evaluation for the growth of rice and off-seasons crops as rice based cropping system on paddy fields of central Guilan, Iran. Indian J Sci Technol 6(10):5395–5403

Stephen J, Shabanamol S, Rishad KS, Jisha MS (2015) Growth enhancement of rice (Oryza sativa) by phosphate solubilizing Gluconacetobacter sp.(MTCC 8368) and Burkholderia sp.(MTCC 8369) under greenhouse conditions. 3. Biotech 5(5):831–837

Tan KZ, Radziah O, Halimi MS et al (2015) Assessment of plant growth-promoting rhizobacteria (PGPR) and rhizobia as multi-strain biofertilizer on growth and N2 fixation of rice plant. Aust J Crop Sci 9:1257–1264

Tang MJ, Zhu Q, Zhang FM, Zhang W, Yuan J, Sun K et al (2019) Enhanced nitrogen and phosphorus activation with an optimized bacterial community by endophytic fungus Phomopsis liquidambari in paddy soil. Microbiol Res 221:50–59

Toju H, Okayasu K, Notaguchi M (2019) Leaf-associated microbiomes of grafted tomato plants. Sci Rep 9(1):1–11

Toure Y, Ongena MARC, Jacques P, Guiro A, Thonart P (2004) Role of lipopeptides produced by Bacillus subtilis GA1 in the reduction of grey mould disease caused by Botrytis cinerea on apple. J Appl Microbiol 96(5):1151–1160

Trân Van V, Berge O, Ngô Kê S, Balandreau J, Heulin T (2000) Repeated beneficial effects of rice inoculation with a strain of *Burkholderia vietnamiensis* on early and late yield components in low fertility sulphate acid soils of Vietnam. Plant Soil 218:273–284

Truyens S, Weyens N, Cuypers A, Vangronsveld J (2015) Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ Microbiol Rep 7(1):40–50

Umashankari J, Sekar C (2011) Comparative evaluation of different bioformulations of PGPR cells on the enhancement of induced systemic resistance (ISR) in Rice P. oryzae pathosystem under upland condition. Curr Bot 2:12–17

Valenzuela-Ruiz V, Robles-Montoya RI, Parra-Cota FI, Santoyo G, del Carmen Orozco-Mosqueda M, Rodríguez-Ramírez R, de Los Santos-Villalobos S (2019) Draft genome sequence of Bacillus paralicheniformis TRQ65, a biological control agent and plant growth-promoting bacterium isolated from wheat (Triticum turgidum subsp. durum) rhizosphere in the Yaqui Valley, Mexico. 3 Biotech 9(11):1–7

WFE (2022). https://www.weforum.org/agenda/2022/03/visualizing-the-world-s-biggest-rice-producers/. Accessed 6 Oct 2022

Woo SL, Pepe O (2018) Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. Front Plant Sci 9:1801. https://doi.org/10.3389/fpls.2018.01801

Yanni YG, Rizk RY, Corich V, Squartini AK, Ninke S, Philip Hollingsworth G, Orgambide F, de Bruijn J, Stoltzfus D, Buckley TM, Schmidt PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between Rhizobium leguminosarum bv. trifolii and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A, Corich V, Giacomini A et al (2001) The beneficial plant growth-promoting association of Rhizobium leguminosarum bv. trifolii with rice roots. Funct Plant Biol 28(9):845–870

Yanni Y, Zidan M, Dazzo F, Rizk R, Mehesen A, Abdelfattah F, Elsadany A (2016) Enhanced symbiotic performance and productivity of drought stressed common bean after inoculation with tolerant native rhizobia in extensive fields. Agric Ecosyst Environ 232:119–128. https://doi.org/10.1016/j.agee.2016.07.006

Yoshida S (1981) Fundamentals of rice crop science. Int Rice Res Inst

Yu X, Ai C, Xin L, Zhou G (2011) The siderophore-producing bacterium, Bacillus subtilis CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. Eur J Soil Biol 47 (2):138–145

Zubaidah E, Nurcholis M, Wulan SN, Kusuma A (2012) Comparative study on synbiotic effect of fermented rice bran by probiotic lactic acid bacteria Lactobacillus casei and newly isolated Lactobacillus plantarum B2 in wistar rats. APCBEE Procedia 2:170–177

Chapter 9
Strategies to Evaluate Microbial Consortia for Mitigating Abiotic Stress in Plants

Sarita Sharma, Zalak R. Rathod, Ritika Jain, Dweipayan Goswami, and Meenu Saraf

Abstract Abiotic stress is the most significant constraint to agricultural productivity. Crop plants must deal with adverse external pressures caused by environmental conditions through their internal biological systems, leading to a loss in development, growth, and productivity. Plant-associated microbes are crucial to crop yields. Although numerous studies have shown that single bacteria can benefit plants, it is becoming increasingly clear that when a microbial consortium—two or more associating microorganisms are implicated, synergistic or additive results can be predicted. Microbial consortia, which are being assessed as a strategy for applications in a range of fields, must be characterized and managed. In this review, we propose a step-by-step technique for identifying whether the plant growth-promoting microorganisms (PGPMs) included can form viable microbial consortia for future application, and if so, how to establish the ideal combinations. To determine the optimal consortia combinations, different techniques were used, in which diverse PGPMs with host growth-supporting features were explored to evaluate if they could function in cohesion and offer a cumulative effect toward better plant growth promotion. To evaluate the valuable microbial consortia, tests for compatibility, response to external stimuli (pH, temperature), generation time, a unique and rapid plant bioassay, and pot experimentation strategies should be employed. Scanning electron microscopy (SEM) and transmission electron microscope (TEM) methods can be employed to confirm the presence of microbial consortia on the roots of plants. The microbial consortium found in the root microbiome stimulates plant growth by regulating the synthesis of phytohormones, osmolytes, organic acids, increased nutrient intake, and an enhanced antioxidant system, all of which help plants to cope with stress. In this review, we cover the numerous strategies that can be used to develop the most competent consortia and their prospective application in managing abiotic stress.

S. Sharma · Z. R. Rathod · R. Jain · D. Goswami · M. Saraf (✉)
Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India
e-mail: msaraf@gujaratuniversity.ac.in

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_9

Keywords Microbial-consortia · PGPMs · Rapid plant bioassay · SEM · TEM · Abiotic Stress

9.1 Introduction

Since the onset of civilization, agriculture has been the most important source of income (Gouda et al. 2018). Food security has been one of society's primary issues for a long time, and any element that threatens it has been one of society's challenges. With an increasing population rate and an unsustainable traditional agricultural system, farmers and the government are struggling with how to produce enough food to fulfill global demand (Prajapati et al. 2022a, b; Khoshru et al. 2020). According to the FAO, agricultural land covers 38.47% of the world's land area, and while 28.43% of that land is arable, only 3.13% is permanently used for crop production. The issue has deteriorated as 20–25% of land worldwide is degraded each year, with another 5–10 million hectares destroyed each year. The movement of nutrients, energy, and carbon between soil organic matter, the soil environment, the aquatic ecosystem, and the atmosphere has a significant impact on agricultural productivity, water quality, and climate change (Gouda et al. 2018).

Abiotic stresses are major constraints of plant growth and development, which in turn affects crop yield, food quality, and global food security. Under stress conditions, numerous parameters such as biochemistry, molecular biology, and physiology of plants are affected. The use of chemical pesticides and inorganic fertilizers causes environmental pollution and degradation of soil fertility. During the stress period, the plant releases certain exudates that can act as a signaling mechanism to alter or create a healthy rhizosphere soil community (Shaikh et al. 2022; Prajapati et al. 2022a, b).

A well-studied and sustainable alternative for improving plant growth and soil fertility is the application of plant growth-promoting bacteria (PGPB) as biofertilizers, which possess functional traits that regulate the growth, development, and productivity of crops. These growth-promoting effects are due to the improvement of the availability and biosynthesis of several limiting macro- and micronutrients, as well as crop protection against stressful environmental conditions. Plant growth-promoting microorganism (PGPM) is a term that applies to all microorganisms (e.g., bacteria, actinomycetes, fungi, and algae) that have a beneficial effect on plant growth through the action of either direct or indirect mechanisms (e.g., mineral nutrition, ethylene reduction, disease suppression). PGPMs have a significant role in sustainable agriculture. They increase the production of various crops, improve soil fertility, promote diversity and interaction with other beneficial microorganisms, inhibit the growth and infective action of potential pathogens, and generally maintain the sustainability of the systems (Prajapati et al. 2022a, b; Santoyo et al. 2021).

The application of microbial consortia to agricultural fields is an innovative natural approach, which can help plants tolerate different stress conditions and enhance plant growth as compost is made up of diverse microbial consortia that can function in different temperature segments (Sathiavelu 2021).

9.2 Strategies for the Development of Microbial Consortia/Rhizobacterial Consortia

9.2.1 What Are Microbial Consortia?

Rhizobacteria that stimulate plant growth are a symbiotic association between plants and microbes found in the rhizosphere that boost plant growth (Rochlani et al. 2022). The roots are referred to as rhizomes, and the surrounding environment is referred to as spheres. The rhizosphere is the zone of soil that surrounds a plant's root system. The zone, which is around 1 mm wide, has no defined edges. Rhizobacteria are bacteria found in the rhizosphere that can create an environment for roots (Rochlani et al. 2022; Jha and Saraf 2015). The varied microbial communities of the rhizosphere enable the formation of microorganisms that can stimulate plant growth under abiotic conditions via direct and indirect mechanisms (Rochlani et al. 2022; Shaikh et al. 2022; Saraf et al. 2017).

Currently, agriculture is heavily dependent on mineral fertilizers and inorganic pesticides (inorganic), and the impact of their continuous application is reflected in deteriorating soil health and increased resistance to pests and pathogens (Prajapati et al. 2022a, b). In the past 40 years, usage of nitrogen fertilizers has increased by sevenfold and pesticide usage by threefold. In the future, these trends will continue unabated, as the application threefold of both inorganic fertilizers and pesticides is expected to increase by an additional threefold by 2050, which will cause unprecedented damage to the agroecosystem (Sekar et al. 2016). Engineering the plant rhizomicrobiome is an alternative approach to increasing soil health and enhancing plant productivity (Pindi and Satyanarayana 2012). Microbial interaction in the rhizosphere provides plants with multiple plant growth-promoting traits and different stress-tolerant traits apart from enhancing their own population and function (Sekar et al. 2016; Keswani et al. 2014). The inconsistency in the performance of a single microbial product in field application has emphasized the need for co-inoculation or consortia of the microbial products (Santoyo et al. 2021).

Although numerous studies have shown that single microorganisms can benefit plants, it is becoming increasingly clear that when a microbial consortium (mixed culture)—two or more interacting microorganisms—is involved, additive or synergistic results can be assumed. This is owing, in part, to the fact that multiple species can perform a range of activities in an ecosystem like the rhizosphere. The use of mixed cultures of beneficial microorganisms as soil inoculants is based on the principles of natural ecosystems, which are sustained by the quality and quantity of their inhabitants and specific ecological parameters, i.e., the greater the diversity and number of inhabitants, the higher the order of their interaction and the more stable the ecosystem (Higa 1994). The mixed culture technique is essentially an attempt to apply these ideas to natural systems such as agricultural soils in order to alter the microbial balance in favor of enhanced plant growth, productivity, and protection (Santoyo et al. 2021; Higa 1994;).

Beneficial plant growth stimulation mechanisms include increased nutrient availability, phytohormone modulation, biocontrol, and biotic and abiotic stress tolerance exerted by various rhizosphere microbial players such as plant growth-promoting bacteria (PGPB) and fungi such as *Trichoderma* and *Mycorrhizae* (Prajapati et al. 2022a, b; Santoyo et al. 2021).

The influence of different PGPR strains on plants has been thoroughly investigated in recent years, leading to the commercialization of a significant number of microbial inoculums (Santos Villalobos et al. 2018; Reed and Glick 2013). The construction of bacterial consortia has received interest as a feasible technique for sustainable food production to improve the beneficial capabilities exhibited by these bacteria. In rare circumstances, a consortium of several strains of the same species can display improved activity and be considered. Due to their coverage of a varied set of plant growth promotion and biological regulatory mechanisms, bacterial consortia have been shown to boost beneficial traits in plants as compared to individual strains (Ju et al. 2019). The adoption of these consortia is a viable technique for improving agricultural crops under drought (Joshi et al. 2020), salinity (Sharma et al. 2022c; Nawaz et al. 2020), heavy metal (Prajapati et al. 2022a, b), nutrient uptake (Rana et al. 2012), pests, and phytopathogenic diseases (Villa-Rodriguez et al. 2019). Furthermore, some bacterial consortiums can fix nitrogen, convert some inaccessible nutrients into assimilable forms, produce phytohormones, and chelate iron, all of which are important in maintaining soil quality and health; these can also mitigate the negative effects of some conventional nonsustainable farming techniques (Shaikh et al. 2022).

Rhizobacterial consortia are classified into two types: simple and complex. The fermentation method or protocol (generation of a large population of bacteria to be later made into an inoculant), in which strains are grown individually or in combination with other species/strains in a suitable medium for all PGPR species (Bashan and Prabhu 2020), is the difference. This is an essential stage because a greater number of species often results in a greater number of interactions between strains, resulting in changes in metabolite secretions. The effectiveness of bacterial consortia in field conditions, on the other hand, is reliant on the type and function of strains utilized, where some elements demand special consideration, such as tolerance to severe climatic conditions, survival, and persistence in the soil after inoculation (Gosal and Kaur 2017; Verbruggen et al. 2013;).

The source of the strain isolation influences the selection of these strains because consortium members must grow in the environmental conditions (soil type, host, and climate) where they will be applied. Additionally, when two or more strains form a rhizobacterial consortium, each strain not only competes with the others functionally for plant growth promotion but also complements the others for soil and/or plant establishment (Sharma et al. 2020; Ney et al. 2018; Morriën 2016; Pandey et al. 2012) (Fig.9.1).

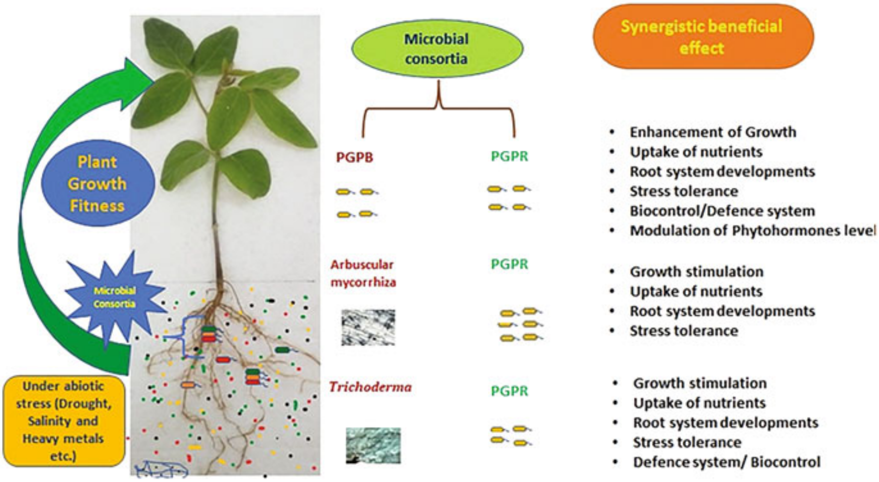


Fig. 9.1 Combining different rhizosphere microorganisms to form microbial consortia can promote plant growth, development, and nutrient uptake while also improving the plant defense system against diseases and enhancing tolerance to various environmental stresses

Though the concept of the consortium is theoretically feasible, developing a consortium is a challenge for researchers due to factors such as the mutual compatibility of microbes, their reliance on one another, and the task of maintaining inoculum potential while not depleting plant resources excessively during mutualism/symbiosis. There is currently no conventional or experimentally confirmed process for screening and choosing promising consortia among a vast number of theoretically feasible consortia. The traditional hit-and-trial strategy yields a vast number of combinations and time-consuming approaches (at least 4 months), which are applied at random, leaving room for scientific improvisation. To address this specific issue, we explored the various methodologies for evaluating rhizobacterial microbial consortia (Fig. 9.2), as outlined below.

9.2.1.1 Step 1: Analysis of Traits of Plant Growth-Promoting Rhizobacteria

Beneficial bacteria and fungi that act as plant growth-promoting microorganisms (PGPMs) can alleviate stress and stimulate plant growth in two ways: indirectly by inducing defense mechanisms against phytopathogens and/or directly by solubilizing mineral nutrients (nitrogen, phosphate, potassium, iron, and so on), producing plant growth-promoting substances (e.g., phytohormones), and secreting specific enzymes (e.g., 1-aminocyclopropane1-carboxylate deaminase).

To prepare possible rhizobacteria consortia, all selected rhizobacteria could evaluate for plant growth-enhancing properties. The following are plant growth-enhancing attributes:

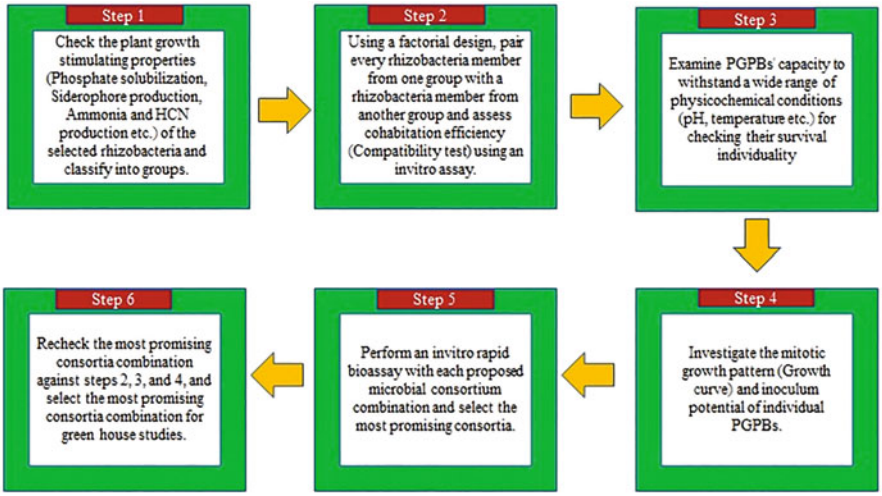


Fig. 9.2 The "strategies" for selecting the most promoting consortia of plant growth-promoting bacteria

(a) Phosphate solubilization: The ability of rhizobacteria to solubilize insoluble phosphates has been investigated using Pikovskaya's medium (Pikovskaya 1948). Each rhizobacterial culture spot could subsequently be inoculated in the center of Pikovskaya's media agar plates with tricalcium phosphate [$Ca_3(PO_4)_2$] as an insoluble phosphate source. Rhizobacteria that can dissolve insoluble phosphates will generate halos. Using the diameter of clearing halo zones, the P solubilization index (PSI) is determined using the formula below (Rathod and Saraf 2021a; Jain et al. 2020).

$$\text{Phosphate solubilization index (PSI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

(b) Siderophore production: Iron [Fe (III)] is required by all organisms as a cofactor for numerous critical metabolic activities. Siderophores are low-molecular-weight organic ligands secreted by soil microorganisms that bind to iron and release it for microbial absorption. Iron shortage in metal-stressed soils can be remedied using siderophores generated by various bacterial genera. Siderophores increase IAA production by chelating hazardous metal species, and IAA has been shown to benefit metal remediating plants. The CAS agar method is used to determine siderophore production (Schwyn and Neilands 1987). In the dark blue medium, the formation of a bright zone with yellowish (hydroxamate), pinkish (catecholate), and whitish (carboxylate) colors indicated the production of siderophore (Saraf et al. 2017).

(c) Hydrogen cyanide and ammonia production: The production of HCN and ammonia is regarded as an indirect plant growth enhancer. A volatile chemical with antifungal qualities is HCN. In addition to helping the host plant meet its nitrogen needs, ammonia production can help prevent disease invasion. HCN's strong toxicity against phytopathogens makes it a popular biocontrol agent in agricultural systems. However, HCN is also used to chelate metal ions and therefore indirectly contributes to the availability of phosphate (Mahmud et al. 2021). Alström and Burns (1989) found that the synthesis of HCN by rhizobacterial culture could be determined by the color change of filter paper. A change in the color of the filter paper from yellow to light brown, brown, or reddish-brown had been recorded as a weak (+), moderate (++), or strong (+++) production of HCN. NH_3 production could be determined by the method described by Cappuccino and Sherman (1992). The formation of yellow to brown precipitate showed the presence of NH_3 (Trivedi et al. 2018; Jha and Saraf 2011).

(d) Indole acetic acid production (IAA): The synthesis of indole acetic acid was determined using the method described by Bric et al. (1991). The pink color that developed after adding Salkowsky's reagent to cell-free supernatant was spectrophotometrically measured (Shah et al. 2020; Patel et al. 2012).

(e) Biocontrol activity: The agar diffusion method can be used to assess antibacterial and antifungal activity (Sharma et al. 2022b; Thakkar and Saraf 2015).

9.2.1.2 Step 2: Compatibility Efficiency Studies

Individual plant growth-promoting rhizobacteria in the consortia must cultivate in synchrony to exert synergistic effects on plant growth. For that, a paired-wise growth performance study could be conducted in vitro to examine the presence of any antagonism among individual members of the two and more plant growth-boosting rhizobacteria groups. The compatibility efficiency assay has been constructed so that every PGPM member in group I received challenges with every other PGPM member in group II and group III and more. Overnight-grown broth cultures of the relevant PGPMs (one from each group I and II) could be streaked in two halves of nutrient agar. After incubation at optimum temperature, all plates were evaluated for the presence of any zones of inhibition at the colony borders where the two cultures intersected. If the counterpart did not show any zone of inhibition, it suggests the absence of any diffusible toxins or volatile substances that could cause antagonism against each other (Rathod et al. 2020; Rathod and Saraf 2021b; Prasad and Babu 2017). This study provides proof that the tested plant growth-boosting rhizobacterial consortia are growing in a mutually noninhibitory manner, paving the way for further research on microbial consortia.

9.2.1.3 Step 3: Sensitivity to Physical and Chemical Conditions

Temperature responses might have been measured in the 20–45 °C range, while pH can be studied at optimal temperatures in the 5–11 range (with unit interval). In both trials, an aliquot of overnight-developed plant growth-promoting rhizobacterial cultures has been employed as inoculum. Each experiment was carried out in triplicate to ensure that the results appeared reproducible. Rhizobacteria growth has been assessed spectrophotometrically after overnight incubation at various temperatures and pH levels (Sharma and Saraf 2022).

9.2.1.4 Step 4: PGPR Growth and Mitotic Behavior

Generation times of co-habiting rhizobacteria in microbial consortia would ensure a balance in the relative inoculum density of distinct isolates. If one microbial consortia member develops faster, it may deplete the medium's nutrients and provide unsuitable growth circumstances for the other members of the consortia. The same mitotic growth behavior of PGPR consortia supports co-survival ability and their attractiveness as prospective candidates for consortia creation. At the most optimum temperature and pH conditions, the growth kinetics of each plant growth-promoting rhizobacteria can be studied. Overnight-developed plant growth-promoting rhizobacteria culture was inoculated in triplicate in nutrient broth and incubated in a BOD incubator at 150 rpm. Then, at regular intervals, culture broth aliquot could be collected and a growth curve could be produced with time (in hours) on the x-axis and absorbance (OD measured at 600–610 nm) on the y-axis (Sharma et al. 2021; Jha and Saraf 2012). The generation time can be calculated using standard methods. The generation time is the amount of time it takes for the cells (or population) to divide (Todar 2015).

$$\mathrm{G\ (generation\ time)} = \frac{t(\mathrm{time,\ in\ minute\ or\ hours})}{n\ (\mathrm{number\ of\ generations})}$$

$n = 3.3\mathrm{logb/B}$

$$\mathrm{G\ (generation\ time)} = \frac{t(\mathrm{time,\ in\ minute\ or\ hours})}{3.3\log b/B}$$

t = time interval in hours or minute.

B = number of bacteria at the beginning of a time interval.

b = number of bacteria at the end of the time interval.

n = number of generations (number of times the cell population doubles during the time interval).

9.2.1.5 Step 5: Design of Microbial Consortia

Microbial consortia combinations can be studied for their plant growth stimulating efficiency using a two- and three-factorial design approach, ensuring that each consortium comprises one member from group I (perhaps rhizobacteria) with two members from groups II and III (may be endophyte) and may be between different rhizobacteria. Under aseptic conditions, equal amounts of overnight-grown cultures of the various PGPMs (~10^8–10^9 cfu/mL) are mixed together to form consortia combinations, which would then be employed for application in agriculture crop and selecting consortia combinations that show significant increments in vegetative growth parameters as compared with noninoculated crop (Sharma et al. 2022c; Mishra and Sundari 2017; Jha and Saraf 2012).

9.2.1.6 Step 6a: Rapid Plant Bioassay

Mishra and Sundari (2017) created a quick plant bioassay technique that has been utilized to assess the microbial community. It is an in vivo plant growth study in which seeds are surface sterilized according to standard protocol, coated with the corresponding consortia combinations, and placed for germination. Mishra and Sundari (2017) established a novel experimental setup termed the "tube-in-tube" approach in their laboratory using germinated seeds with healthy radicals and plumules. The cap of the sterilized Eppendorf tube (1.5 mL) has been removed, and the bottom could be subsequently cut to create an opening. Germinated seeds were then transferred aseptically into this Eppendorf tube, and the assembly could then be placed in an autoclaved glass test tube (50 mL capacity), giving rise to the term "tube-in-tube" (Fig. 9.3).

It is an in vivo plant growth study in which seeds can surface sterilized as per standard protocol and coated with the respective consortia combinations (detailed in seed germination paragraph) and placed for germination. Germinated seeds with healthy radicals and plumules could be selected and transferred to the novel experimental setup called the "tube-in-tube" method developed by Mishra and Sundari (2017) in their laboratory. The cap of the sterilized Eppendorf tube (1.5 mL) could be removed and bottom cut to make an aperture. Germinated seeds could be transferred aseptically into this Eppendorf, and the assembly could place in an autoclaved glass test tube (50 mL capacity), thus drawing its name "tube-in-tube" method. The test tube may hold 10 mL of half-strength modified Melin–Norkrans medium, free of glucose and malt. The entire "tube-in-tube" system could be then closed with sterile cotton to maintain aseptic conditions and incubated for 10 days at 30 2 °C with no sunshine regulation. To keep the root system in the dark, the bottom portion of the complete test tube rack carrying the setup was wrapped in a black sheet of paper (Fig. 9.2). SEM has been used to visually confirm bacterial attachment with plant roots after harvest. The influence of various PGPR consortia on plant growth may be assessed using four parameters: root length (RL), shoot length (SL), root dry weight (RDW), and shoot dry weight (SDW). The geometrical mean for the different combinations of RL and SL could be determined, and deviations among replicates could be reported as standard deviations (Mishra and Sundari 2017). This rapid plant bioassay technique ("tube-in-tube method") proved to be effective for screening a large set of consortia combinations in a short span of time (Fig.9.3).

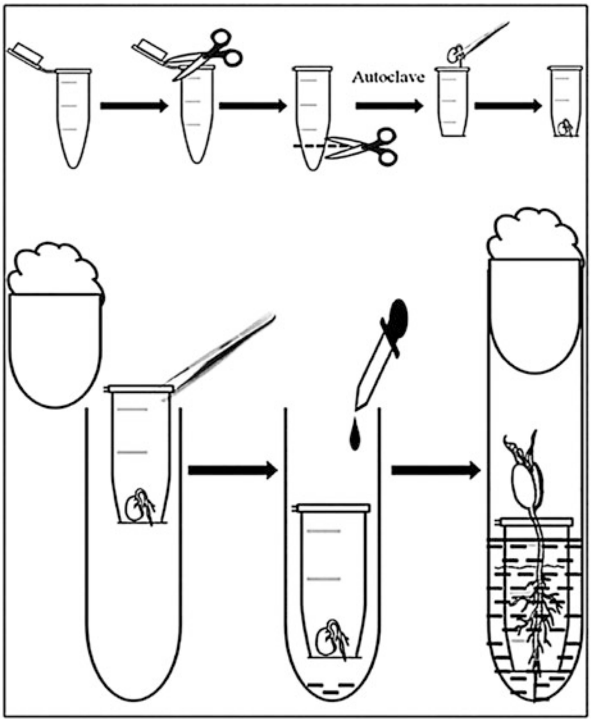


Fig. 9.3 Rapid plant bioassay ("tube-in-tube") approach: (**a**) autoclave Eppendorf (1.5 mL); (**b**, **c**) Eppendorf cap, and bottom removed; (**d**, **e**) Eppendorf germinated seedling; *f*, Eppendorf in an autoclaved test tube with seedling; (**g**) a glass test tube containing medium; (**h**) plugged tube-in-tube system to preserve aseptic state for plant growth (Mishra and Sundari 2017)

9.2.1.7 Step 6b: Pot Experiments

Rhizobacterial consortiums with promising plant growth-boosting properties are evaluated for seed germination. Seeds are surface sterilized with 0.2 percent $HgCl_2$ for 2 min before being washed in sterile distilled water for 10 min. Seedlings for 7–8 h in YEMA/nutrient broth with a pre-screened rhizobacteria consortium combination in log phase containing approximately 10^8–10^9 CFU/mL are held at optimal temperature in a shaker. Control seeds are immersed in a sterile medium. The seeds are then dried aseptically in laminar air flow overnight before being employed in pot experiments. For pot experiments, only sterile soil should be used. Standard protocols could be used to analyze physicochemical parameters. Transfer the sterile soil to pots; the amount of soil used for pot studies is determined by the size of the pots. Standard germination (percentage) of seeds can be counted until no further germination occurs (Rathod et al. 2021; Jha and Saraf 2012). Seedling vigor indices could be determined using the formula proposed by Abdul-Baki and Anderson (1973) as follows: -

$$\text{Seedling Vigour Index (SVI)} = \text{Total Seedling Length (cm)} \times \text{Germination Percentage (\%)}$$

After 1 month, the vegetative growth parameters are assessed to assess the influence of microbial consortia on plant growth in comparison to noninoculated pots. Confirm the presence of microbial consortia on the roots of plants using scanning electron microscopy (SEM) and transmission electron microscope (TEM) techniques.

By applying step-to-step strategies, the best and most manageable number of consortia are thus shortlisted for further field trials to improve productivity in a sustainable manner.

9.3 Microbial Consortia on Plant Roots: Scanning Electron Microscopy (SEM)/Transmission Electron Microscopy (TEM)

Basically, SEM is used in microbiology for analyzing the organism's morphological structure and measurement of size. Nowadays, it is widely used for the observation of microorganisms adhering on plant parts (Root, Shoot, etc.). Trivedi and Saraf (2019) studied endophytes from the *Ricinus communis* plant's stem, leaves, and root. Despite being an excellent tool for investigating ultrastructure, scanning electron microscopy (SEM) is less frequently used than transmission electron microscopy for microbes such as viruses or bacteria. SEM could be used for visual confirmation of bacterial association with plant roots. Olivares et al. (2017) observed the *H. seropedicae* strain HRC54 attached with humic acid plates on the sugarcane leaf surface. Kim and Krcmcr (2005) utilized SEM techniques to detect IAA-producing bacteria viz. *Pseudomonas putida*, *Bacillus megaterium*, *Bradyrhizobium japonicum*, etc., which had been isolated by the IAA screening method based on an in situ membrane assay (Bric et al. 1991) (Fig. 9.4).

Mishra and Sundari (2017) developed the microbial consortia with *Pseudomonas* and diazotrophs applied to *Sorghum bicolor* plant as plant growth-promoting consortium (PGPC). Colonization pattern of primary tomato roots by *Pseudomonas fluorescens* SEM aids in evaluating consortium formation and activity on plants to identify specific microorganisms present in the plant when the consortium can measure multiple microorganisms, as well as their size and structure.

Transmission electron microscopy (TEM) is used for seen internal structure by sending an electron beam across a sample. As a result, an image of the sample's internal structure is created up to 50 million times from its original size. TEM, on the other hand, produces a two-dimensional image. The organism having the ability to accumulate metal has been seen in TEM (Avendaño et al. 2016). Trivedi and Saraf (2019) examined selenium accumulation in endophytic selenobacteria using transmission electron microscopy (TEM) (Fig. 9.5). Sodium selenite had decreased and

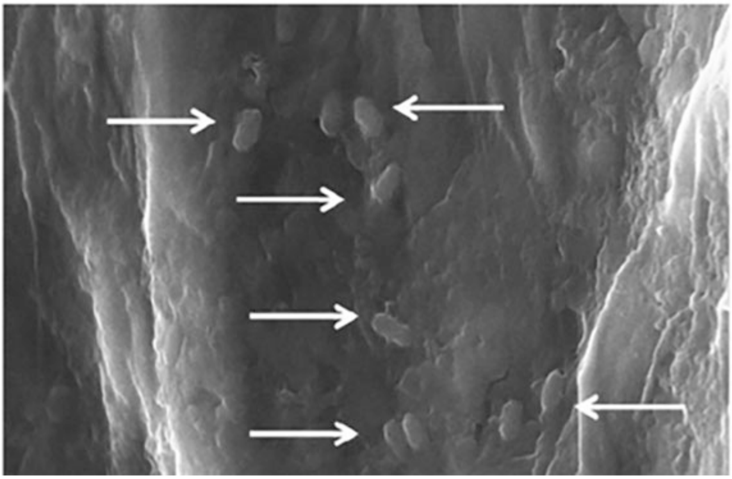


Fig. 9.4 SEM of PGPC association with *Sorghum bicolor* plant roots (Mishra and Sundari 2017)

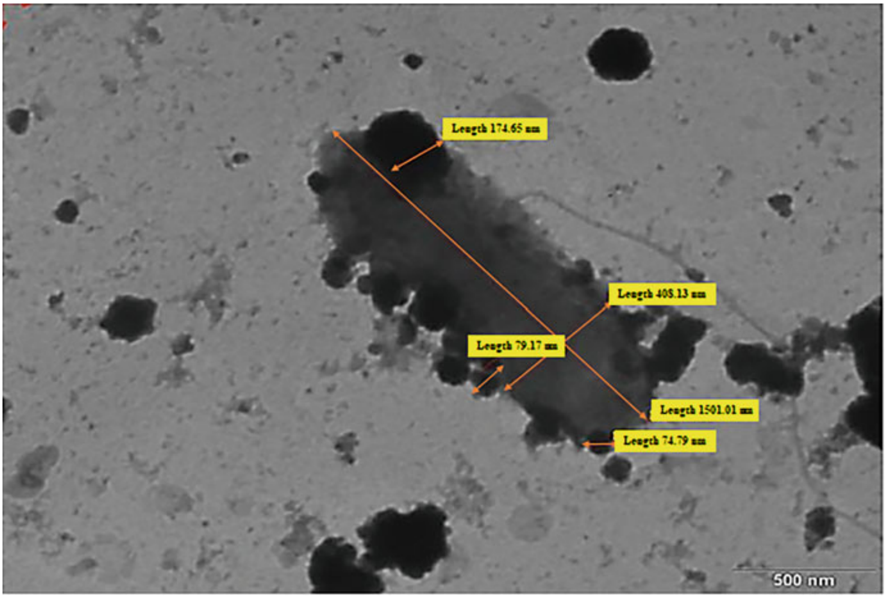


Fig. 9.5 Transmission electron microscopy (TEM) of selenium particle accumulation around endophytic selenobacterial isolates *Parburkholderia megapolitana* sp. *MGT9*. (Trivedi and Saraf 2019)

accumulated in the bacterial cell because of the accumulation of elemental selenium. The bacterial cell revealed red coloring because the reduced form of sodium selenite is red in color. In the presence of selenite, most of the selenium build-up occurred in the internal cell membrane of bacteria with wavelengths of 174.65 nm, 74.79 nm, and 76.32 nm, according to TEM imaging (Trivedi et al. 2020).

Transmission electron microscopy (TEM) micrographs showed the spherical NPs, which had an average diameter of 12 nm. By mixing it with an aqueous solution of $AgNO_3$, *Argemone mexicana* leaf extract serves as a capping and reducing agent in the creation of AgNPs. Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants were explained by Alabdallah and Hasan (2021). The use of TEM is beneficial in the field of microbiology because it can be used to identify and measure the structure and size of microorganisms that can bind or chelate metals, especially in contaminated sites and waste treatment facilities for dump yards. TEM images can also be used to resolve metal-contaminated fields or accumulate micro and macro metals in crops.

9.4 Role of Microbial Consortia as Efficient Biofertilizer

Microbial inoculants are mixtures that contain live algae, fungus, and bacteria, whether in alone or in a consortium, to boost plant growth and increase agricultural output. Beneficial microbes (algae, fungus, and bacteria alone or in a consortium) in biofertilizers improve soil chemical and biological attributes by fixing nitrogen, cellulolytic activity, iron, or phosphate (Mahmud et al. 2021; Seenivasagan and Babalola 2021). Microbes mostly as biofertilizers accomplish beneficial actions such as phosphorus solubilization, nitrogen fixation, siderophore formation, hydrogen cyanide, and ammonia synthesis, and the production of plant growth chemicals. Because of the presence of these bacteria, plants have antagonistic effects on a variety of phytopathogens (Rochlani et al. 2022; Jha and Saraf 2012). They inhabit the rhizosphere, whether applied to seed, plant surfaces, roots, or soil, and through their biological activity, they improve nutrient bioavailability, boost plant growth, and increase soil microflora. As a result, they are preparations that quickly restore soil fertility (Mahmud et al. 2021; Seenivasagan and Babalola 2021; Jha and Saraf 2015). They are critical elements of integrated nutrient management (INM) strategies for increasing soil productivity and sustainability while also preserving the environment because by being pollution-free, cost-effective, and a source of renewable nutrients to plants to replenish synthetic fertilizers in a sustainable production system (Yadav and Sarkar 2019). According to Panda (2011), the impact of bio-fertilizers on crop improvement ranges from 35% to 65% (Mahmud et al. 2021). The continual application of biofertilizer to the land for 3–4 years can retain fertility due to the efficacy of parental inoculums, which can successfully maintain plant growth and multiplication. They improve the texture, pH, and other characteristics of the soil. Biofertilizers are low-cost, sustainable sources of plant nutrients that are supplemental artificial fertilizers. In comparison to chemical fertilizers, biofertilizers are more environmentally friendly; they can be created from natural sources, are less likely to cause damage, and aid in the development of healthy soil. To some extent, plants are cleansed of chemical fertilizers that are precipitated

(Seenivasagan and Babalola 2021). Depending on their capabilities, such as delivering nutrients to plants and acting as natural pest deterrents, a wide range of microorganisms can be used as biological fertilizers at the industrial level (Rochlani et al. 2022). When considering biofertilizer as a modern agricultural tool, its use is critical as a component of integrated nutrient management, a reduction in the use of hazardous chemicals, a cost-effective source of renewable energy for plants, and a source of renewable energy for plants in sustainable agriculture (Seenivasagan and Babalola 2021).

9.5 Mechanisms as Biofertilizer

Biofertilizers are classified into several categories based on their functional capabilities, such as nitrogen-fixing biofertilizers, phosphate biofertilizers, micronutrient biofertilizers, and plant growth-promoting rhizobacteria, among others. Nitrogen-fixing biofertilizers increase soil nitrogen levels by absorbing atmospheric nitrogen and releasing it to plants. *Azotobacter*, *Nostoc*, *Rhizobium*, and *Azospirillum* are a few examples (Itelima et al. 2018). Phosphate biofertilizers are divided into two types: phosphorous solubilizing biofertilizers (PSB) and phosphorus mobilizing biofertilizers (PMB). PSB dissolves insoluble phosphate from organic and inorganic sources. *Bacillus*, *Pseudomonas*, *Penicillium*, *Aspergillus*, and other bacteria are examples (Etesami et al. 2017). Phosphorus is transferred from the soil to the root cortex via PMB. Arbuscular Mycorrhiza is one example (AM fungi). Micronutrient biofertilizers include silicate and zinc solubilizer bacteria. In soil, these bacteria break down silicates and aluminum silicates. *Bacillus* sp. is one example. Plant growth-promoting rhizobacteria (PGPR) are bacteria that live in the rhizosphere (Upadhyay et al. 2019). The rhizosphere is a thin layer of soil surrounding the roots characterized by high levels of biochemical activities and composed of plants, bacteria, fungi, and soil constituents. They boost plant growth by functioning as bioprotectants, biostimulants, and nutrient enhancers (Fig. 9.6).

The mechanism of action refers to the biological and chemical process by which microorganisms contained in biofertilizers exert their effects on the plant's rhizosphere. Plant growth rhizobacteria can execute a variety of mechanisms that increase plant growth and development, eventually leading to sustainable agriculture methods. Direct mechanisms of these rhizospheric bacteria can increase plant growth by increasing nutrient intake via nitrogen fixation, phosphate solubilization, phytohormone production, and exopolysaccharide production, resulting in sustainable and eco-friendly agri-science perspective. These microorganisms also have an indirect role in plant protection by producing antibiotics, hydrogen cyanide, siderophores, and other biocontrol chemicals (Rochlani et al. 2022; Prajapati et al. 2022a, b; Panchal et al. 2022). Surprisingly, these relationships between plant–root and microbial communities have been labeled as symbiosis. As the former decomposes unavailable nutrients into an available form, the latter benefit from root exudates such as carbohydrates, proteins, sugars, vitamins, mucilage, amino acids, and

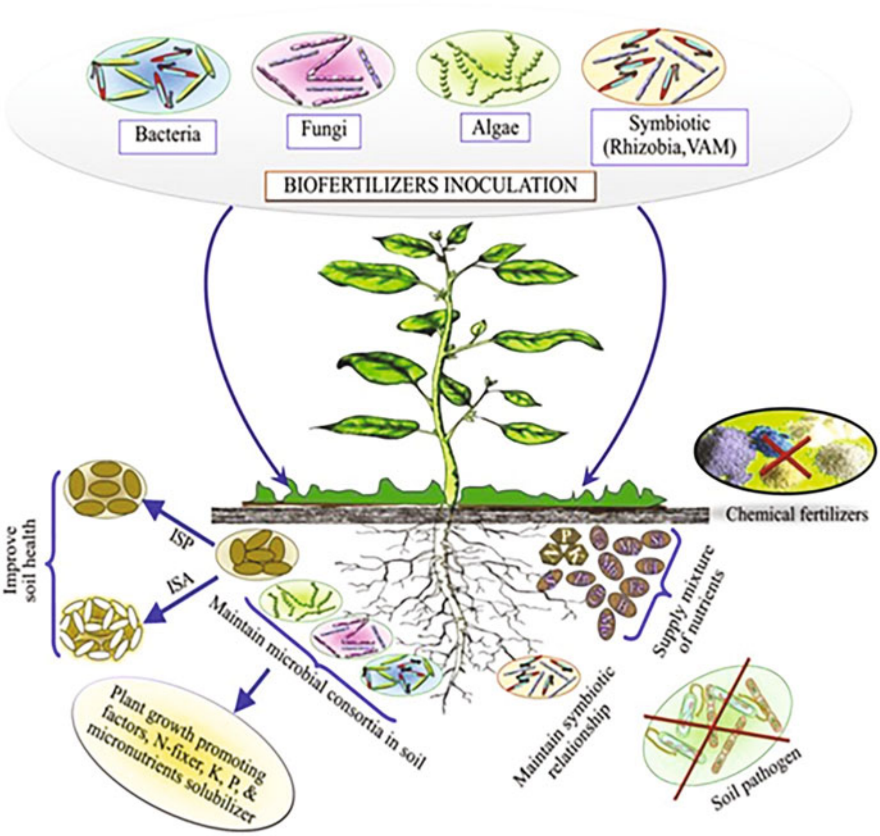


Fig. 9.6 The effect of biofertilizers on plant growth and soil health. [*VAM* vesicular-arbuscular mycorrhiza, *ISA* increased soil aggregation, *ISP* increased soil porosity] (Mahmud et al. 2021). https://www.researchgate.net/figure/Schematic-representation-Influence-of-biofertilizers-on-plant-growth-performance-and_fig1_353131148

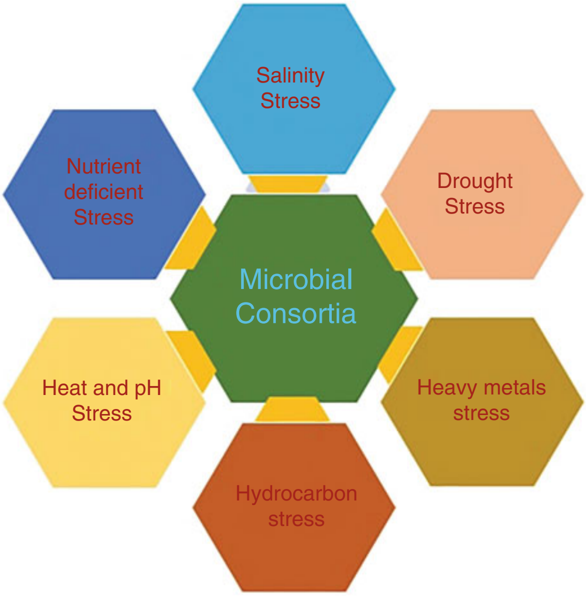
organic acids (Vives-Peris et al. 2020), which modify biochemical properties of the rhizosphere by acting as a messenger between the microbes and the plants (Shaikh et al. 2022; Vives-Peris et al. 2020).

9.6 Role of Microbial Consortia to Remediate Abiotic Stress

9.6.1 Abiotic Stress Affecting Crop

There are various types of abiotic stress that affect soil and ultimately crop productivity. These stresses are salinity stress (increase in salts concentration in soil), drought stress (insufficient water availability to plants), heavy metal stress

Fig. 9.7 Microbial consortia as biofertilizer to remediate abiotic stress



(excessive harmful metals in soil), temperature stress (very high and very low temperature), and nutrients stress (insufficient nutrients in the soil) (Fig. 9.7). In this review, we describe three major soil stress: drought, salinity, and heavy metals. Drought and salt stress have a complex relationship that affects almost every element of a plant's life. Both these stresses have the most detrimental effects on agriculture (de Oliveira et al. 2013). Stress causes disturbance in photosynthesis, resulting in leaf senescence, the formation of excessive reactive oxygen species (ROS), nutritional deprivation, and the breakdown of cellular organelles and metabolism, all of which result in diminished plant growth (de Oliveira et al. 2013). Another key soil stress is heavy metal stress, which is getting more intense because of a variety of anthropogenic influences (Glick 2010). Unchecked population growth and the industrial revolution are accumulating toxic metals and organic wastes in soil, rendering it unfit for agricultural techniques and detrimental to all living things (Glick 2010).

One of the most common abiotic factors impacting crop plants is water deprivation. Drought stress occurs when the amount of water available in the soil diminishes. Drought produces a variety of harmful consequences on plants that are multifaceted in their effects. From seed germination to maturity and senescence, the plants respond to drought stress at physiological, biochemical, and molecular levels (Tiwari et al. 2017). Because plants need to use groundwater, their root length increases under mild drought stress (Forni et al. 2017), while extremely dry conditions can slow root growth. However, PGPR under stress conditions modifies root architecture and boosts plant nutrient absorption and water drawing ability (Shaikh et al. 2022; Kasim et al. 2013). These rhizobacteria could be able to grow under stressful conditions and provide a beneficial effect on plants to cope with stressful environments (Jain et al. 2020; Jain and Saraf 2021; Bilal et al. 2018). ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, production of exopolysaccharide (EPS) (Panchal et al. 2022) and volatile organic compounds (VOCs), osmolyte and antioxidant production, enhanced mineral nutrient uptake, phytohormone production, and modulation are among the mechanisms proposed by PGPM to overcome drought stress in plants. The PGPRs are bestowed through these pathways, either singly or jointly, to counteract drought stress in plants (Gontia-Mishra et al. 2020). Microbial consortia of *Pseudomonas putida* NBRIRA + *Bacillus amyloliquefaciens* NBRISN13 mitigates drought stress in chickpea by enhancing physiological parameters such as shoot length, root length, and fresh and dry weight of root and shoot, modulates defense enzymes such as superoxide dismutase, catalase, lipid peroxidase, and enhance soil enzymes activity and microbial diversity in the rhizosphere region under drought stress (Kumar et al. 2016).

Soil salinity is defined a salt level that exceeds the plant's requirements. When the electrical conductivity (EC) in the soil surrounding the root zone reaches 4 dS/m (40 mM NaCl), the soil becomes saline (Egamberdieva et al. 2017). Excessive salt concentrations causing low water availability create drought-like conditions and result in altering the physicochemical features of soil and interfering with nutrient uptake, rendering nutrients unavailable to plants. Salt stress affects plant growth, photosynthetic capacity, CO_2 assimilation, and nitrogen content and leads to ion toxicity, which results in oxidative stress (Liu et al. 2015). However, the combination of compatible yet dissimilar genera of microbes can greatly boost plant growth under saline circumstances and may aid in salinity amelioration (Kapadia et al. 2021). Microbial consortia provide a variety of essential tasks under salinity conditions, including promoting plant growth, acting as osmoprotectants, antioxidants, and biocontrol agents, and reducing stress in the soil. Microbial consortia of four rhizobacteria strains *Bacillus* sp. + *Delftia* sp. + *Enterobacter* sp. + *Achromobacter* sp. helps to overcome the salinity stress in tomato. Consortia alleviate salt stress in tomatoes by increasing plant growth parameters, chlorophyll content, mineral uptake, accumulation, and transportation to a different part of the plant (Kapadia et al. 2021).

Heavy metals (HMs) are described as elements with metallic characteristics and a wide molecular weight range, which includes transition metals. Metal concentrations in soil have risen considerably because of the industrial revolution and human activities (Dabhi et al. 2021; Sharma et al. 2021). Plant metabolism and growth are harmed by the abundant HM in soil, which is absorbed and translocated to numerous organs of plants (Cheng 2003). Excess metals in soil have an adverse effect on soil characteristics and fertility, making it unfit for agricultural uses (Khan et al. 2012). Due to well-known plant growth-enhancing mechanisms such as hormone production (IAA, GA), siderophore generation, nitrogen fixation, and phosphate solubilization, PGPR can be used to help phytoremediation contaminated sites (Ojuederie and Babalola 2017). Heavy metal tolerance and accumulation by plants may be significantly influenced by heavy metal-resistant bacteria living in the rhizosphere. Rhizobacteria isolated from the landfill site and mining areas are able to tolerate heavy metal stress (Sharma and Saraf 2022). Application of rhizobacteria consortia of *Bacillus cereus* MG257494.1, *Alcaligenes faecalis* MG966440.1, and *Alcaligenes faecalis* MG257493.1 shows tolerance against heavy metals (Cu, Pb, Cd, and Zn), and their application on sorghum mitigate heavy metal stress by increasing the dehydrogenase activity, decreasing metal accumulation in plant parts and soil, also regulating bioaccumulation factor (BAF) of heavy metals (Abou-Aly et al. 2021). Some of the applications of microbial consortia under abiotic stress are listed in Table 9.1.

9.7 Conclusions

Microbial consortium is part of the plant microbiome that interacts synergistically to promote plant growth and health through the production of metabolites with antibiotic activity and by solubilizing nutrients and making them available to the plant, forming nodules to fix nitrogen, and producing plant-growth-stimulating phytohormones or enzymes that degrade ethylene precursors, such as ACC deaminase. This review presents a consortium screening protocol as a step-to-step strategy to develop microbial consortia to construct, evaluate, and shortlist the most potent microbial consortia. The review described a factorial design involving two and more representative groups and has many PGPRs to facilitate the selection of the most auspicious combinations for larger greenhouse trials before developing bio-inoculants. In vivo, rapid plant bioassays are obligatory to evaluate the performances of microbial consortia even when the isolates exhibit similar preference to physiological growth conditions, synergy in co-culture, and high mitotic activity. SEM and TEM help in the evaluation of the development of consortium and their activity on the plant to identify the microorganism, especially present in the plant where the consortium can measure multiple microorganisms, their size, and structure too. Biofertilizers have been used to boost crop production by augmenting the plant's available nutrients through the organic matter decomposition process. Two main reasons necessitate the use of biofertilizers in today's crop production. The first is to increase the use of biofertilizers, which results in the corresponding increase in crop yield, and the second is the long-term use of synthetic fertilizers degrading the soil besides other threats to our health and environment. The efficacy of biofertilizers can be enhanced by sound knowledge and long-time practical experience in a diverse soil type. Application of microbial inoculants, especially consortia, will be one of the solutions to alleviate plant abiotic stress, and enhanced plant growth and productivity under stress conditions have been reported. The directed use of microbial consortia will facilitate the production of plants in a more sustainable way that, eventually, will not depend on agrochemicals.

Table 9.1 Abiotic stress alleviation by microbial consortia

PGPR strain	PGPR traits	Stress	Crop	Effect on plants	References
Consortia of *Aspergillus* sp. S_{11} and S_{17}	Phosphate solubilization and siderophore production	Salinity	Chickpea	Increase in the vegetative parameter such as plant height, no. of lateral roots and leaves, chlorophyll content in chickpea	Urja and Meenu (2010)
Rhizobium tropici CIAT 899 + *P. polymyxa* DSM36		Drought	Common bean	Increase in the production of phytohormones, nodulation rate, nitrogen content, and overall growth of common bean	Figueiredo et al. (2008)
Azospirillum brasilense + *Azotobacter chroococcum*	IAA production and ACC deaminase activity	Heavy metal	Wheat	Consortia of lead-tolerant microbes improve grain yield, proline content, and membrane integrity, while significantly reducing the production of MDA and H_2O_2	Janmohammadi et al. (2013)
Azospirillum + arbuscular mycorrhizal		Drought	Rice	Enhanced stomatal conductance, physiological parameter, and biomass production of rice	Ruíz-Sánchez et al. (2011)
Consortia of four Cr-tolerant PGPR strains RZB-03, RZB-04, BB-A1, and BB-G7		Heavy metal	Mung bean	Increased root length, shoot length, biomass, and chlorophyll content of mung bean	Singh et al. (2010)
Pseudomonas putida NBRIRA + *Bacillus amyloliquefaciens* NBRIS N13	ACC deaminase activity, minerals solubilization, hormone production, biofilm formation, siderophore activity	Drought	Chickpea	Increase in the plant growth parameter, modulates the defense enzymes, soil enzymes, and microbial diversity	Kumar et al. (2016)
*Enterobacter*sp.12+*Enterobacter* sp. 126 + *Serratia* sp. 73	IAA production and ACC deaminase activity	Salinity	Wheat	Increased seedling emergence, shoot and root growth, biomass, and SOD activity	Barra et al. (2016)
Bacillus cereus Y5 + *Bacillus* sp. Y14 + *Bacillus subtilis* Y16		Salinity	Wheat	Improved the gas exchange photosynthetic rate, transpiration rate,	Shahzad et al. (2017)

(continued)

Table 9.1 (continued)

PGPR strain	PGPR traits	Stress	Crop	Effect on plants	References
				stomatal conductance, relative water contents; also enhanced root growth to absorb more soil for nutrient and water uptake, thus increasing the yield of wheat	
Ochrobactrum pseudogrignonense RJ12 + *Pseudomonas* sp. RJ15 + *Bacillus subtilis* RJ46	ACC deaminase activity, IAA, siderophore and HCN producer, phosphate solubilizer	Drought	*Vigna mungo* L. (black gam) and *Pisum sativum* L. (pea)	Inoculation with consortia increase seed germination, root and shoot length, and plant biomass. It also reduced ACC accumulation in plants by downregulating the expression of the ACC-oxidase gene	Saikia et al. (2018)
Bacillus sp. SR-2-1 + *Bacillus* sp. SR-2-1/1	P-solubilization, IAA, and ACC deaminase	Salinity	Potato	Enhance RWC while decreasing antioxidant enzyme activity and MDA content, also regulate Na+/K+ efflux, and higher production of auxin in the rhizosphere improves tuber yield	Tahir et al. (2019)
AM fungi (*Funnelliformis mosseae*, *Claroideoglomus etunicatum*), + *Azotobacter chroococcum*, + *Azospirillium lipoferum*		Drought	Juglans regia L. (walnut.)	Use of consortia decrease the negative effects of drought stress on seedlings by improving growth and nutrient acquisition and increasing the proline, peroxidase activity, phenol, soluble sugar, and starch content	Behrooz et al. (2019)
Acinetobacter pittii + *Acinetobacter oleivorans* + *Acinetobacter calcoaceticus* + *Comamonas testosterone*	Solubilize the insoluble forms of phosphate, potassium, and zinc, and fix N_2 gas	Salinity	Durum wheat	Enhancing the PQ ratio, complete quenching of chlorophyll fluorescence, and the portion of light absorbed by PSII antenna	Yaghoubi Khanghahi et al. (2020)

Pseudomonas putida P45 + *Bacillus amyloliquefaciens* B17; *Pseudomonas putida* P7 + *Paenibacillus favisporus* B30	IAA, gibberellic acid, P-solubilization,	Drought	Sorghum	Improve the seed and Stover yield of kharif sorghum and improve macro- and micronutrients in the soil	Kakde et al. (2020)
B. japonicum USDA 110 + *P. putida* NUU8		Drought	Soybean	Improves growth nutrient uptake and nutrient contents in soybean and soil and also enhance the activities of soil enzymes such as protease and acid and alkaline monophosphoeserase	Jabborova et al. (2021)

Acknowledgments Thanks are due to our DST-FIST Sponsored Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Gujarat, India.

Conflict to Interest The authors declare no conflict of interest.

References

Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria[1]. Science 13(6):630–633. https://doi.org/10.2135/cropsci1973.0011183X001300060013x

Abou-Aly HE, Youssef AM, Tewfike TA, El-Alkshar EA, El-Meihy RM (2021) Reduction of heavy metals bioaccumulation in sorghum and its rhizosphere by heavy metals-tolerant bacterial consortium. Biocatal Agric Biotechnol 31:101911. https://doi.org/10.1016/j.bcab.2021.101911

Alabdallah NM, Hasan MM (2021) Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants. Saudi J Biol Sci 28(10):5631–5639. https://doi.org/10.1016/j.sjbs.2021.05.081

Alström S, Burns RG (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol Fertil Soils 7(3):232–238. https://doi.org/10.1007/BF00709654

Avendaño R, Chaves N, Fuentes P, Sánchez E, Jiménez JI, Chavarría M (2016) Production of selenium nanoparticles in *Pseudomonas putida* KT2440. Sci Rep 6(1):1–9. https://doi.org/10.1038/srep37155

Barra PJ, Inostroza NG, Acuña JJ, Mora ML, Crowley DE, Jorquera MA (2016) Formulation of bacterial consortia from avocado (Persea americana Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Appl Soil Ecol 102:80–91. https://doi.org/10.1016/j.apsoil.2016.02.014

Bashan Y, Prabhu SR, de Bashan LE, Kloepper JW (2020) Disclosure of exact protocols of fermentation, identity of microorganisms within consortia, formation of advanced consortia with microbe-based products. Biol Fertil Soils 56(4):443–445. https://doi.org/10.1007/s00374-020-01464-x

Behrooz A, Vahdati K, Rejali F, Lotfi M, Sarikhani S, Leslie C (2019) Arbuscular mycorrhiza and plant growth-promoting bacteria alleviate drought stress in walnut. HortScience 54(6): 1087–1092. https://doi.org/10.21273/HORTSCI13961-19

Bilal S, Shahzad R, Khan AL, Kang SM, Imran QM, Al-Harrasi A et al (2018) Endophytic microbial consortia of phytohormones-producing fungus *Paecilomyces formosus* LHL10 and bacteria *Sphingomonas* sp. LK11 to *Glycine max* L. regulates physio-hormonal changes to attenuate aluminum and zinc stresses. Front Plant Sci 9:1273. https://doi.org/10.3389/fpls.2018.01273

Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57(2):535–538. https://doi.org/10.1128/aem.57.2.535-538.1991

Cappuccino JG, Sherman N (1992) Biochemical activities of microorganisms. Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co., San Francisco, CA, p 76

Cheng S (2003) Effects of heavy metals on plants and resistance mechanisms. Environ Sci Pollut Res 10(4):256–264. https://doi.org/10.1065/espr2002.11.141.2

Dabhi J, Solanki A, Patel R, Sharma S, Saraf MS (2021) Bioremediation of heavy metals: a brand new methodology to sustainable agriculture. Int J Innovat Res Sci Eng Technol 10(6): 6031–6049. https://doi.org/10.15680/IJIRSET.2021.1006033

de Oliveira AB, Alencar NLM, Gomes-Filho E (2013) Comparison between the water and salt stress effects on plant growth and development. Respon Organ Water Stress 4:67–94. https://doi.org/10.5772/54223

Egamberdieva D, Davranov K, Wirth S, Hashem A, Abd-Allah EF (2017) Impact of soil salinity on the plant-growth–promoting and biological control abilities of root associated bacteria. Saudi J Biol Sci 24(7):1601–1608. https://doi.org/10.1016/j.sjbs.2017.07.004

Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects a review. J Soil Sci Plant Nutr 17(4):897–911. https://doi.org/10.4067/S0718-95162017000400005

Figueiredo MV, Burity HA, Martinez CR, Chanway CP (2008) Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. Appl Soil Ecol 40(1):182–188. https://doi.org/10.1016/j.apsoil.2008.04.005

Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410(1):335–356. https://doi.org/10.1007/s11104-016-3007-x

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28(3):367–374. https://doi.org/10.1016/j.biotechadv.2010.02.001

Gontia-Mishra I, Sapre S, Deshmukh R, Sikdar S, Tiwari S (2020) Microbe-mediated drought tolerance in plants: current developments and future challenges. In: Plant microbiomes for sustainable agriculture. Springer, Cham, pp 351–379

Gosal SK, Kaur J (2017) Microbial inoculants: a novel approach for better plant microbiome interactions. In: Probiotics in agroecosystem. Springer, Singapore, pp 269–289

Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol Res 206: 131–140. https://doi.org/10.1016/j.micres.2017.08.016

Higa T (1994) Effective microorganisms: a new dimension for nature farming. In Proceedings of the second international conference on Kyusei nature farming. US Department of Agriculture, Washington, DC, pp 20–22

Itelima JU, Bang WJ, Onyimba IA, Oj E (2018) A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. J Microbiol Biotechnol Rep 2(1):22–28. http://hdl.handle.net/123456789/1999

Jabborova D, Kannepalli A, Davranov K, Narimanov A, Enakiev Y, Syed A et al (2021) Co-inoculation of rhizobacteria promotes growth, yield, and nutrient contents in soybean and improves soil enzymes and nutrients under drought conditions. Sci Rep 11(1):1–9. https://doi.org/10.1038/s41598-021-01337-9

Jain R, Sharma S, Rathod ZR, Shah RK, Lucie KM, Saraf MS (2020) Analyzing the role of phosphate solubilizing bacteria isolated from Rhizospheric soil of Cyamopsis in improving agricultural productivity. Biosci Biotechn Res Commun Spec Issue 13:16–20. https://doi.org/10.21786/bbrc/13.1specialissue/01

Jain R, Saraf M (2021) Exploring the abiotic and biotic stress tolerance potential of rhizobacteria isolated from cyamopsis. J Adv Sci Res 12(03):190–194. https://doi.org/10.55218/JASR.202112327

Janmohammadi M, Bihamta MR, Ghasemzadeh F (2013) Influence of rhizobacteria inoculation and lead stress on the physiological and biochemical attributes of wheat genotypes. Cerc Agron Moldova 46(1):49–67

Jha CK, Saraf M (2011) In vitro evaluation of indigenous plant growth promoting rhizobacteria isolated from *Jatropha curcas* rhizosphere. Int J Genet Eng Biotechnol 2(1):91–100

Jha CK, Saraf M (2012) Evaluation of multispecies plant-growth-promoting consortia for the growth promotion of *Jatropha curcas* L. J Plant Growth Regul 31(4):588–598. https://doi.org/10.1007/s00344-012-9269-5

Jha CK, Saraf M (2015) Plant growth promoting rhizobacteria (PGPR): a review. E3 J Agric Res Dev 5(2):0108–0119. https://doi.org/10.13140/RG.2.1.5171.2164

Joshi B, Chaudhary A, Singh H, Kumar PA (2020) Prospective evaluation of individual and consortia plant growth promoting rhizobacteria for drought stress amelioration in rice (*Oryza sativa* L.). Plant Soil 457(1):225–240. https://doi.org/10.1007/s11104-020-04730-x

Ju W, Liu L, Fang L, Cui Y, Duan C, Wu H (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. Ecotoxicol Environ Saf 167:218–226. https://doi.org/10.1016/j.ecoenv.2018.10.016

Kakde NA, Manjunath M, Hanwate GR, Gourkhede PH (2020) Influence of microbial consortia on yield of kharif sorghum (*Sorghum bicolor* L.) and soil parameters under rainfed conditions. Indian J Dryland Agric Res Dev 35(2):54–56

Kapadia C, Sayyed RZ, El Enshasy HA, Vaidya H, Sharma D, Patel N et al (2021) Halotolerant microbial consortia for sustainable mitigation of salinity stress, growth promotion, and mineral uptake in tomato plants and soil nutrient enrichment. Sustainability 13(15):8369. https://doi.org/10.3390/su13158369

Kasim WA, Osman ME, Omar MN, El-Daim A, Islam A, Bejai S, Meijer J (2013) Control of drought stress in wheat using plant-growth-promoting bacteria. J Plant Growth Regul 32(1):122–130. https://doi.org/10.1007/s00344-012-9283-7

Keswani C, Mishra S, Sarma BK, Singh SP, Singh HB (2014) Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. Appl Microbiol Biotechnol 98(2):533–544. https://doi.org/10.1007/s00253-013-5344-5

Khan MS, Zaidi A, Wani PA (2012) Chromium–plant-growth-promoting rhizobacteria interactions: toxicity and management. In: Toxicity of heavy metals to legumes and bioremediation. Springer, Vienna, pp 67–88

Khoshru B, Moharramnejad S, Gharajeh NH, Asgari Lajayer B, Ghorbanpour M (2020) Plant microbiome and its important in stressful agriculture. In: Plant microbiome paradigm. Springer, Cham, pp 13–48

Kim SJ, Krcmcr RJ (2005) Scanning and transmission electron microscopy of root colonization of *morningglory* (*lpomoea* spp.) seedlings by rhizobacteria. Symbiosis

Kumar M, Mishra S, Dixit V, Kumar M, Agarwal L, Chauhan PS, Nautiyal CS (2016) Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). Plant Signal Behav 11(1):e1071004. https://doi.org/10.1080/15592324.2015.1071004

Liu W, Li RJ, Han TT, Cai W, Fu ZW, Lu YT (2015) Salt stress reduces root meristem size by nitric oxide-mediated modulation of auxin accumulation and signaling in Arabidopsis. Plant Physiol 168(1):343–356. https://doi.org/10.1104/pp.15.00030

Mahmud AA, Upadhyay SK, Srivastava AK, Bhojiya AA (2021) Biofertilizers: a nexus between soil fertility and crop productivity under abiotic stress. Curr Res Environ Sustain 3:100063. https://doi.org/10.1016/j.crsust.2021.100063

Mishra N, Sundari SK (2017) Asix-step-strategy'to evaluate competence of plant growth promoting microbial consortia. Curr Sci 113(1):63–70. http://www.jstor.org/stable/26163908

Morriën E (2016) Understanding soil food web dynamics, how close do we get? Soil Biol Biochem 102:10–13. https://doi.org/10.1016/j.soilbio.2016.06.022

Nawaz A, Shahbaz M, Imran A, Marghoob MU, Imtiaz M, Mubeen F (2020) Potential of salt tolerant PGPR in growth and yield augmentation of wheat (*Triticum aestivum* L.) under saline conditions. Front Microbiol 11:2019. https://doi.org/10.3389/fmicb.2020.02019

Ney L, Franklin D, Mahmud K, Cabrera M, Hancock D, Habteselassie M, Newcomer Q (2018) Examining trophic-level nematode community structure and nitrogen mineralization to assess local effective microorganisms' role in nitrogen availability of swine effluent to forage crops. Appl Soil Ecol 130:209–218. https://doi.org/10.1016/j.apsoil.2018.06.015

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12):1504. https://doi.org/10.3390/ijerph14121504

Olivares FL, Busato JG, de Paula AM, da Silva LL, Aguiar NO, Canellas LP (2017) Plant growth promoting bacteria and humic substances: crop promotion and mechanisms of action. Chem Biol Technol Agric 4(1):1–13. https://doi.org/10.1186/s40538-017-0112-x

Panchal R, Prajapati K, Prajapati M, Sharma S, Saraf MS (2022) Bacterial exopolysaccharides: types, its biosynthesis and their application in different fields. Acta Sci Biotechnol 3(3):1–9

Panda SC (2011) Organic farming for sustainable agriculture, third edn. Kalyani Publisher, New Delhi

Pandey P, Bisht S, Sood A, Aeron A, Sharma GD, & Maheshwari DK (2012). Consortium of plant-growth-promoting bacteria: future perspective in agriculture. Bacteria agrobiol: plant probiotics 185–200

Patel D, Jha CK, Tank N, Saraf M (2012) Growth enhancement of chickpea in saline soils using plant growth-promoting rhizobacteria. J Plant Growth Regul 31(1):53–62. https://doi.org/10.1007/s00344-011-9219-7

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17:362–370

Pindi PK, Satyanarayana SDV (2012) Liquid microbial consortium-a potential tool for sustainable soil health. J Biofertil Biopestici 3:124. https://doi.org/10.4172/2155-6202.1000124

Prajapati K, Prajapati M, Panchal R, Sharma S, Saraf MS (2022a) Application of microorganisms for bioremediation of heavy metals. Acta Sci Environ Sci 1(1):01–09

Prajapati M, Panchal R, Prajapati K, Sharma S, Saraf MS (2022b) Rhizobacterial - plant interaction approaches that enhance plant growth under abiotic stress. Acta Sci Agric 6(5):67–74. https://doi.org/10.31080/ASAG.2022.06.1136

Prasad AA, Babu S (2017) Compatibility of *Azospirillum brasilense* and *Pseudomonas fluorescens* in growth promotion of groundnut (*Arachis hypogea* L.). An Acad Bras Cienc 89:1027–1040. https://doi.org/10.1590/0001-3765201720160617

Rana A, Saharan B, Nain L, Prasanna R, Shivay YS (2012) Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. Soil Sci Plant Nutr 58(5):573–582. https://doi.org/10.1080/00380768.2012.716750

Rathod ZR, Saraf MS (2021a) Effect of endophytes as phosphate solubilizing bacteria on Vigna radiata plant. Vidhya J Gujarat Univ 16(2):38–50. ISSN: 2321-1520

Rathod ZR, Saraf MS (2021b) Effect of endophytic consortium on *Pisum sativum* and *Vigna angularis* plant analyst with FTIR spectroscopy. Acta Sci Microbiol 4(12):129–140. https://doi.org/10.31080/ASMI.2021.04.0969

Rathod ZR, Dharmpal MA, Mishra AY, Jain R, Patel BV, Saraf MS (2020) Compatibility studies on different endophytes of *Citrus limon* antagonistic to bacterial with plant pathogen. Adv Biores 11(3):126–129. https://doi.org/10.15515/abr.0976-4585.11.3.126129

Rathod ZR, Sharma S, Saraf MS (2021) Endophytic bacterial effects on seed as enhancement in germination with mobilization of reserves and microbial disinfection in *Citrus limon* (L) by biopriming under salinity stress. Curr Trends Biomed Eng Biosci 20(2):556033. https://doi.org/10.19080/CTBEB.2021.20.556033

Reed MLE, Glick BR (2013) Applications of plant growth-promoting bacteria for plant and soil systems. Applications of microbial engineering. Taylor and Francis, Enfield, CT, pp 181–229

Rochlani A, Dalwani A, Shaikh NB, Shaikh N, Sharma S, Saraf MS (2022) Plant growth promoting rhizobacteria as biofertilizers: application in agricultural sustainability. Acta Sci Microbiol 5(4):12–21. https://doi.org/10.31080/ASMI.2022.05.1028

Ruíz-Sánchez M, Armada E, Muñoz Y, de Salamone IEG, Aroca R, Ruíz-Lozano JM, Azcón R (2011) Azospirillum and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. J Plant Physiol 168(10):1031–1037. https://doi.org/10.1016/j.jplph.2010.12.019

Saikia J, Sarma RK, Dhandia R, Yadav A, Bharali R, Gupta VK, Saikia R (2018) Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Sci Rep 8(1):1–16. https://doi.org/10.1038/s41598-018-21921-w

Santos Villalobos SDL, Parra Cota FI, Herrera Sepúlveda A, Valenzuela Aragón B, Estrada Mora JC (2018) Colección de microorganismos edáficos y endófitos nativos para contribuir a la seguridad alimentaria nacional. Rev Mex Cien Agríc 9(1):191–202. https://doi.org/10.29312/remexca.v9i1.858

Santoyo G, Guzmán-Guzmán P, Parra-Cota FI, Santos-Villalobos SDL, Orozco-Mosqueda M, Glick BR (2021) Plant growth stimulation by microbial consortia. Agronomy 11(2):219. https://doi.org/10.3390/agronomy11020219

Saraf MS, Sharma S, Thakkar A, (2017) Production and optimization of siderophore from plant growth promoting rhizobacteria. Scholar Press 1-85. EAN:978-3-330-65139-5

Sathiavelu A (2021) A review on the role of compost microbes in the abiotic stress tolerance in plants. SPAST Abstracts 1(01) https://spast.org/techrep/article/view/1296

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160(1):47–56. https://doi.org/10.1016/0003-2697(87)90612-9

Seenivasagan R, Babalola OO (2021) Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product. Biology 10(11):1111. https://doi.org/10.3390/biology10111111

Sekar J, Raj R, Prabavathy VR (2016) Microbial consortial products for sustainable agriculture: commercialization and regulatory issues in India. In: Agriculturally important microorganisms. Springer, Singapore, pp 107–132

Shah RK, Raval VH, Saraf M (2020) Biosynthesis and purification of indole-3-acetic acid by halotolerant rhizobacteria isolated from little Runn of Kachchh. Biocatal Agric Biotechnol 23: 101435. https://doi.org/10.1016/j.bcab.2019.101435

Shahzad S, Khan MY, Zahir ZA, Asghar HN, Chaudhry UK (2017) Comparative effectiveness of different carriers to improve the efficacy of bacterial consortium for enhancing wheat production under salt affected field conditions. Pak J Bot 49(4):1523–1530

Shaikh NB, Shaikh N, Rochlani A, Dalwani A, Sharma S, Saraf MS (2022) Rhizobacteria that promote plant growth and their impact on root system architecture, root development, and function. Acta Sci Microbiol 5(4):53–62. https://doi.org/10.31080/ASMI.2022.05.1035

Sharma S, Saraf MS (2022) Isolation, screening and biochemical characterizations with multiple traits of heavy metal tolerant rhizobacteria from mining area and landfill site. Adv Biores 13(1): 147–156. https://doi.org/10.15515/abr.0976-4585.13.1.147156

Sharma S, Shah RK, Rathod ZR, Jain R, Lucie KM, Saraf MS (2020) Isolation of heavy metal tolerant rhizobacteria from Zawar mines area, Udaipur, Rajasthan, India. Biosci Biotechnol Res Commun 13(1):233–238. https://doi.org/10.55218/JASR.202112327

Sharma S, Rathod ZR, Saraf MS (2021) Elucidate the influence of heavy metal on bacterial growth isolated from a mining location and a waste dump: using their inducible mechanism. Curr Trends Biomedical Eng Biosci 20(2):556034. https://doi.org/10.19080/CTBEB.2021.20.556034

Sharma S, Rathod ZR, Saraf MS (2022b) Exploring the biotic stress tolerance potential of heavy metal tolerate rhizobacteria isolated from mines area and landfill site. Acta Sci Microbiol 5(2): 31–37. https://doi.org/10.31080/ASMI.2022.05.1000

Sharma S, Rathod ZR, Saraf MS (2022c) Salinity Management in *Glycine max* L. using cytokinin from rhizobacteria isolated from mines and dump sites. Curr Trends Biomedical Eng Biosci 20(5):556047. https://doi.org/10.19080/CTBEB.2022.20.556047

Singh NK, Rai UN, Tewari A, Singh M (2010) Metal accumulation and growth response in *Vigna radiata* L. inoculated with chromate tolerant rhizobacteria and grown on tannery sludge amended soil. Bull Environ Contam Toxicol 84(1):118–124. https://doi.org/10.1007/s00128-009-9875-5

Tahir M, Ahmad I, Shahid M, Shah GM, Farooq ABU, Akram M, Zakir A (2019) Regulation of antioxidant production, ion uptake and productivity in potato (*Solanum tuberosum* L.) plant inoculated with growth promoting salt tolerant *Bacillus strains*. Ecotoxicol Environ Saf 178:33–42. https://doi.org/10.1016/j.ecoenv.2019.04.027

Thakkar A, Saraf M (2015) Role of volatile metabolites from *T. citrinoviride* in biocontrol of phytopathogens. Int J Res Chem Environ 5(1):86–95. http://ijrce.org/issue/jan%202015/12.pdf

Tiwari S, Prasad V, Chauhan PS, Lata C (2017) Bacillus amyloliquefaciens confers tolerance to various abiotic stresses and modulates plant response to phytohormones through osmoprotectants and gene expression regulation in rice. Front Plant Sci 8:1510. https://doi.org/10.3389/fpls.2017.01510

Todar K (2015) Textbook of bacteriology

Trivedi G, Saraf M (2019) Application of microbial endophytes isolated from plants of semi-arid region, PhD thesis, Gujarat University, Ahmedabad, Gujarat, India

Trivedi G, Patel P, Saraf M (2018) Amelioration of drought tolerant plant growth promoting endophytic bacteria from *Ricinus communis*. Int J Res Adv Technol 6(10):2814–2824

Trivedi G, Patel P, Saraf M (2020) Synergistic effect of endophytic selenobacteria on biofortification and growth of *Glycine max* under drought stress. S Afr J Bot 134:27–35. https://doi.org/10.1016/j.sajb.2019.10.001

Upadhyay SK, Saxena AK, Singh JS, Singh DP (2019) Impact of native ST-PGPR (*Bacillus pumilus*; EU927414) on PGP traits, antioxidants activities, wheat plant growth and yield under salinity. Climat Chang Environ Sustain 7(2):157–168. https://doi.org/10.5958/2320-642X.2019.00021.8

Urja P, Meenu S (2010) Role of single fungal isolates and consortia as plant growth promoters under saline conditions. Res J Biotechnol 5(3):5–9

Verbruggen E, van der Heijden MG, Rillig MC, Kiers ET (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol 197(4):1104–1109. https://doi.org/10.1111/j.1469-8137.2012.04348.x

Villa-Rodriguez E, Parra-Cota F, Castro-Longoria E, López-Cervantes J, de los Santos-Villalobos S (2019) *Bacillus subtilis* TE3: a promising biological control agent against *Bipolaris sorokiniana*, the causal agent of spot blotch in wheat (*Triticum turgidum* L. subsp. durum). Biol Control 132:135–143. https://doi.org/10.1016/j.biocontrol.2019.02.012

Vives-Peris V, de Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39(1):3–17. https://doi.org/10.1007/s00299-019-02447-5

Yadav KK, Sarkar S (2019) Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. Environ Ecol 37(1):89–93

Yaghoubi Khanghahi M, Strafella S, Crecchio C (2020) Changes in photo-protective energy dissipation of photosystem II in response to beneficial bacteria consortium in durum wheat under drought and salinity stresses. Appl Sci 10(15):5031. https://doi.org/10.3390/app10155031

Part III
Contributions to Ecosystem and Crop Production

Chapter 10
Co-inoculation of Rhizobacteria in Common Bean (*Phaseolus vulgaris*) Production in East Africa

H. Korir, S. C. Kipngetich, and N. W. Mungai

Abstract Plant growth-promoting microorganisms (PGPMs) have been shown as an important component of agricultural sustainability. The use of PGPMs is an environmentally friendly approach to increasing the yields of crops through their various direct and indirect mechanisms. It has been proven that the benefits of these PGPMs can be tapped by understanding the intrinsic crop–PGPM relationship and harnessing it for crop improvement. This review has examined work done on bean–rhizobia–PGPM globally, with specific examples from East Africa. The mode of action of PGPMs, their effect on nodulation, growth, and yield, considerations for the formulation of mixed consortia inoculations, and commercialization of such inoculants are discussed. Overall, many studies show a synergistic effect of rhizobia–PGPM in controlled and field environments. The response is influenced by abiotic factors including soil moisture stress, temperature, salinity, and biotic factors. Formulation of bacterial inoculants in East Africa is mostly peat-based, and none of the commercially available inoculants are made of rhizobia–PGPM consortia. The findings of this review indicate the opportunity for commercial exploitation of rhizobia–PGPM consortia for bean production in East Africa.

Keywords *Phaseolus vulgaris* · PGPM · Rhizobia · Consortia · Co-inoculation

10.1 Introduction

Common bean (*P. vulgaris*) is an important crop globally produced on all continents. It occupies an area of seven million hectares in Africa annually. East Africa leads with 71% of the total acreage. The average production of beans in farmer's fields is generally low at less than 1000 kg ha^{-1} compared to the potential yield of 2000–3000 kg ha^{-1}. Research on the use of rhizobia either alone or in combination

---

H. Korir · S. C. Kipngetich · N. W. Mungai (✉)
Department of Crops, Horticulture and Soils, Egerton University, Njoro, Kenya
e-mail: Korir.1303117@student.egerton.ac.ke; nmungai@egerton.ac.ke

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_10

with other plant growth-promoting microorganisms (PGPMs) is an alternative for improving common bean production yield.

Common beans can form nodules with different rhizobia species and symbiotic biovars. The most common species that can form nodulation belongs to *Rhizobium*, *Ensifer*, and other genera such as *Bradyrhizobium*; it has also been reported that species belonging to *Paraburkholderia* and *Cupriavidus* are able to form nodules in *Phaseolus vulgaris* (Shamseldin and Velázquez 2020). Understanding the diversity of endosymbionts capable of nodule formation in *P. vulgaris* and its high promiscuity is crucial in assessing native strains that are effective and competitive to ensure the success of inoculation. Inoculation with rhizobia is common for many grain legumes to enhance symbiotic efficiency, growth, and yields. *P. vulgaris* is generally considered a poor nitrogen fixer compared to other grain legumes. The low symbiotic ability is attributed to the lack of effective rhizobia in soil, where *P. vulgaris* is new, with slow nodule formation on the root system, and in other instances, the existence of a large pool of vastly competitive but less effective native population of rhizobia in soil (Hungria et al. 2013). Recent efforts to produce improved germplasm have resulted in varieties with demonstrated superior nitrogen-fixing ability (Hungria et al. 2000, 2003). To further enhance the symbiotic efficiency between *P. vulgaris* and PGPMs, co-inoculation is increasingly reported in controlled and field trials.

PGPMs influence plant growth in different ways: by enhancing nitrogen uptake, production of growth promotors (auxin, cytokinin), solubilization of minerals, especially phosphorous, chelation of metal ions by siderophores, and improving resistance to biotic stresses. PGPMs have shown the ability to suppress plant pathogens through several mechanisms including release of antimicrobial agents, competing for nutrients, by siderophores, metabolizing growth-inhibitory hormones, and improving mineral nutrition or plant resistance to biotic stresses (Kudoyarova et al. 2019; Stajković et al. 2011).

Co-inoculation is the combined application of PGPMs from different genera and species to increase nodulation, growth, biomass, and plant tolerance to various biotic and abiotic conditions and yields (Shamseldin and Velázquez 2020). The goal of co-inoculation is to harness the synergy of the consortia of microbiota for the benefit of the plant. Studies report varied responses, some showing synergy of co-inoculation, through improved growth and yields, others reporting similar results for single and co-inoculation (neutral), and while others reporting possible competition resulting in lower performance for consortia (Korir et al. 2017; Jesus et al. 2018; Gabre et al. 2020; Steiner et al. 2020; Cardoso and Ferreira 2021). Co-inoculation of *B. japonicum* and *P. putida* has a synergistic effect and improves soybean nodulation and growth and can be used to formulate a consortium of biofertilizers for sustainable production (Jabborova et al. 2021). Co-inoculation response is dependent on several factors, including abiotic conditions such as temperature and moisture and biotic factors including germplasm, pest, and diseases. This paper will examine the documented evidence of this synergistic effect (or lack thereof), how it works, the conditions under which it works best, and examine some of the factors that influence this relationship and opportunities for commercial production.

10.2 General Overview of the Modes of Action of PGPMs

There has been increased research interest and commercialization of PGPMs for enhanced plant growth and yields (Agrawal et al. 2014). PGPMs function in several mechanisms to promote plant growth both directly and indirectly. These mechanisms include nutrient acquisition by plants, alleviation of abiotic stress conditions, acting as biocontrol agents against pathogens, and production of plant growth-promoting substances. These modes of action have been elaborated in the sub-sections below.

10.2.1 Nutrient Acquisition

Nitrogen (N) is one of the major nutrients required by plants for their growth and development. The availability of N for use by plants has been studied extensively in relation to PGPMs. The most studied is nitrogen fixation through the legume–rhizobia symbiosis, whereby rhizobia convert nitrogen trapped in the molecular or atmospheric form (N_2) into biologically useful forms (Backer et al. 2018). In addition to the symbiotic nitrogen fixation, nonsymbiotic nitrogen-fixing bacteria such as *Bacillus*, *Azotobacter*, *Alcaligenes*, *Azospirillum*, *Clostridium*, *Enterobacter*, and *Klebsiella* (Ahmad et al. 2005; Bhattacharyya and Jha 2012) are able to convert N_2 into plant usable forms.

Second to N, phosphorous (P) is an essential plant nutrient. Most of the P is however in insoluble forms and is slowly released for plant uptake since it majorly exists in complexes of aluminum, iron, or calcium phosphates (Mullen 2005; Goldstein and Krishnaraj 2007). For P to be available, it must be solubilized. A number of PGPMs have the ability to solubilize P (Sharma et al. 2013) through an array of mechanisms, including their capacity to reduce pH by excreting organic acids (such as citrate, gluconate, lactate, and succinate) and protons (Mullen 2005; Martínez-Viveros et al. 2010). Some PGPMs possess the ability to make iron available for the crops through the secretion of siderophores that bind and transport iron from the soil and into the bacterial cells that ultimately get absorbed by the plant roots (DalCorso et al. 2013; Saha et al. 2016), thereby improving plant nutrition (Souza et al. 2015). Other studies have shown the capacity of some PGPMs to solubilize zinc, potassium, and sulfur into plant-usable forms (He et al. 2010; Glick 2012; Gahan and Schmalenberger 2015; Ramesh et al. 2014).

10.2.2 Alleviation of Abiotic Stress: Soil Moisture and Salinity

Inadequate water supply to plants is a major challenge to crop production, especially in rain-fed agriculture. With the changing climate, crops need to adapt to conditions of drought. PGPMs residing in the rhizosphere of crops can confer tolerance/resistance of the plant to drought/water stress conditions. PGPMs have been reported to produce exopolysaccharides that have water-holding and cementing properties (Grover et al. 2011; Tewari and Arora 2014) and are therefore important in maintaining soil moisture and structure. Additionally, the PGPMs can induce drought tolerance through the synthesis of plant hormones such as auxin (Vurukonda et al. 2016). These hormones increase root growth, thereby leading to increased nutrient and water uptake, thereby helping plants overcome environmental stresses (Chibeba et al. 2015; Vurukonda et al. 2016). Cytokinin produced by the PGPMs increases abscisic acid, leading to the closure of the stomata during drought, thereby reducing water loss through the leaves (Arora et al. 2020; Figueiredo et al. 2008).

Closely related to water stress, soil salinity is an abiotic stress plants must deal with. Salinity has adverse effects on plant growth and development by reducing osmotic potential and creating an ionic imbalance, causing sodium and chloride toxicity. PGPMs produce exopolysaccharides that bind the Na^+ cation and thereby decrease the Na^+ content and alleviate salt stress in plants (Egamberdieva 2011). Glick (2005) also reported that PGPMs lower the levels of the stress hormone, ethylene, making crops more tolerant to salt stress. Inoculation of common beans with strains of *Rhizobium* PGPM led to a considerable yield despite being grown in a salt-stressed field (Yanni et al. 2016).

10.2.3 Biological Control Against Pathogens

Competition for space and nutrients with the phytopathogens, antagonism through the production of antifungal antibiotics, volatile organic molecules production, and synthesis of cell wall degrading molecules are among the mechanisms through which PGPMs suppress the pathogens, thereby protecting the crops (Araujo et al. 2005; Robin et al. 2008; Singh et al. 2008; Kai et al. 2009; Zhao et al. 2014). As biocontrol agents, *Rhizobium*, *Bacillus*, and *Pseudomonas* sp. isolated from root nodules and rhizosphere of common bean proved to be successful against a wide range of plant pathogens such as *Sclerotinia sclerotiorum*, *Colletotrichum* sp., *Macrophomina phaseolina*, *Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani* (Kumar and Dubey 2012). In common beans, PGPMs have been reported to reduce the incidence of diseases in crops. For instance, Sabaté et al. (2017) reported that after emergence, the control had 100% incidence of the pathogen, while the seedlings from the inoculated treatment had only 38%, implying that inoculation with the PGPMs significantly reduced the incidence of *M. phaseolina* by 62% compared to the nontreated seeds. Corrêa et al. (2014) reported that *B. cereus* DFs093 reduced the severity to 28% and progress (14%) of the *M. phaseolina* pathogen on common beans grown in soils infected with the pathogen.

10.2.4 Production of Growth Regulators/Promoters

A wide range of processes in plant development and plant growth is controlled by exogenous IAA in which a low amount of IAA can stimulate primary root elongation, increase root hair formation, and stimulate the formation of lateral roots (Spaepen et al. 2007). As a result, plants have greater access to water and soil nutrients as bacterial IAA increases both the root surface area and length. In a study by Sabaté et al. (2017), *Bacillus amyloliquefaciens* B14 isolated from soils belonging to the common bean-producing regions synthesizes the phytohormone indole-3-acetic acid (IAA). The IAA is known to influence cell elongation, root growth, plant growth promotion, and differentiation of tissues (Babalola 2010), making it a very important metabolite for plant growth promotion. A greenhouse study showed that common beans inoculated with *Azospirillum brasilense* Sp245 increased their root growth through the production of phytohormones (Remans et al. 2008). Other studies have reported the release of plant growth-promoting substances such as IAA and siderophores by the PGPMs (Kudoyarova et al. 2019; Zahir et al. 2004), leading to improved growth and yield of crops. PGPMs also produce cytokinins, gibberellins, ethylene, and ACC deaminase that are involved in root initiation, cell division, cell enlargement, and various plant physiological processes (Kaur and Sharma 2013; Perez-Montano et al. 2014).

10.3 *P. vulgaris* Growth Response to Co-inoculation with PGPMs

Plant growth-promoting rhizobacteria have been shown to increase the performance of rhizobia in nodulation and nitrogen fixation of common beans leading to increased plant growth and grain yields. Greenhouse and field studies with PGPM strains have validated enhanced nodulation and nitrogen fixation in common beans (Sánchez et al. 2014). Additionally, common beans have been reported as a nonselective plant host and are able to perceive nodulation signals from different strains of rhizobia such as *R. tropici* (Cardoso and Ferreira 2021; Jesus et al. 2018; Gabre et al. 2020; Hungria et al. 2013; Steiner et al. 2020), *R. etli* bv. *phaseoli* (Shamseldin and Werner 2007; Silva et al. 2003), *R. gallicum* bv. *phaseoli*, *R. giardinii* bv. *giardinii*, *R. leguminosarum* bv. *phaseoli*, and *R. leguminosarum* bv. viciae (Mhamdi et al. 2002), and this may promote nitrogen fixation and plant growth and development. Therefore, co-inoculation of common beans with an array of rhizobia strains and various PGPMs can be employed to improve the agronomic performance of common beans. This could be attributed to the multistrain synergistic effect caused by the diverse strains of rhizobia and PGPMs applied to beans during inoculation. This section highlights the various studies on the co-inoculation of common beans with rhizobia consortia with other PGPM strains in East Africa (Table 10.1).

Table 10.1 Selected consortia of PGPMs used in common bean production in East Africa

PGPM consortia	Country	Condition/soil characteristics	Mode of action	Reference
Rhizobium and *Azospirillum*	Kenya	Field experiment of two common bean varieties under low and high phosphorus levels	Nutrient acquisition: phosphorous	Muthamia et al. (2013)
Rhizobium and *Trichoderma*	Malawi	Phosphorus deficient, acid soil	Acquisition of nutrients: phosphorous	Mweetwa et al. (2016)
Consortia of indigenous rhizobia	Kenya	Greenhouse	Growth promotion	Ouma et al. (2016)
Bacillus and *Rhizobium*	Kenya	Greenhouse	Biocontrol against root-knot nematodes	Karanja et al. (2007)
Rhizobium and *Trichoderma*	Zambia	Greenhouse experiment using an acidic soil	Acquisition of nutrients: phosphorous	Chilombo (2019)
Native *Rhizobium* consortia + commercial rhizobia	Kenya	Greenhouse study using sterilized soil	Nutrient acquisition	Menge et al. (2018a)
Rhizobia and other nodule-associated bacteria (NAB)	Kenya	Greenhouse study using autoclaved vermiculite	Growth promotion	Wekesa et al. (2016)
Rhizobia strains and *Bacillus* strains	Kenya	Greenhouse study using P-deficient soil	Acquisition of nutrients (N and P)	Korir et al. (2017)
Multi-strains	Kenya	Greenhouse-controlled environment with sterile vermiculite	Growth promotion	Gicharu et al. (2013)
Native consortium + exotic rhizobia	Kenya	Greenhouse study using sterilized soil	Growth promotion, nutrient acquisition	Menge et al. (2018b)
Consortium inoculation with rhizobia	Ethiopia	Drought stress using drought-tolerant and -susceptible common bean varieties	Alleviation of water stress	Eticha (2021)

10.3.1 Effect of Co-inoculation with PGPM Consortia on Common Bean Growth Promotion

Co-inoculation of common beans with *Rhizobium* and *Trichoderma* has been shown to improve the nodulation of legumes, both in terms of the number of nodules and their effectiveness. A study in Malawi by Mweetwa et al. (2016) reported nodule effectiveness of up to 88% when common bean plants were co-inoculated with *Rhizobium* and *Trichoderma*. The authors reported that there was no significant difference between un-amended controls and the single inoculations with either *Rhizobium* or *Trichoderma*. A similar study by Chilombo (2019) showed that single inoculation with *Trichoderma* or *Rhizobium* did not have a significant difference from the control. However, *Rhizobium* + *Trichoderma* co-inoculation showed a significant increase in the number of nodules per plant. The same trend was also reported in terms of nodule effectiveness, suggesting that *Trichoderma* increases the number of nodules by increasing infection sites through the extension of the root system of the plant.

Inoculation of crops with mixed inoculum is a strategy that can be used to improve the efficiency of bacterial strains. In Kenya, higher nodule numbers and nodule dry weights were reported when common beans were inoculated with a consortium of indigenous rhizobia strains compared to the inoculation with commercial rhizobia (CIAT 899) strain (Ouma et al. 2016). Additionally, the authors found that significantly highest shoot dry weight was achieved when the common bean was co-inoculated with a mixture of the indigenous rhizobia strains and the CIAT 899 than when CIAT 899 was singly applied. Overall, it was shown that the native isolates, especially the consortium, were more effective than the commercially available CIAT 899 in improving the performance of common beans (Ouma et al. 2016). Studies on the mixed inoculation of native *Rhizobium* consortia and exotic/commercial rhizobia strains have been carried out by other researchers in Kenya. A field study by Menge et al. (2018a) showed that the highest and significant nodule dry weight and shoot dry weight were achieved when the common bean was co-inoculated with a mix of native consortium + commercial rhizobia compared to inoculation with single commercial rhizobia strains. A greenhouse study by Menge et al. (2018b) showed that co-inoculation of common beans with a mixture of native and exotic rhizobia isolates significantly recorded the highest number of nodules and nodule dry weight. The study results showed that a significantly higher shoot dry weight was observed upon co-inoculation with a mixture of native consortium + exotic rhizobia when compared with single exotic and native rhizobia inoculations. A greenhouse and field study by Gicharu et al. (2013) showed that co-inoculation of climbing common bean varieties with a consortium of rhizobia strains produced significantly higher nodule numbers and yield than the control treatment. A study by Karanja et al. (2007) showed that the highest shoot dry weight was recorded in the K194 + USDA 2674 and K67 + CIAT 899 co-inoculations, while the least was recorded in the controls. All the isolates of *Bacillus subtilis* and their combinations with *Rhizobium* strain USDA 2674 caused an increase in shoot dry weight.

Co-inoculation of common beans with rhizobia and other nodule endophytes can improve the synergy of rhizobia to improve crop performance. One such study by Wekesa et al. (2016) carried out in Western Kenya showed that the number of pods per plant inoculated with a mixture of rhizobia and other nodule-associated bacteria (NAB) was significantly higher than that inoculated with rhizobia alone, and no significant difference was noted on the number of pods per plant between the uninoculated control and single rhizobia inoculation. The authors reported similar trends with the number of seeds per pod and the weight of pods per plant with co-inoculation with rhizobia and other NAB recording significantly higher values than single rhizobia inoculation and the control. The total shoot dry matter of plants inoculated with rhizobia and other NAB was significantly higher than the uninoculated and those inoculated with rhizobia alone. The authors reported no significant difference between the shoot dry weight for common beans inoculated with rhizobia alone and the uninoculated ones (Wekesa et al. 2016). From these documented studies, the use of rhizobia + PGPM enhanced nodulation, shoot weight, and grain yield parameters under controlled and field environments.

10.3.2 PGPM Consortia Inoculation on Nutrient Acquisition for Common Beans

The study results by Menge et al. (2018a) showed that the multi-strain mixture of both native consortium + exotic rhizobia recorded the highest shoot nitrogen of 3.40%. The authors reported significantly higher phosphorus content due to co-inoculation with a consortium of native rhizobia. Menge et al. (2018b) also reported that co-inoculation of common bean with multi-strain consortia of both native consortium and exotic rhizobia recorded the highest shoot nitrogen. The PGPMs belonging to the genus *Bacillus* have been reported to be an efficient phosphate solubilizer. With P nutrition being crucial in the nodule formation by rhizobia (Remans et al. 2007), co-inoculation with PGPMs possessing P solubilization in soils is vital. A greenhouse study by Korir et al. (2017) showed that co-inoculation of rhizobia strains with PGPMs enhanced the growth of common beans in phosphorous-deficient soil. The authors reported an increased number of nodules and nodule weight due to co-inoculation of rhizobia strains with *Paenibacillus polymyxa* and *Bacillus megaterium* in respect to inoculation with *Rhizobium* alone in common beans. Furthermore, findings from the study showed that co-inoculation of common beans with *Rhizobium* and PGPMs significantly increased shoot and root dry weights of plants in comparison to *Rhizobium* inoculation alone and the uninoculated control. Furthermore, a study carried out by Muthamia et al. (2013), to test the efficiency of *Rhizobium* and *Azospirillum* co-inoculation in low and high phosphorous (P) soils, showed that there was an improvement in biomass and grain yield both at low P and high P soils in both bean varieties under study. The improvements were higher than any individual *Rhizobium* or *Azospirillum*. Comparison of soil P status showed that co-inoculation led to a higher grain yield improvement in the low P soils than in high P soil.

10.3.3 Co-inoculation Effect of PGPM Consortia on Biological Control of Root-Knot Nematodes

Attack by root-knot nematodes has an adverse effect on the growth and development of common beans. Control of the root-knot nematodes can be achieved by biological control using PGPMs. A study by Karanja et al. (2007) showed that co-inoculation of common beans with rhizobia and *Bacillus* can be used to control the number and damage by the root-knot nematodes. They reported that soils treated with *Bacillus* isolate K194 in combination with each of *Rhizobium* isolates (USDA 2674 and CIAT 899) recorded the lowest number of juveniles of the root-knot nematode recovered. Co-inoculation of the common bean with both *Rhizobium* and *Bacillus* resulted in less damage by nematodes compared to those that were inoculated with rhizobia alone (Karanja et al. 2007).

10.3.4 Alleviation of Moisture Stress in Common Beans by Co-inoculation with PGPM Consortia

Plant adaptation to abiotic stresses can be achieved through mixed inoculation with rhizobia consortia. One of the abiotic stresses affecting crops is water stress due to drought conditions. A study carried out in Ethiopia showed that consortium inoculation significantly improved the growth of drought-susceptible common bean variety under water-deficit conditions than plants inoculated with rhizobia alone and the uninoculated plants (Eticha 2021). Results from this study showed that co-inoculation increased the leaf biomass under drought stress more than single *Rhizobium* inoculation and control. Similarly, the author reported a significant increase in the root dry weight increased due to co-inoculation in both drought-tolerant and -susceptible common bean varieties than noninoculated control plants (Eticha 2021).

10.4 Formulation and Survival of the PGPM Biofertilizers

The efficacy of co-inocula depends on the appropriate selection of the strain, cellular concentration, method of inoculation, and plant genotype (Fukami et al. 2016). An effective PGPM mixed inocula formulation should exhibit rhizobacterial strains that have a highly competitive ability, a wider array of modes of action, high feasibility for mass multiplication, compatibility with other rhizobacteria, and high rhizosphere competence and tolerance to abiotic stresses (Tabassum et al. 2017). The carrier material used in the formulation of biofertilizer is essential because it will determine the survival of PGPM strains in the field, in addition to protecting the PGPMs from stress and improving their shelf life (Hungria et al. 2005; Mishra and Arora 2016). In the Eastern Africa region, different formulations and carrier materials have been used in the preparation of PGPM biofertilizer. In Sudan, for example, charcoal was reported and used as the main carrier for rhizobium-based inoculants among the other locally available carrier materials (Elsalahi et al. 2016). Charcoal was reported to be readily available and in abundance in addition to its high water-holding capacity and low contamination levels (Elsalahi et al. 2016). However, the partially sterilized charcoal carrier has a short shelf life of 2 months from the time of manufacture (Elshafie and Elhussein 1991). In Uganda, the PGPM inoculants containing *Rhizobium* strains have been formulated using sterile peat as carrier material infused with yeast extract mannitol agar broth (Chianu et al. 2011). Other carrier materials used in the formulation of PGPM inoculants are filter mud (Odame 1997; Bala et al. 2011), vermiculite (Balume et al. 2013), and in liquid form with the different PGPMs mixed in a 1:1 ratio (Muthamia et al. 2013).

Mixed PGPM strains in biofertilizers survive through synergistic effects with other microbes (Enebe and Babalola 2018; Maheshwari 2012). A study by Kumar et al. (2016) reported a synergistic effect of *P. putida* and *B. amyloliquefaciens*, and there was no alteration of the microbial population with their application. The competition also enhances the survival of some PGPM strains in mixed biofertilizers. Da Conceição et al. (2018) showed that co-inoculation of *Bradyrhizobium* and *R. tropici* was competitive, with *R. tropici* competing with *Bradyrhizobium* for nodule infection. Dardanelli et al. (2008) observed a positive effect on co-inoculation of *Rhizobium tropici CIAT899* and *Rhizobium etli* ISP42 with *Azospirillum brasilense* on the expression of nod-gene and nodulation and benefited the plant by enhancing root branching and acetylene reduction activates. Elkoca et al. (2010) noted that triple inoculation with *Bacillus megaterium*, *Bacillus subtilis*, and *Rhizobium leguminosarum* significantly increased macro and micronutrients uptake and improved the yield of *P. Vulgaris*. Babu et al. (2015) showed that co-inoculation of *Rhizobium* spp. and PGP agent significantly improved nodulation, shoot and root dry matter, and grain yield in chickpea, and they further observed that that inoculation of isolates of PGPR and cyanobacteria improved growth of chickpea, pea, and lentil with an increase in hydrolytic enzyme activity. Another aspect of the survival of microorganisms in inoculants is associated with the storage temperature and water activity of the inoculants, as this will influence the shelf life of the PGPM inoculants (Goudar et al. 2017). The Eastern Africa region is characterized by high temperatures, and this adversely affects the survival of rhizobia in the packaged inoculants and in the inoculated seeds in the field (Deshmukh et al. 2014).

10.5 Commercialization of PGPM Strains' Biofertilizers

Although *Rhizobium* inoculants have been in existence for more than a century, their production in African countries is still at a pilot scale, mostly for purposes of research purposes, having no strategy to promote commercial production (Laetitia and Lesueur 2013). In East Africa, Kenya has a well-established use and production of biofertilizers. Biofix, a product produced by MEA Fertilizer Limited and licensed by the University of Nairobi, MIRCEN, is the main product in the market, which is fully commercialized. The species used in biofix for common bean production is *Rhizobium tropici* strain CIAT 899 (Balume et al. 2013). In Uganda, biofertilizer is produced in two plants, Mahavani Limited and Makerere University. Biofertilizer legumes and Bio-N fix are the products produced at Madhavani Limited and Makerere University, respectively. The strains in these products are *B. japonicum* and *Rhizobium tropici* for soybean and common bean, respectively (Bala et al. 2011). In Ethiopia, National Soil Research Laboratories, Microbiology Unit, produces inoculants commercially for common bean cultivation. Rwanda Agricultural Board (Rwanda) and Sokoine University of Agriculture (Tanzania) had rhizobial production units (Chianu et al. 2011). Currently, the production of inoculants in Rwanda has not been entirely due to inadequate equipment and trained staff. At Sokoine University, inoculant production stopped when external funding was exhausted (Bala et al. 2011). The MEA fertilizer limited produces 220,000 kg of biofix per year, while Rwanda produces 44,500 kg of RAB inoculant (Woomer et al. 2014). Sudan's Biopesticides and Biofertilizers Department is the only *Rhizobium* inoculants producer in Sudan (Elsalahi et al. 2016). A summary of the efforts to produce and commercialize PGPMs is shown in Table 10.2. As observed from Table 10.2, all of the biofertilizers produced for distribution to farmers and research are formulated using one microorganism. Documents showing commercial production of biofertilizers containing consortia of the PGPM strains are not available.

10.6 Conclusions

The use of rhizobia–PGPM consortia for bean production has resulted in a synergistic effect on nodulation, growth and yield parameters, resistance to nematodes, and better adaptability to low soil moisture and high soil salinity. New bean varieties have been developed with a higher ability to fix nitrogen. Similarly, research on the effectiveness of native rhizobia applied singly or in consortia with other PGPMs underscores the inherent benefit of the bean–rhizobia–PGPM symbiosis. Questions on how to ensure consistent enhanced growth and yield because of symbiotic effectiveness across different environments still abound. Future research should be conducted into how different organisms interact in a consortium and how to establish the best combination for different agroecosystems, minimize competition and redundancy, and ensure synergy. Research also should consider the best formulation

Table 10.2 Status on the commercialization of PGPM biofertilizers in East Africa

PGPM strain(s)	Name of Biofertilizer	Country	Manufacturer	Target crop(s)	Reference(s)
Rhizobium tropici strain CIAT 899	BIOFIX	Kenya	MEA Ltd	Common bean	Balume et al. (2013); Odame (1997)
Rhizobium tropici	RAB inoculant	Rwanda	Rwanda Agricultural Board	Common bean	Chianu et al. (2011) https://oneacrefund.org/documents/73/Nitrogen_Fixation_In_Beans_Ag_Innovations.pdf
B. japonicum	NITROSUA	Tanzania	Sokoine University of Agriculture	Soybean and lucerne	Chianu et al. (2011)
B. japonicum and *Rhizobium tropici*	Bio-N fix	Uganda	Madhavani Ltd. and Makerere University	Soybean and common bean	Chianu et al. (2011)
Rhizobium leguminosarum biovar phaseoli strain HB-429	Not mentioned	Ethiopia	National Soil Research Laboratories, Microbiology Unit, Addis Ababa	Common bean	Assefa et al. (2018); Hailemariam and Asfaw (2015)
Rhizobium leguminosarum	Not mentioned	Sudan	Biopesticides and Biofertilizers Department	Common bean	Elhassan et al. (2010); Elsalahi et al. (2016)

approaches for different PGPM consortia and ensure ease of possible commercialization. Consideration of abiotic factors such as ambient and soil temperature, salinity, and moisture stress may lead to sustainable utilization of the bean–rhizobia–PGPM symbiosis.

Acknowledgments Not applicable.

Conflict of Interest The authors declare no conflict of interest.

Glossary

Abiotic factors Nonliving components such as water, soil, and atmosphere that affect the activity of soil microbes.

Biofertilizer Substances that contain microorganisms, which when added to the soil increase its fertility and promote plant growth.

Biological control Management of plant disease by inhibiting plant pathogens or improving plant immunity through the effects of beneficial microorganisms.

Biotic factors The living components that affect the functioning of soil microbes such as plants and other soil organisms.

Carrier materials Any substance that can be used to deliver viable microbial cultures from the laboratory to the field.

Co-inoculation Inoculation of plants with a mixture of two or more plant growth-promoting microbiota.

Competitiveness The ability of the introduced bacteria to colonize the roots and nodules of plants in the presence of native soil population.

Microbial consortia Two or more bacterial or microbial groups living symbiotically.

Formulation Preparation of inoculants in the carrier materials.

Plant growth promoters Chemicals that promote cell division, cell enlargement, flowering, fruiting, and seed formation in plants. Examples are auxins, gibberellins, and cytokinins.

Growth regulators Chemical substances act as intercellular messengers that influence the growth and differentiation of plant cells, tissues, and organs.

Indole acetic acid A chemical that is synthesized by microbes and plants, and it plays a role in both root and shoot development.

Microbial inoculants Agricultural amendments that use beneficial rhizospheric or endophytic microbes to promote plant health.

Inoculation The application of inoculants to the soil, seeds, or plant.

Mechanisms of action The biotic activities of the soil microbes in the soils that lead to the stimulation of plant growth through mobilizing nutrients in soils, production of numerous plant growth regulators, protection plants from phytopathogens, improving soil structure, and bioremediating the polluted soils.

Nutrient acquisition The mechanism by which plants capture the elements that are essential for their growth.

Plant growth-promoting microbiota The soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development.

Rhizobia Bacteria found in soil that helps in fixing nitrogen in leguminous plants.

Siderophores Small molecular iron-chelators that are produced by microbes.

Stress alleviation Mitigation of plants against harmful biotic and abiotic factors.

Symbiosis Any kind of relationship or interaction between two dissimilar organisms, each of which benefits from the other.

Symbiotic efficiency The ability of soil microbes to plant growth-promoting microbiota to form a beneficial relationship with the plant and promote its growth and development.

Synergy Microbial interaction in which both or all the microbial populations involved get benefited by supporting each other's growth and proliferation.

References

Agrawal PK, Agrawal S, Kundan R, Bhatt M (2014) Application and perspective of plant growth promoting rhizobacteria in development of sustainable agriculture. Int J Curr Res 6(10): 9044–9051

Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent pseudomonas in the presence and absence of tryptophan. Turkish J Biol 29:29–34

Araujo FF, Henning AA, Hungria M (2005) Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development. World J Microbiol Biotechnol 21:1639–1645

Arora NK, Fatima T, Mishra I, Verma S (2020) Microbe-based inoculants: role in next green revolution. In: Shukla V, Kumar N (eds) Environmental concerns and sustainable development. Springer, Singapore, pp 191–245

Assefa F, Keneni K, Demissie N (2018) Overview of rhizobial inoculants research and bio fertilizer production for increased yield of food Legumes in Ethiopia. Ethiopian J Crop Sci (Special Issue) 1:255–271

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570

Babu S, Prasanna R, Bidyarani N, Nain L, Shivay YS (2015) Synergistic action of PGP agents and Rhizobium spp. for improved plant growth, nutrient mobilization and yields in different leguminous crops. Biocatalys Agric Biotechnol 4(4):456–464

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473. https://doi.org/10.3389/fpls.2018.01473

Bala A, Karanja N, Murwira M, Lwimbi L, Abaidoo R, Giller, KE (2011) Production and use of Rhizobial inoculants in Africa. N2Africa

Balume IK, Keya SO, Karanja NK, Woomer PL (2013) Improving shelf life of legume inoculants in East Africa. In: Joint proceedings of the 27th soil science society of East Africa and the 6th African soil science society conference

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPM): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Cardoso RM, Ferreira EPB (2021) Assessment of consortia inoculation effects on the agronomical performance of the common bean. Commun Soil Sci Plant Anal 52(16):1971–1980. https://doi.org/10.1080/00103624.2021.1908319

Chianu JN, Nkonya EM, Mairura FS, Chianu JN, Akinnifesi FK (2011) Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: a review. Agron Sustain Dev 31:139–154

Chibeba AM, Guimarães MF, Brito OR, Nogueira MA, Araujo RS, Hungria M (2015) Coinoculation of soybean with *Bradyrhizobium* and *Azospirillum* promotes early nodulation. Am J Plant Sci 6(10):1641–1649

Chilombo G (2019) Response of common bean and groundnut to co-inoculation of *Trichoderma* and Rhizobia in an acid soil. MSc. Dissertation, University of Zambia, Lusaka, Zambia. http://dspace.unza.zm/bitstream/handle/123456789/6868/Main%20Document.pdf?sequence=1&isAllowed=y. Accessed 15 Oct 2021

Corrêa BO, Shafer JT, Moura AB (2014) Spectrum of biocontrol bacteria to control leaf, root and vascular diseases of dry bean. Biol Control 72:71–75

Da Conceição JE, de Almeida LR, do Amaral BR, da Silva AOO, Araújo AP (2018) Co-inoculation of Brady*Rhizobium* stimulates the symbiosis efficiency of *Rhizobium* with common bean. Plant Soil 425(1):201–215

DalCorso G, Manara A, Furini A (2013) An overview of heavy metal challenge in plants: from roots to shoots. Metallomics 5:1117–1132

Dardanelli MS, de Cordoba FJF, Espuny MR, Carvajal MAR, Díaz MES, Serrano AMG et al (2008) Effect of Azospirillum brasilense coinoculated with rhizobium on Phaseolus vulgaris flavonoids and Nod factor production under salt stress. Soil Biol Biochem 40(11):2713–2721

Deshmukh VV, Mane SS, Ingle RW, Totawar MV, Joshi MS (2014) Shelf life study of Bradyrhizobium japonicum isolates of Vidarbha region. Indo-Am J Agric & Vet Sci 2(1):31–38

Egamberdieva D (2011) Survival of *Pseudomonas extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 in the rhizosphere of common bean (*Phaseolus vulgaris*) under saline conditions. Plant Soil Environ 57:122–127

Elhassan GA, Abdelgani ME, Osman AG, Mohamed SS, Abdelgadir BS (2010) Potential production and application of biofertilizers in Sudan. Pak J Nutr 9(9):926–934

Elkoca E, Turan M, Donmez MF (2010) Effects of single, dual and triple inoculations with Bacillus subtilis, Bacillus megaterium and Rhizobium leguminosarum bv. Phaseoli on nodulation, nutrient uptake, yield and yield parameters of common bean (Phaseolus vulgaris l. cv.'elkoca-05'). J Plant Nutr 33(14):2104–2119

Elsalahi RH, Mohamed SS, Sherif AM, Osman AG (2016) Rhizobium biofertilizer (Okadin) production and future prospects in Sudan. Environ Nat Resour Int J (ENRIJ) 1(1):1–12

Elshafie AE, Elhussein AA (1991) An evaluation of *Rhizobium* survival in two carriers new to Sudan. Exp Agric 27(3):319–321. https://doi.org/10.1017/S0014479700019037

Enebe MC, Babalola OO (2018) The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. Appl Microbiol Biotechnol 102(18):7821–7835

Eticha A (2021) Consortium effect ff rhizobium strains to regulate physiological and biochemical response in common bean (Phaseolus Vulgaris) during water stress. PhD. Thesis dissertation. Adama Science and Technology University, Adama, Ethiopia. http://213.55.101.23/bitstream/handle/123456789/1653/Eticha%20Abdisa%20Bayisa.pdf?sequence=1&isAllowed=y. Accessed 15 Oct 2021

Figueiredo MVB, Burity HA, Martinez CR, Chanway CP (2008) Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *rhizobium tropici*. Appl Soil Ecol 40:182–188

Fukami J, Nogueira MA, Araujo RS, Hungria M (2016) Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. AMB Express 6(1):1–13

Gabre VV, Venancio WS, Moraes BA, Furmam FDG, Galvão CW, Gonçalves DRP, Etto RM (2020) Multiple effect of different plant growth promoting microorganisms on beans (*Phaseolus vulgaris* L.) crop. Braz Arch Biol Technol. https://doi.org/10.1590/1678-4324-solo-2020190493

Gahan J, Schmalenberger A (2015) Arbuscular mycorrhizal hyphae in grassland select for a diverse and abundant hyphospheric bacterial community involved in sulfonate desulfurization. Appl Soil Ecol 89:113–121

Gicharu G, Gitonga N, Boga H, Cheruiyot R, Maingi J (2013) Effect of inoculating selected climbing bean cultivars with different rhizobia strains on nitrogen fixation. Inter J Microbiol Res 1:25–31

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:17

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012: 1–15

Goldstein AH, Krishnaraj PU (2007) Phosphate solubilizing microorganisms vs. phosphate mobilizing microorganisms: what separates a phenotype from a trait? In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Dordrecht, pp 203–213

Goudar G, Sreenivasulu G, Kumbar B, Nagaraj H (2017) Plant growth promotional activity of newly developed formulation of Azospirillum on maize. Int J Curr Microbiol App Sci 6 (12):370–380

Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240

Hailemariam A, Asfaw B (2015) Benefits and prospects of bio fertilizer in Ethiopian agriculture. In: Proceedings of the Second Cereals and Legumes Group Meeting 26th February 2015, Addis Ababa, Ethiopia

He CQ, Tan GE, Liang X, Du W, Chen YL, Zhi GY, Zhu Y (2010) Effect of Zn-tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. Appl Soil Ecol 44:1–5

Hungria M, Andrade DS, Chueire LMO, Probanza A, Guttierrez-Mañero FJ, Megías M (2000) Isolation and characterization of new efficient and competitive bean (*Phaseolus vulgaris* L.) rhizobia from Brazil. Soil Biol Biochem 32:1515–1528

Hungria M, Campo RJ, Mendes IC (2003) Benefits of inoculation of the common bean (Phaseolus vulgaris) crop with efficient and competitive *Rhizobium tropici* strains. Biol Fert Soils 39:51–61

Hungria M, Loureiro MF, Mendes IC, Campo RJ, Graham PH (2005) Inoculant preparation, production and application in nitrogen fixation in agriculture, forestry, ecology, and the environment. Springer, Dordrecht, pp 223–253

Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol Fert Soils 49:791–801

Jabborova D, Kannepalli A, Davranov K, Narimanov A, Enakiev Y, Syed A et al (2021) Co-inoculation of rhizobacteria promotes growth, yield, and nutrient contents in soybean and improves soil enzymes and nutrients under drought conditions. Sci Rep 11:1–9

Jesus EDC, Leite RDA, Bastos RDA, Aragão OODS, Araújo AP (2018) Co-inoculation of Bradyrhizobium stimulates the symbiosis efficiency of Rhizobium with common bean. Plant Soil 425(1):201–215

Kai M, Haustein MF, Petri A, Scholz B, Piechulla B (2009) Bacterial volatiles and their action potential. Appl Microbiol Biotechnol 81:1001–1012

Karanja N, Mutua G, Kimenju J (2007) Evaluating the effect of *Bacillus* and *Rhizobium*. bi-inoculant on nodulation and nematode control in *Phaseolus vulgaris* L. In: Bationo A, Waswa B, Kihara J, Kimetu J (eds) Advances in integrated soil fertility management in sub-Saharan Africa: challenges and opportunities. Springer, Dordrecht

Kaur N, Sharma P (2013) Screening and characterization of native Pseudomonas sp. as plant growth promoting rhizobacteria in chickpea (*Cicer arietinum* L.) rhizosphere. Afr J Microbiol Res 7: 1465–1474

Korir H, Mungai NW, Thuita M, Hamba Y, Masso C (2017) Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front Plant Sci 8:441. https://doi.org/10.3389/fpls.2017.00141

Kudoyarova G, Arkhipova T, Korshunova T, Bakaeva M, Loginov O, Dodd IC (2019) Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. Front Plant Sci 10:1368. https://doi.org/10.3389/fpls.2019.01368

Kumar P, Dubey RC (2012) Plant growth promoting rhizobacteria for biocontrol of phytopathogens and yield enhancement of *Phaseolus vulgaris*. J Curr Perspect App Microbiol 1:6–38

Kumar M, Mishra S, Dixit V, Kumar M, Agarwal L, Singh P, Chauhan PS, Nautiyal CS (2016) Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). Plant Signal Behav 11(1):e1071004. https://doi.org/10.1080/15592324.2015.1071004

Laetitia H, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97(20):8859–8873

Maheshwari DK (ed) (2012) Bacteria in agrobiology: plant probiotics. Springer Science & Business Media, Berlin

Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319

Menge EM, Njeru EM, Koskey G, Maingi J (2018a) Rhizobial inoculation methods affect the nodulation and plant growth traits of host plant genotypes: a case study of common bean *Phaseolus vulgaris L.* germplasms cultivated by smallholder farmers in eastern Kenya. Adv Agric Sci 6(03):77–94

Menge EM, Njeru EM, Maingi J (2018b) Does Rhizobial inoculation functionality vary with host plant genotype? A case study of common bean *Phaseolus vulgaris L.* germplasms grown by smallholder farmers in Eastern Kenya. Proceeding of the 1st Annual International Conference held on 17th–19th April 2018, Machakos University, Kenya

Mhamdi R, Laguerre G, Aouani ME, Mars M, Amarger N (2002) Different species and symbiotic genotypes of field rhizobia can nodulate *Phaseolus vulgaris* in Tunisian soils. FEMS Microbiol Ecol 41(1):77–84

Mishra J, Arora NK (2016) Bioformulations for plant growth promotion and combating phytopathogens: a sustainable approach in bioformulations for sustainable agriculture. Springer, New Delhi

Mullen MD (2005) Phosphorus in soils: biological interactions. In: Hillel D, Rosenzweig C, Powlson D, Scow K, Singer M, Sparks D (eds) Encyclopedia of soils in the environment, vol 3. Academic Press/Elsevier, Ltd., Oxford, pp 210–215

Muthamia JGN, Kimani PK, Chemingw'a G, Esilaba AO (2013) Enhancing nitrogen fixation and productivity of dry beans in phosphorus and nitrogen limited in Central Kenya through *Rhizobium, Azospirillum* rhizobacteria-*Rhizobium* co-inoculation. Joint proceedings of the 27th soil science Society of East Africa and the 6th African soil science society

Mweetwa AM, Chilombo G, Gondwe BM (2016) Nodulation, nutrient uptake and yield of common bean inoculated with *Rhizobia* and *Trichoderma* in an acid soil. J Agric Sci 8:61–71

Odame H (1997) Bio fertilizer in Kenya: research, production and extension dilemmas. Biotechnol Devt Monitor 30:20–23

Ouma EW, Asango AM, Maingi J, Njeru EM (2016) Elucidating the potential of native rhizobial isolates to improve biological nitrogen fixation and growth of common bean and soybean in smallholder farming systems of Kenya. Inter J Agron 016:4569241. https://doi.org/10.1155/2016/4569241

Perez-Montano F, Villegas CA, Bellogin RA, Cerro PD, Espuny MR, Guerrero IJ, Lopez-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169:325–336

Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP (2014) Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of Central India. Appl Soil Ecol 73:87–96

Remans R, Croonenborghs A, Torres Gutierrez R, Michiels J, Vanderleyden J (2007) Effects of plant growth-promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant P nutrition. In: Bakker PAHM, Raaijmakers JM, Bloemberg G, Höfte M, Lemanceau P, Cooke BM (eds) New perspectives and approaches in plant growth-promoting rhizobacteria research. Springer, Dordrecht

Remans R, Beebe S, Blair M et al (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). Plant Soil 302:149–161

Robin A, Vansuyt G, Hinsinger P, Meyer JM, Briat JF, Lemanceau P (2008) Iron dynamics in the rhizosphere: consequences for plant health and nutrition. Adv Agron 99:183–225

Sabaté DC, Brandan CP, Petroselli G, Erra-Balsells R, Carina MA (2017) Decrease in the incidence of charcoal root rot in common bean (*Phaseolus vulgaris* L.) by *Bacillus amyloliquefaciens* B14, a strain with PGPR properties. Biol Control 13:1–8

Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2016) Microbial siderophores and their potential applications: a review. Environ Sci Pollut Res 23:3984–3999

Sánchez AC, Gutiérrez RT, Santana RC, Urrutia AR, Fauvart M, Michiels J, Vanderleyden J (2014) Effects of co-inoculation of native *Rhizobium* and *Pseudomonas* strains on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. Eur J Soil Biol 62:105–112

Shamseldin A, Velázquez E (2020) The promiscuity of *Phaseolus vulgaris* L. (common bean) for nodulation with rhizobia: a review. World J Microbiol Biotechnol 36(5):63. https://doi.org/10.1007/s11274-020-02839-w

Shamseldin A, Werner D (2007) Presence of *rhizobium Etli bv. Phaseoli* and *Rhizobium Gallicum bv. Gallicum* in Egyptian soils. World J Microbiol Biotechnol 23:285–289

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:1–14

Silva CP, Vinuesa E, Esperanza EL, Martínez-Romero VS (2003) *Rhizobium etli* and *Rhizobium gallicum* nodulate common bean (Phaseolus vulgaris) in a traditionally managed milpa plot in Mexico: population genetics and biogeographic implications. Appl Environ Microbiol 69(2): 884–893

Singh N, Pandey P, Dubey RC, Maheshwari DK (2008) Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World J Microbiol Biotechnol 24:1669. https://doi.org/10.1007/s11274-008-9680-z

Souza R, de Ambrosini A, Passaglia LMP (2015) Plant growth promoting bacteria as inoculants in agricultural soils. Gene Ther Mol Biol 38:401–419

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Stajković O, Delić D, Josic D, Kuzmanović Đ, Rasulić N, Knežević-Vukčević J (2011) Improvement of common bean growth by co-inoculation with *rhizobium* and plant growth-promoting bacteria. Rom Biotechnol Lett 16(1):5919–5926

Steiner F, da Silva Oliveira CE, Zoz T et al (2020) Co-inoculation of common bean with *rhizobium* and *Azospirillum* enhance the drought tolerance. Russ J Plant Physiol 67:923–932

Tabassum B, Khan A, Tariq M, Ramzan M, Khan MSI, Shahid N, Aaliya K (2017) Bottlenecks in commercialisation and future prospects of PGPR. Appl Soil Ecol 121:102–117. S0929139316305479. https://doi.org/10.1016/j.apsoil.2017.09.030

Tewari S, Arora NK (2014) Multifunctional exopolysaccharides from *Pseudomonas aeruginosa* PF23 involved in plant growth stimulation, biocontrol and stress amelioration in sunflower under saline conditions. Curr Microbiol 69:484–494

Vurukonda SSKP, Vardharajula S, Shrivastava M, Skz A (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24

Wekesa CS, Okun D, Juma K et al (2016) Abundance and symbiotic potential of common bean (Phaseolus vulgaris) nodule associated bacteria in Western Kenya soil. MAYFEB J Agric Sci 1: 1–9

Woomer PL, Huising J, Giller KE, et al (2014) N2Africa final report of the first phase: 2009–2013. www.N2Africa.org. Accessed 23 Oct 2021

Yanni Y, Zidan M, Dazzo F et al (2016) Enhanced symbiotic performance and productivity of drought stressed common bean after inoculation with tolerant native rhizobia in extensive fields. Agric Ecosys Environ 232:119–128

Zahir ZA, Muhammad A, Frankenberger WT (2004) Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv Agron 81:97–168

Zhao Y, Selvaraj JN, Xing F, Zhou L, Wang Y, Song H (2014) Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum*. PLoS One 9:e92486

Chapter 11
Management of Sustainable Vegetable Production Using Microbial Consortium

Habtamu Mekonnen, Lamenew Fenta, Mulugeta Kibret, and Kindu Geta

Abstract To meet the high food demand of the alarmingly growing world population, chemical fertilizers and pesticides are extensively used. Uses of chemical fertilizers and pesticides accompanied by improper farm management practices and application of agrochemicals and pesticides have caused loss of soil fertility and plant health. The exploitation of beneficial soil microorganisms as a substitute for chemical fertilizers in the production of food is one potential solution to this challenge. Since soil microorganisms are inexpensive and eco-friendly, they have been used repeatedly for maximizing the production of many crops across different agronomic practices. So, the application of effective microbes (EMs) solely or in a consortium is becoming an alternative to chemical fertilizers and pesticides for enhancing vegetable production under sustainable agriculture. Microorganisms, such as plant growth-promoting rhizobacteria and mycorrhizal fungi, have demonstrated their ability in the formulation of biofertilizers in the agricultural sector, providing plants with nutrients required to enhance their growth, increase yield, manage abiotic and biotic stress, and prevent phytopathogens attack. This chapter, hence, focuses on the important role performed by beneficial soil microorganisms in the consortium as a cost-effective, nontoxic, and eco-friendly approach in the management of the rhizosphere to promote vegetables' growth and yield.

Keywords Biostimulant · Effective microbes · Microbial consortia · Sustainable agriculture

---

H. Mekonnen (✉) · M. Kibret
Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia

L. Fenta
Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia

Department of Biology, Assosa University, Assosa, Ethiopia

K. Geta
Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia

Debre Tabor University, Debre Tabor, Ethiopia

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_11

11.1 Introduction

The role of soil microorganisms in sustainable agriculture has gained big attention over the last two decades. Sustainable agriculture is a unique modern farming practice that promotes soil health and wholesome crop yield and reduces pollution of cultivable soils. In addition, it is a strategic agro-biotechnological approach where the present societal food demands can be met without compromising food security for the future generation. Due to the ever-growing human population, steps to curb the concept of food security are required, which can intensify crop yield per unit area production capacity (Glick 2012).

In the agricultural sector, vegetables are the most vital part involved in nutritional security due to their short life cycle, high yield, nutritional enrichment, and economic viability and also provide employment opportunities to rural citizens (Mekonnen and Kibret 2021). Currently, ensuring the demand for vegetable production and feeding the increasing population, the worldwide application of chemical fertilizers in the form of synthetic fertilizers and pesticides is causing fertility loss and environmental pollution. Pesticides are used for controlling pests and insects but pose some negative influences on the ecosystem. Excessive use of chemical fertilizers and pesticides cause several illness and death in humans (Verma et al. 2014). Moreover, improper farm management practices and application of agrochemicals cause loss of soil fertility and health. In addition, the pathogens have a detrimental impact on crop productivity. However, sustainable agricultural practices must be essential for providing better food, fuel, and fiber for a healthy nation. Therefore, to minimize the consistent application of expensive and disruptive chemicals in vegetable production, viable and practically applicable alternative strategies need to be developed. To this end, researchers have begun to direct their interests toward ensuring agrarian sustainability using beneficial soil microorganisms. In this regard, the advent of microbial preparation often called biofertilizers involving many useful soil microbes has provided an effective solution to high-input agrochemicals. Hence, the use of nonpathogenic rhizosphere microbes to enhance vegetable production is currently considered a safe, viable, and low-cost alternative to synthetic chemicals (Zaidi et al. 2017). Since soil microorganisms are inexpensive and eco-friendly, they have been used repeatedly for maximizing the production of several crops across different agronomic practices. The application of effective microbes (EMs) individually or in a consortium as a mixed culture is considered for enhancing vegetable production under sustainable agriculture (Kumar et al. 2019).

EMs are naturally coexisting microbes useful and applied to agriculture as biofertilizers or microbial inoculants to enhance plant growth and manage the soil ecosystem. Some of the well-known microbial consortia include lactic acid bacteria (LAB), photosynthetic bacteria, actinobacteria, mycorrhiza, and yeasts. These microbes are physiologically well-matched and coexist in a provided nutritional medium (Naik et al. 2020). In the agriculture system, beneficial microbial inoculants majorly include plant growth-promoting bacteria (PGPB) and VAM (vesicular arbuscular mycorrhizal) fungi. They function via various direct and indirect mechanisms (Toyota and Watanabe 2013). Even though considerable research work has been conducted to explain the impact of rhizosphere microbes in the enhancement of vegetable crops, very few efforts have been made to systematize the information that could benefit progressive vegetable farming communities. Considering the importance of beneficial soil microbes and the success achieved so far, efforts herein have been directed to highlight the impact of microbial consortia or individually on the quality and yield of vegetables grown in different agronomic regions of the world. Furthermore, efforts are also made to explore the challenges of the current development in using microbial consortium as the best tool for sustainable vegetable production (Fig. 11.1).

11.2 Role of Microbial Consortium in Sustainable Vegetable Production

Sustainability in agriculture is the long-term maintenance of soil productivity using natural resources without degrading the environment. Maintenance and preservation of natural resources including the functional and diverse microbial population in the soil is an essential component of sustainable agriculture (Kumari et al. 2020). Microbial consortium (MC) involves the symbiotic interactions of two or more microbial groups for improved crop growth (Clark et al. 2009). It is hard to find microbes that occur, survive, and persist as single cells, strains, or even single species in bulk soil, in the proximity of plant root canopy, as phyllosphere, or even as endophytes (Santoyo et al. 2021a, b). The associations of microbes enhance the turnover of soil organic matters and mobilize nutrients for the growth of plants and fixation of nitrogen. Despite their intrinsic diversity, MCs tend to respond to the environmental stressors as a unique organism, because they can have more chances of survival than any microbial strain living in isolation to adopt one or more of their components to the stressor and can take advantage of internal beneficial interactions among members (Nuti and Giovannetti 2015). Since each of a given ecosystem's physiological functions can be carried out by more than one microbial species, the functional biodiversity and the possibility of replacement among different microbial components play a significant role in maintaining an active life of the ecosystem. As a result of the intrinsic advantages, MC acceptability and applicability by practitioners have increased unlike the use of a single strain, as demonstrated by Sarkar and Chourasia (2017). Under this study, the metabolic versatility of the microbial community for the treatment of organic wastes was used. Besides, the process of co-metabolism is another interactive advantage of MC over single inoculum. This usually manifests when a specific microbial group produces a specific metabolite, which often serves as a potentially limiting nutrient for another microbial population within the consortium. This essentially helps to ensure the complete mineralization of by-products, which could constitute an environmental nuisance to both

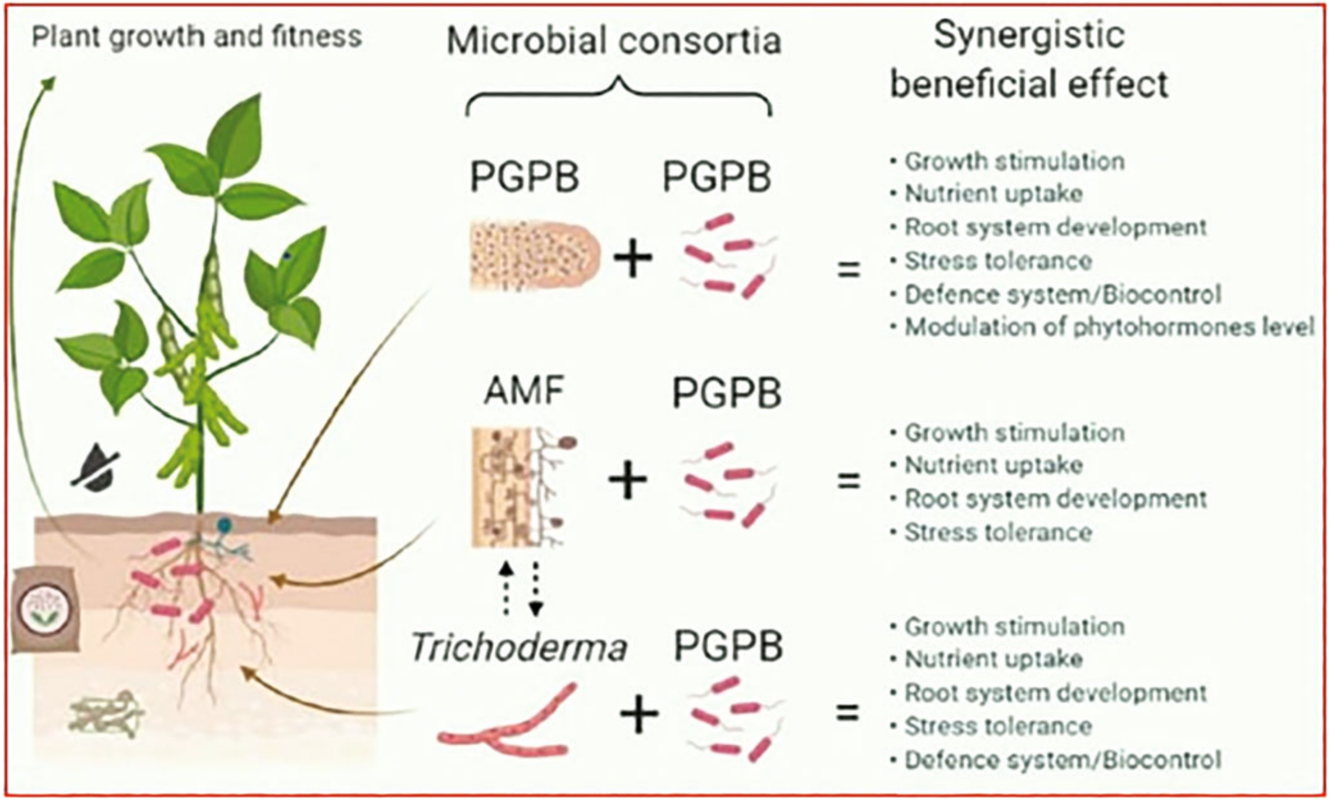


Fig. 11.1 Microbial consortia: plant-growth-promoting bacteria (PGPB), arbuscular mycorrhizal fungi (AMF), and fungi (Santoyo et al. 2021a, b)

microbiota and plants while optimizing the arable function of the soil. Furthermore, the balance among the different components of an MC will ultimately consist of a continuous shift between actively growing (i.e., viable and culturable) cells and nondividing (i.e., viable but nonculturable) cells of the various components of the total population. It is known that different microbial populations communicate with each other within the given environment (e.g., through "quorum sensing") by exchanging precise chemical signals (Nuti and Giovannetti 2015). The powerful features of natural consortia have inspired the interest in engineering synthetic consortia for the application of industrial biotechnology (Minty et al. 2013).

Increasing global use of chemical fertilizers often beyond crop and soil's requirement thresholds; hence, there is a need for researchers and agriculturists to find ways to limit the intense applications of agrochemicals and to significantly reduce their impacts on the soil ecosystem (Sun et al. 2015; Liu et al. 2017). Consequently, the integration of natural biotechnological advantages of soil microbes in vegetable crop production systems would undoubtedly mitigate plant growth challenges and will brighten the global desire for sustainable agriculture (Odoh 2017).

11.3 Types of Microbial Consortia

Under natural conditions, plants communicate with microbial species, with each other to give rise to networks that can have beneficial or harmful effects on plant growth, thus shaping a plant microbiome (Mendes et al. 2011; Carrión et al. 2019). The application of different plant growth and health-promoting microorganisms (PGPMs) with diverse mechanisms of action provides a wide spectrum of benefits for the plant, including direct stimulation of its growth and health of the plant, which leads to better production (Sharma et al. 2018; Behera et al. 2020; Bradáčová et al. 2020; Dheeman et al. 2020).

11.3.1 Bacteria–Bacteria Consortia

The impact of numerous PGPB strains on plants has been well investigated. A bacterial consortium generally constitutes two or more compatible bacteria of different species in a synergistic or additive interaction (Stockwell et al. 2011; Panwar et al. 2014; Sarma et al. 2015). In some cases, a mixture of different strains of the same species can exhibit enhanced activities and be considered a consortium. In the review conducted by Singh et al. (2019), bacterial interactions within a consortium are classified into three types based on the effects they have on each other: (i) stimulatory or positive, (ii) inhibitory or negative, or (iii) neutral. The positive interactions generally create a network to support individual members through cross-feeding, where one bacterium utilizes the metabolic products produced by another consortium member. Mutualism, protocooperation, and commensalism are examples of positive associations. Negative interactions lead to the suppression of bacterial members in a consortium, destroying the community structure and its functioning (Moënne-Loccoz et al. 2015); they include amensalism, predation, parasitism, and competition. Amensalism is a type of unidirectional interaction where the growth of one of the members is affected by the production of toxic compounds by its partner (Roell et al. 2019). In neutral interactions, members of the consortium do not influence each other. This interaction occurs when two species consume different substances (nutritional divergence), and neither produces compounds inhibitory to other members of the consortium (Singh et al. 2019). In agriculture, however, members that interact positively in a mutualistic way are desirable for stable performance over prolonged cultivation to obtain the expected positive effect when applied to a crop (Liu et al. 2012; Mahmud et al. 2020).

Bacterial consortium communication is highly dependent on molecular signals; among them, quorum sensing (Qs) plays a significant role in bacterial compatibility in consortium formulations. Among several signal molecules, the acyl-homoserine lactone (AHL) signal molecules are the most well-known in the bacterial consortium (Mukherjee and Bassler 2019). Other important signaling compounds reported in bacterial consortia are volatile organic compounds (VOCs), which are implicated in both bacteria–bacteria and plant–bacteria communication. These compounds include terpenoids, alkanes, alkenes, ketones, sulfur-containing compounds, and alcohols that act as low-molecular-weight (<300 Da), low-boiling-point, high-vapor-pressure, and lipophilic signal molecules (Fincheira and Quiroz 2018).

11.3.2 Fungus–Bacteria Consortia

According to Deveau et al. (2018) bacteria–fungi interaction (BFI) is intrinsically modulated by the behavioral properties of either or both of the interacting partners. Usually, during their coexistence, there exist intimate biophysical and metabolic associations leading to the development of bacterial–fungi interdependency. Central to BFIs is the communication between the bacterium and the fungus. According to Frey-Klett et al. (2011), the best-known and most extensively studied category of bacterial–fungal communication is antibiosis, a chemical warfare that is typified by the diffusion of deleterious and often chemically complex molecules from one partner to the other. In their review, they have mentioned a variety of mechanisms, including the inhibition of key cellular functions such as cellular respiration (e.g., hydrogen cyanide and fusaric acid), cell wall synthesis (e.g., penicillin and butyric acid), and transport systems (e.g., β-phenylethanol), while others impair the integrity of cell membranes (e.g., hydrolytic enzymes, cyclic lipopeptides, and polymyxin B). For example, exposure to phenazines and phloroglucinols produced by certain *Pseudomonas* isolates induces the expression of several ABC transporters in the fungal phytopathogen *Botrytis cinerea*, which is thought to prevent the intrahyphal accumulation of antifungal metabolites (Schoonbeek et al. 2002; Schouten et al.

2008). In addition, a *B. cinerea* laccase was found to be responsible for the production of reactive species that detoxify 2,4-diacetylphloroglucinol (Schouten et al. 2008).

The other molecules with more subtle effects than antibiotics during BFIs are signaling molecules. Adams et al. (2009) found that some bacterial metabolites stimulate fungal hyphal growth. They stated that *Streptomyces* sp. AcH505 shows enhanced production of the secondary metabolite auxofuran during its interaction with *Amanita muscaria*, which promotes the extension of the fungal mycelium. Further unidentified volatile substances produced by some bark beetle-associated bacteria stimulate the growth of their symbiotic fungi. Bacterial peptidoglycans have been shown to induce *Candida albicans* hyphal growth, while the presence of the *C. albicans* metabolite farnesol can modulate the expression of virulence genes in *Pseudomonas aeruginosa* by influencing bacterial quorum sensing (Cugini et al. 2010).

Moreover, bacterial–fungal communication may also occur via modifications of the physicochemical properties of their environment. A common effect is an alteration of the pH, although some microorganisms (e.g., streptococci, lactobacilli, and *Candida*) occupy environments under a broad range of pH conditions, most of them are susceptible to acidic pH below 4 (O'May et al. 2005). Thus, alterations in pH can affect microbial community structure by either promoting or inhibiting the growth of acid-sensitive organisms. On cheese surfaces, for example, yeast lactate metabolism and the production of alkaline metabolites such as ammonia cause deacidification that favors the growth of less-acid-tolerant bacterial strains that are essential for cheese ripening (Corsetti et al. 2001). Similarly, the presence of the alkalinizing yeast *Geotrichum candidum* enhances the growth of *Salmonella* on tomato fruit surfaces (Wade et al. 2003). In addition to its effects on microbial growth, environmental pH also influences other microbial processes; for example, the rate of synthesis of the secondary metabolite aflatoxin by *Aspergillus parasiticus* is higher under acidic growth conditions, while an alkaline medium increases the production of penicillin by *Aspergillus nidulans* (Calvo et al. 2002).

Besides the role of diffusible molecules in BFIs, migration and physical contact are also important processes in the establishment of BFIs. Chemotaxis (directed movement) of bacteria toward fungi and fungi-derived molecules has been demonstrated in several instances; for example, both detrimental and beneficial *Pseudomonas* species exhibit taxis toward fungal mycelial exudates (Deveau et al. 2010). Cell–cell contact between fungi and bacteria can result in important changes to their physiology and interactions. Adhesive interactions mediated by polysaccharides for the attachment by *Pseudomonas* bacteria with antifungal activity onto the hyphae of the button mushroom *Agaricus bisporus* have been reported (Frey-Klett et al. 2011). A role for extracellular polysaccharides in the attachment of bacterial species to arbuscular mycorrhizal fungi has also been reported, while in the brewing industry, the co-flocculation of the fission yeast *Schizosaccharomyces pombe* with the Gram-positive lactic acid bacterium *Pediococcus damnosus* appears to be mediated in part by yeast cell surface mannose and galactose residues (Bianciotto et al. 2001; Peng et al. 2001).

Nutritional interactions between fungi and bacteria are important to many BFIs. Trophic competition between fungi and bacteria is well documented in the plant root environment (rhizosphere), where bacterial competition for nutrients such as C, N, or Fe can be an effective biocontrol mechanism against fungal root pathogens. Examples of bacterial–fungal trophic competition in other environments include competition for C substrates during the decomposition of leaves the uptake and release of nutrients by yeast during wine fermentation, which greatly affects the growth of malolactic bacteria and competition between the feed additive *S. cerevisiae* CNCM I-1077 and rumen bacteria in an in vitro rumen system (Chaucheyras-Durand et al. 2005). Consortium treatment of *Trichoderma viride* and *Bacillus thuringiensis* showed early blight disease suppression and a high yield of potatoes. Besides, a combination of *P. fluorescens* and *T. viride* reduced early blight disease incidence and increased the yield of potatoes compared to the control under field conditions. This study reported that seed treatment with a combination of *P. fluorescens* and *T. viride* and also between *B. thuringiensis* and *T. viride* can become part of the integrated management of early blight disease of potato and plant growth promotion (Abdullahi et al. 2016).

11.4 Microbial Consortium as Plant Biostimulants

According to Du Jardin (2015), the widely accepted definition of plant biostimulants are substance(s) and/or microorganisms when applied to plants or the soil rhizosphere stimulate the natural processes to enhance nutrient uptake efficiency and tolerance to abiotic stress and improve nutritional quality and crop production. They are often included in agricultural management practices aimed at reducing chemical inputs, increasing productivity, and recovering the natural equilibrium of the ecosystems. PGPR is being tried as consortia and is more effective than single inoculation in solanaceous vegetables (Gupta and Kaushal 2017). A study conducted by Messele et al. (2017) reported that the growth parameters of pepper plants significantly increased the height and dry weight of local pepper varieties in the dual inoculation of *Bacillus* and *Trichoderma* spp. to local pepper varieties compared to the individual one. Besides, the combination of *Bacillus* spp. and *Trichoderma* spp. gave the highest records of growth parameters, fruit yields, and plant nutrient content on tomatoes than individual ones in pot experiments (Morsy et al. 2009).

Plant-growth-promoting microorganism is a term that applies to all microorganisms (e.g., bacteria, actinobacteria, fungi, and algae) having a beneficial effect on plant growth through the action of either direct or indirect mechanisms (Santoyo et al. 2021a, b). They increase the production of various crops, improve soil fertility, promote diversity and interaction with other beneficial microorganisms, inhibit the growth and infective action of potential pathogens, and maintain the sustainability of the ecosystem (Abhilash et al. 2016; Jiménez-Vázquez et al. 2020). In general, PGPMs are based on interactions of single microorganisms with plants, evaluating different parameters of growth and plant health, such as the length or weight of the plant or its tissues, chlorophyll content, or the nutritional content of its tissues or fruits (Khan et al. 2019; Mahmud et al. 2020). This has led to a better understanding of plant–microbe interactions where a multiplicity of microbial species can exist. Using a mixture of two or more compatible microorganisms of different species (or strains) can facilitate beneficial additive or synergistic results since the lack of activities in one added microbe can be found through the action of the other (Nadarajah 2019). The addition of microbial consortia, therefore, can restructure and stimulate plant-growth-promoting mechanisms in both optimal conditions and under different types of biotic and abiotic stress (Woo and Pepe 2018). Microbial consortia between bacteria, *Trichoderma*, and arbuscular mycorrhizal fungi to stimulate plant growth have been found to significantly increase agricultural productivity (Santoyo et al. 2021a, b).

11.5 Microbial Consortium as a Biocontrol Agent (BCA)

Plant growth-promoting microorganisms either live together with non-PGPR strains in soil or the rhizosphere in different combinations (Vacheron et al. 2013). Considering this community-based living style of PGPR strains, the current trend is to mix biocontrol agents (BCAs) of diverse microbial species having PGP traits to achieve desired agricultural outcomes. The application of microbial consortium consisting of efficient strains for biological control may be a superior technique compared to the application of individual microbes for managing plant diseases. Moreover, the application of microbes in a consortium may improve the efficacy, reliability, and consistency of the microbes under diverse soil and environmental conditions (Stockwell et al. 2011). Microbial strains that have no suppressive effect on other microbial strains when cocultured in a common medium (Jain et al. 2012; Singh et al. 2013), in the consortium may, therefore, have an enhanced impact on PGP or disease suppression. The use of different species of microbes in combination may further have the advantage of enhancing biocontrol efficacies as different microbes occupy different niches in the rhizosphere and thereby restrict competition among them. Additionally, diversity in biocontrol mechanisms offered by each microbial component may also help in enhancing disease suppressiveness. Some earlier studies showed that different microorganisms namely *Trichoderma*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Glomus*, etc. were used to develop microbial consortia (Duffy et al. 1996; Jetiyanon 2007; Kannan and Sureendar 2009; Srivastava et al. 2010). Duffy et al. (1996) showed that *Trichoderma koningii* when applied with some fluorescent Pseudomonad strains suppressed the take-all pathogen of wheat greater than *T. koningii* alone. Similarly, Jetiyanon (2007) showed that a mixture of the *Bacillus* strains IN937a and IN937b increased superoxide dismutase and peroxidase activities by 25–50% in tomato and pepper against some soil and seed-borne pathogens. Srivastava et al. (2010) also showed that the combined application of *Trichoderma*, fluorescent *Pseudomonas,* and *Glomus* suppressed Fusarium wilt incidence in tomatoes under field conditions by more than 50% over a single application of *Glomus*.

The rationale behind the selection of the microbes used in consortia is their ability to fix atmospheric N, solubilize phosphorous in the soil, produce phytohormones, and have antagonistic activities against the pathogens. Later on, microbes capable of inducing systemic resistance (Bakker et al. 2013) as well as enhancing nutrient use efficiency were also included in the microbial mixtures (Pandey et al. 2018, 2021; Dhiman et al. 2019). These microbes can do their work individually. However, when compatible strains of these microbes are applied together as a consortium, crops are expected to get a combined benefit of high N and P availabilities for uptake leading to better plant health and yield. Combining antagonist bioagents may further facilitate the disease-free growth of the plants. Therefore, applying microbes as a consortium has great potential, particularly in modern agriculture, where minimization of chemical fertilizers and pesticides is one of the priorities. Enhanced N and P uptake along with biocontrol of soil-borne pathogens were reported in chickpeas when *Rhizobium*, PSB, and *Trichoderma* were applied as a consortium (Rudresh et al. 2005). According to Messele et al. (2017), the dual culture technique on *Colletotrichum* isolates from pepper showed that isolates of *Bacillus* and *Trichoderma* in combination showed the greatest pepper anthracnose disease reduction compared to pathogen-inoculated plants. Moreover, through the biocontrol potential of *P. fluorescens*-Bak150 and *Trichoderma viride*-ES1 against potato late blight pathogen caused by *P. infestans* under greenhouse conditions, it was found that that *P. fluorescens* and *T. viride* significantly reduced the disease compared to the untreated controls (Zegeye et al. 2011). However, in most of these studies conducted earlier, the fate of the microbes inoculated as consortia in the rhizosphere was not assessed and a greater emphasis should therefore be given to this aspect for better utilization of microbial consortia in enhancing their efficacies.

11.6 Microbial Consortium-Mediated Plant Defense

Living plants always remain in contact with a variety of beneficial, nonbeneficial, and pathogenic microorganisms, including bacteria and fungi. They must make out the identities of the microbes and activate their respective mechanisms either to attract the desired microbial species or to keep away the unwanted ones. In the case of pathogenic microbes, plants activate their effective and quick defense mechanisms to protect themselves by arresting the growth of pathogens. Perception and identification of pathogens are, therefore, the key points in the activation of effective and rapid plant defense responses (Ponce de León and Montesano 2013). Plants have developed several mechanisms to resist different biotic and abiotic stresses during evolution. When plant pathogenic microorganisms encounter plants, defense responses are triggered similarly as it is stimulated by beneficial microorganisms. However, the duration and level of activation of the defense responses are much more when the plants are bio-primed with beneficial rhizospheric microbes (Shoresh et al. 2010).

Scholars have developed some successful and effective microbial consortia against soil-borne pathogens such as *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. They demonstrated some of the host-mediated defense responses involved in the suppression of plant diseases by microbial consortia consisting of potential BCAs viz., *Trichoderma harzianum*, *B. subtilis*, and *P. aeruginosa* against *S. sclerotiorum* in pea plants (Jain et al. 2012)) and *P. aeruginosa*, *T. harzianum*, and *Mesorhizobium* sp. against *S. rolfsii* in chickpea plants (Singh et al. 2013). The triple microbial consortium consisting of compatible strains of *P. aeruginosa* PJHU15, *T. harzianum* TNHU27, and *B. subtilis* BHHU100 enhanced the defense parameters of the treated pea plants up to 1.4–2.3-folds when challenged with *S. sclerotiorum* while plants treated with the individual microbes showed an only 1.1–1.7-fold increment in the same parameters compared to untreated counterparts. The microbial consortium activated the antioxidant enzyme activities and the phenylpropanoid pathway leading to the accumulation of total phenolics, proline, and (PR) proteins after the pathogen challenge. Phenol accumulation increased up to 1.4–4.6-folds in plants treated with the triple microbial consortium compared to plants treated with either single or dual microbial consortia (Jain et al. 2012). Similarly, co-inoculation of *Azospirillum* and *P. fluorescens* had a synergistic effect on the yield of cotton with protection against *Rhizoctonia bataticola*, the causal agent of root rot disease in cotton (Marimuthu et al. 2013). However, the rhizosphere population of the microbes declined with plant maturity by nearly 50%. The consortium of two PGPR strains, *P. fluorescens* Aur 6 and *Chryseobacterium balustinum* Aur 9, was used for the integrated management of blast disease of rice (Lucas et al. 2009). It was observed that disease severity reduced to half when the microbial strains were used in combination compared to their single application under field conditions. Similarly, co-inoculation of *T. harzianum* Tr6 and *Pseudomonas* sp. Ps14 enhanced the effect of ISR via MYB72 (independent from SAR) in cucumber by activating different signaling pathways against *Botrytis cinerea* (Alizadeh et al. 2013). The co-inoculation treatment with Tr6 and Ps14 reduced the disease index by more than 40% as compared to the single microbial treatments. The consortium of *Paecilomyces lilacinus* KIA and *Rhizobium* sp. also proved very effective in reducing the multiplication of the root-knot nematode caused by *Meloidogyne javanica* in chickpea roots. While the *P. lilacinus* KIA strain could parasitize females and eggs of nematodes, the *Rhizobium* strain could produce antibiotics and phytoalexins. The combined effects of the microbial mixture thus led to better growth inhibition of the nematodes and enhanced plant growth (Siddiqui and Akhtar 2009). The number of galls per root system was also lowered by more than 20% when *P. lilacinus* KIA was co-inoculated with the *Rhizobium* strain compared to its single application. However, random mixing of BCAs may also have adverse effects on disease management. Xu et al. (2010) evaluated those three commercial biocontrol products by combining two at a time and observed that the disease incidence of *B. cinerea* in a strawberry was even higher than the application of the individual BCAs. Negative impacts of microbial mixtures were also reported by Bora et al. (2004) and Elliott et al. (2009). Xu et al. (2010) suggested that the antagonistic activities of the BCAs against each other may have a role to play in reducing their performances. Therefore, the compatibility of microbial mixtures is the most important issue to be addressed in the future while developing microbial consortia to improve their biocontrol efficacies. Besides, ISR is only one of the mechanisms that may be mobilized to counteract plant pathogens in an environmentally friendly and durable way. Integrating ISR-triggering PGPR into disease management programs in conjunction with other strategies is yet to be a worthwhile approach to explore (Kaymak 2010).

The results were obtained from experiments involving microbial consortia, and most of the microbes were selected based on their plant growth promotion and disease-suppressive abilities along with a presumption that the consortia will be effective against the tested pathogens. Only in fewer cases, it was evident that the consortia were developed based on either compatibility of the microbes or prior information that the consortia were effective against the target pathogen. Thus, testing for compatibility is important from the point of view that it may minimize the failures of experiments due to microbial incompatibility.

11.7 Microbial Consortium as Biofertilizer

Microbial interactions in soil ecology are dynamic and complex. It is a determinant to increase the yield and productivity of aboveground crop parts (Philippot et al. 2013). This occurs through a process called bio-fertilization—a phenomenon where microbial inoculants are seeded on plant surfaces, seeds, and/or soil to colonize the rhizosphere. This condition enhances growth through the supply and availability of primary nutrients to the plant (Odoh 2017; Kenneth et al. 2019). Biofertilizer is a culture of bacteria, fungi, and algae either alone or in combination that is packed in carrier materials to enhance plant growth. Several support materials are preferably organic in origin (notably chitosan, gelatin, sawdust, k-carrageenan, zeolite, activated carbon, etc.) and are stable bio-carriers used to immobilize these biofertilizers while substantially eliminating environmental perturbations (Nwankwegu and Onwosi 2017). In comparative research evaluating microbial consortia versus single-strain inoculants, Bradáčová et al. (2019) suggested that microbial consortia increase the efficiency of crop production, particularly under challenging environmental conditions. In arable agricultural application, it is considered an essential component for long-term soil fertility and sustainability. Nuti and Giovannetti (2015) in their view suggested that biofertilizers act by nourishing and fortifying the host plant and inducing general pathogenic resistance, irrespective of its origin and nature. Research has focused on halting the overdependence on synthetic fertilizers coupled with the rising depletion of soil functionality (Chatzipavlidis et al. 2013; Bhardwaj et al. 2014). This bioprocess, however, requires optimization to support increased yield and economic viability of small and marginal farmers. The microbial consortium, apart from being able to mobilize nutritionally important elements from nonusable forms through the biological processes (Mazid et al. 2012), secretes fascinating bioactive ligands (Myc and Nod factors) using a transduction pathway (Roberts et al. 2013) for the release of Ca^{2+} in the cytosol (Sieberer et al. 2009).

11.8 Challenges with Microbial Consortium

Though the application of beneficial soil microorganisms to enhance plant productivity is gaining more focus, they have not been widely accepted on a large scale because of the difficulty of reproducing their beneficial effect on plants in a natural environment or field condition (Jacoby et al. 2017). The major challenges with the application of microbial inoculants are a lack of awareness of the eco-friendly importance among the communities of farmers, inadequate promotion and motivation by the agricultural extension worker to the farmers on the use of biofertilizer products, lack of availability of suitable carriers for biofertilizer formulation, problems of storage facilities to prevent contamination, and extreme climatic conditions, which lead to inconsistency in the efficacy of biofertilizers on plant productivity in a natural environment (Fasusi et al. 2021).

Compatible microbial consortia may result in promoting plant growth and development in various crops (Pandey et al. 2012) . As described in the above section, dual microbial inoculation was reported to enhance plant production more than the application of individual strains. However, similar results were not always obtained in co-inoculation treatments. For instance, Walker et al. (2012) showed that co-inoculation treatments involving *Glomus*, *Azospirillum*, and *Pseudomonas* did not increase secondary metabolites in maize roots when results of the co-inoculated treatments were compared with a single application of *Glomus*. Similarly, mycorrhizal association in common beans exhibited a negative effect on nodule development. Several other reports are also available, which demonstrated that certain microbial consortia were unable to show at least significant effects on plants concerning their applications (Ballesteros-Almanza et al. 2010). Similarly, Felici et al. (2008) documented that co-inoculation with *B. subtilis* 101 and *Azospirillum brasilense* Sp245 had no significant effect on plant biomass accumulation concerning control, whereas individual application of the microbes showed an increase in plant biomass. The negative impact of the dual inoculation treatment was attributed to the involvement of independent signaling pathways in the two microbial species. One of the major causes for such contrary results with microbial mixtures may be attributed to the incompatibility of the microbes in the mixture with each other. Thus, while developing a consortia formulation, a combination of compatible strain that differs in the pattern of plant colonization, combination with a broad spectrum of action against different plant pathogens, combinations with different modes of action, and two or more genetically diverse groups of genera to adapt different pH, moisture, temperature, and relative humidity must be considered (Sarma et al. 2015). The use of microbial inoculants must also consider microbial diversity in the rhizosphere and having effective biotechnological applications.

11.9 Conclusive Remarks and Future Perspectives

As stated above, compatible microbial consortia are important in promoting plant growth and plant disease suppression, as well as a biocontrol agent in various crops. Although the development of microbial consortia is important for healthy plant development, equal understanding of how they influence plant metabolism and the mechanisms involved in the interaction of microbes (bacteria–bacteria, bacteria–fungus, and fungus–fungus) are not explored, particularly their molecular interactions. Molecular-based approaches to analyze the impact of introduced isolate on the microbial diversity and community structure and to predict responses to microbial inoculation are also essential. Further studies must also be conducted to address the consequences of the cooperation between microbes in the rhizosphere under field conditions to assess their ecological impacts and biotechnological applications. In this regard, efforts are needed to promote the use of microbial consortia by considering their multifunctional characteristics and quality standards for potential combinations of microbes that must be generated to ease their agricultural application. More insights are also needed into the microbial consortia-regulated expression of transcription factors associated with host defense responses during biotic and abiotic challenges. In addition, compatible strains of microbes are identical, which can positively influence the host physiological and transcriptional regulations for the development of cost-effective products for commercialization. At the same time, it will be a bonus if simple and rapid testing methodologies/kits are developed for the evaluation of effective microbial consortia, which can predict probable impacts of the consortia on host plants through the synergistic acts of the microbes. Beyond this, it has been indicated by some authors that plants can inherit the threat signals perceived by the parents to their offspring through the trans-generational passage of message when the plants are primed with either chemicals or biological materials. The passage of the message is found to be carried forward to the next generation through epigenetically regulated expression of certain genes. This is an area to be explored further, particularly in the context of whether microbes in consortia can induce fine-tuning of genes to get desired outputs in the next generation. Similarly, it was demonstrated that metabolic changes could occur in favor of plants to achieve a specific objective that benefits agriculture. Additionally, metabolites from the culture of microbial mixes could be evaluated for their potential to stimulate crop plants for enhancing the yield and protection from pests and pathogens by specific up-regulation of some desired genes by the metabolites in the absence of the biologically alive microbial cells. Sophisticated bioinformatics tools may also be considered for the design and development of microbial consortia for sustainable agricultural practices.

References

Abdullahi S, Simon S, Babychan M (2016) Effect of bioagents and their consortia in the management of early blight disease of potato. Int J Agri Res Innov Technol 5(3):495–498

Abhilash P, Tripathi V, Edrisi SA, Dubey RK, Bakshi M, Dubey PK et al (2016) Sustainability of crop production from polluted lands. Energy Ecol Environ 1(1):54–65

Adams A, Currie C, Cardoza Y, Klepzig K, Raffa K (2009) Effects of symbiotic bacteria and tree chemistry on the growth and reproduction of bark beetle fungal symbionts. Can J Fores Res 39(6):1133–1147

Alizadeh H, Behboudi K, Ahmadzadeh M, Javan-Nikkhah M, Zamioudis C, Pieterse CM et al (2013) Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps14. Biol Control 65(1):14–23

Bakker PA, Doornbos RF, Zamioudis C, Berendsen RL, Pieterse CM (2013) Induced systemic resistance and the rhizosphere microbiome. Plant Pathol J 29(2):136–143

Ballesteros-Almanza L, Altamirano-Hernandez J, Pena-Cabriales J, Santoyo G, Sanchez-Yanez J, Valencia-Cantero E et al (2010) Effect of co-inoculation with mycorrhiza and rhizobia on the nodule trehalose content of different bean genotypes. Open Microbiol J 4:83–92

Behera B, Das T, Raj R, Ghosh S, Raza MB, Sen S (2020) Microbial consortia for sustaining productivity of Non-legume crops: Prospects and challenges. Agribiol Res 10(1):1–14

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13(1):1–10

Bianciotto V, Andreotti S, Balestrini R, Bonfante P, Perotto S (2001) Extracellular polysaccharides are involved in the attachment of *Azospirillum brasilense* and *Rhizobium leguminosarum* to arbuscular mycorrhizal structures. Eur J Histochem 45(1):39–50

Bora T, Özaktan H, Göre E, Aslan E (2004) Biological control of *Fusarium oxysporum* f. sp. melonis by wettable powder formulations of the two strains of *Pseudomonas putida*. J Phytopathol 152(9):471–475

Bradáčová K, Florea AS, Bar-Tal A, Minz D, Yermiyahu U, Shawahna R et al (2019) Microbial consortia versus single-strain inoculants: An advantage in PGPM-assisted tomato production. Agronomy 9(2):1–23

Bradáčová K, Kandeler E, Berger N, Ludewig U, Neumann G (2020) Microbial consortia inoculants stimulate early growth of maize depending on nitrogen and phosphorus supply. Plant Soil Environ 66(3):105–112

Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66(3):447–459

Carrión VJ, Perez-Jaramillo J, Cordovez V, Tracanna V, De Hollander M, Ruiz-Buck D et al (2019) Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science 366(6465):606–612

Chatzipavlidis I, Kefalogianni I, Venieraki A, Holzapfel W (2013) Status and trends of the conservation and sustainable use of microorganisms in agroindustrial processes. Commission on Genetic Resources for Food and Agriculture, The Food and Agriculture Organization of the United Nations

Chaucheyras-Durand F, Masséglia S, Fonty G (2005) Effect of the microbial feed additive *Saccharomyces cerevisiae* CNCM I-1077 on protein and peptide degrading activities of rumen bacteria grown in vitro. Curr Microbiol 50(2):96–101

Clark NM, Rillig MC, Nowak RS (2009) Arbuscular mycorrhizal fungal abundance in the Mojave Desert: Seasonal dynamics and impacts of elevated CO_2. J Arid Environ 73(9):834–843

Corsetti A, Rossi J, Gobbetti M (2001) Interactions between yeasts and bacteria in the smear surface-ripened cheeses. Int J Food Microbiol 69(2):1–10

Cugini C, Morales DK, Hogan DA (2010) *Candida albicans*-produced farnesol stimulates *Pseudomonas* quinolone signal production in LasR-defective *Pseudomonas aeruginosa* strains. Microbiology 156(10):3096–3107

Deveau A, Brulé C, Palin B, Champmartin D, Rubini P, Garbaye J et al (2010) Role of fungal trehalose and bacterial thiamine in the improved survival and growth of the ectomycorrhizal fungus *Laccaria bicolor* S238N and the helper bacterium *Pseudomonas fluorescens* BBc6R8. Environ Microbiol Rep 2(4):560–568

Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, Bindschedler S et al (2018) Bacterial–fungal interactions: Ecology, mechanisms and challenges. FEMS Microbiol Rev 42(3):335–352

Dheeman S, Baliyan N, Dubey RC, Maheshwari DK, Kumar S, Chen L (2020) Combined effects of rhizo-competitive rhizosphere and non-rhizosphere *Bacillus* in plant growth promotion and yield improvement of *Eleusine coracana* (Ragi). Can J Microbiol 66(2):111–124

Dhiman S, Dubey RC, Baliyan N, Kumar S, Maheshwari DK (2019) Application of potassium-solubilising *Proteus mirabilis* MG738216 inhabiting cattle dung in improving nutrient use efficiency of *Foeniculum vulgare* Mill. Environ Sustain 2(4):401–409

Du Jardin P (2015) Plant biostimulants: Definition, concept, main categories and regulation. Sci Hortic 196:3–14

Duffy BK, Simon A, Weller DM (1996) Combination of Trichoderma koningii with fluorescent pseudomonads for control of talk-all on wheat. Phytopathology (USA) 86:188–194

Elliott M, Shamoun S, Sumampong G, James D, Masri S, Varga A (2009) Evaluation of several commercial biocontrol products on European and North American populations of *Phytophthora ramorum*. Biocontrol Sci Tech 19(10):1007–1021

Fasusi OA, Cruz C, Babalola OO (2021) Agricultural sustainability: Microbial biofertilizers in rhizosphere management. Agriculture 11(2):1–19

Felici C, Vettori L, Giraldi E, Forino LMC, Toffanin A, Tagliasacchi AM et al (2008) Single and co-inoculation of *Bacillus subtilis* and *Azospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community. Appl Soil Ecol 40(2):260–270

Fincheira P, Quiroz A (2018) Microbial volatiles as plant growth inducers. Microbiol Res 208:63–75

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial-fungal interactions: Hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev 75(4):583–609

Glick BR (2012) Plant growth-promoting bacteria: Mechanisms and applications. Scientifica 2012: 1–16

Gupta S, Kaushal R (2017) Plant growth promoting rhizobacteria: Bioresouce for enhanced productivity of solanaceous vegetable crops. Acta Sci Agron 1(3):10–15

Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S (2017) The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. Front Plant Sci 8:1–19

Jain A, Singh S, Kumar Sarma B, Bahadur Singh H (2012) Microbial consortium–mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. J Appl Microbiol 112(3):537–550

Jetiyanon K (2007) Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strains (IN937a and IN937b) against different pathogens. Biol Control 42(2):178–185

Jiménez-Vázquez KR, García-Cárdenas E, Barrera-Ortiz S, Ortiz-Castro R, Ruiz-Herrera LF, Ramos-Acosta BP et al (2020) The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. Plant J 103(5): 1639–1654

Kannan V, Sureendar R (2009) Synergistic effect of beneficial rhizosphere microflora in biocontrol and plant growth promotion. J Basic Microbiol 49(2):158–164

Kaymak HC (2010) Potential of PGPR in agricultural innovations. In: Plant growth and health promoting bacteria. Springer-Verlag, Germany, pp 45–79

Kenneth OC, Nwadibe EC, Kalu AU, Unah UV (2019) Plant growth promoting rhizobacteria (PGPR): A novel agent for sustainable food production. Am J Agric Biol Sci 14:35–54

Khan N, Bano A, Rahman MA, Guo J, Kang Z, Babar MA (2019) Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (*Cicer arietinum* L.) induced by PGPR and PGRs. Sci Rep 9(1):1–19

Kumar A, Patel JS, Meena VS, Srivastava R (2019) Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. Biocatal Agric Biotechnol 20:1–10

Kumari S, Sharma A, Chaudhary P, Khati P (2020) Management of plant vigor and soil health using two agriusable nanocompounds and plant growth promotory rhizobacteria in Fenugreek. 3 Biotech 10(11):1–11

Liu H, Zhou Y, Xiao W, Ji L, Cao X, Song C (2012) Shifting nutrient-mediated interactions between algae and bacteria in a microcosm: evidence from alkaline phosphatase assay. Microbiol Res 167(5):292–298

Liu P, Jia S, He X, Zhang X, Ye L (2017) Different impacts of manure and chemical fertilizers on bacterial community structure and antibiotic resistance genes in arable soils. Chemosphere 188: 455–464

Lucas J, Solano BR, Montes F, Ojeda J, Megias M, Manero FG (2009) Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa*) in Southern Spain. Field Crop Res 114(3):404–410

Mahmud K, Makaju S, Ibrahim R, Missaoui A (2020) Current progress in nitrogen fixing plants and microbiome research. Plan Theory 9(1):1–17

Marimuthu S, Ramamoorthy V, Samiyappan R, Subbian P (2013) Intercropping system with combined application of *Azospirillum* and *Pseudomonas fluorescens* reduces root rot incidence caused by *Rhizoctonia bataticola* and increases seed cotton yield. J Phytopathol 161(6): 405–411

Mazid M, Khan TA, Mohammad F (2012) Role of nitrate reductase in nitrogen fixation under photosynthetic regulation. World J Pharm Res 1(3):386–414

Mekonnen H, Kibret M (2021) The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. Chem Biol Technol 8(1):1–11

Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH et al (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332(6033): 1097–1100

Messele A, Serawit H, Tesfaye A (2017) In vitro and In vivo evaluation of antagonistic microbes against pepper anthracnose (*Colletotrichum capsici* (syd.) *Bisby* and *Butler*). Int J Environ Sci 6(3):87–93

Minty JJ, Singer ME, Scholz SA, Bae C-H, Ahn J-H, Foster CE et al (2013) Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass. Proc Natl Acad Sci 110(36):14592–14597

Moënne-Loccoz Y, Mavingui P, Combes C, Normand P, Steinberg C (2015) Microorganisms and biotic interactions. Environmental microbiology: fundamentals and applications. Springer, Singapore, pp 395–444

Morsy EM, Abdel-Kawi K, Khalil M (2009) Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents gainst *Fusarium solani* on tomato plants. Egyptian J Phytopathol 37(1): 47–57

Mukherjee S, Bassler BL (2019) Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol 17(6):371–382

Nadarajah KK (2019) Microbes: An important resource for sustainable agriculture. In: Microbiome in plant health and disease. Springer, Singapore, pp 53–77

Naik K, Mishra S, Srichandan H, Singh PK, Choudhary A (2020) Microbial formulation and growth of cereals, pulses, oilseeds and vegetable crops. Sustain Environ Res 30:1–18

Nuti M, Giovannetti G (2015) Borderline products between bio-fertilizers/bio-effectors and plant protectants: the role of microbial consortia. J Agric Sci Technol 5:305–315

Nwankwegu AS, Onwosi CO (2017) Bioremediation of gasoline contaminated agricultural soil by bioaugmentation. Environ Technol Innov 7:1–11

Odoh CK (2017) Plant growth promoting rhizobacteria (PGPR): A bioprotectant bioinoculant for sustainable agrobiology. A review. Int J Adv Res Biol Sci 4(5):123–142

O'May GA, Reynolds N, Macfarlane GT (2005) Effect of pH on an in vitro model of gastric microbiota in enteral nutrition patients. Appl Environ Microbiol 71:4777–4783

Pandey P, Bisht S, Sood A, Aeron A, Sharma G, Maheshwari DK (2012) Consortium of plant-growth-promoting bacteria: future perspective in agriculture. In: Bacteria in agrobiology: plant probiotics. Springer-Verlag, Berlin Heidelberg, pp 185–200

Pandey C, Negi YK, Maheshwari DK, Rawat D, Prabha D (2018) Potential of native cold tolerant plant growth promoting bacilli to enhance nutrient use efficiency and yield of *Amaranthus hypochondriacus*. Plant Soil 428(1):307–320

Pandey C, Dheeman S, Prabha D, Negi YK, Maheshwari DK (2021) Plant growth-promoting bacteria: Effective tools for increasing nutrient use efficiency and yield of crops. In: Endophytes: Mineral nutrient management, vol 3. Springer, pp 293–313

Panwar M, Tewari R, Nayyar H (2014) Microbial consortium of plant growth-promoting rhizobacteria improves the performance of plants growing in stressed soils: An overview. In: Phosphate solubilizing microorganisms. pp. 257–285

Peng X, Sun J, Michiels C, Iserentant D, Verachtert H (2001) Coflocculation of *Escherichia coli* and *Schizosaccharomyces pombe*. Appl Microbiol Biotechnol 57(1):175–181

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11(11):789–799

Ponce de León I, Montesano M (2013) Activation of defense mechanisms against pathogens in mosses and flowering plants. Int J Mol Sci 14(2):3178–3200

Roberts NJ, Morieri G, Kalsi G, Rose A, Stiller J, Edwards A et al (2013) Rhizobial and mycorrhizal symbioses in *Lotus japonicus* require lectin nucleotide phosphohydrolase, which acts upstream of calcium signaling. Plant Physiol 161(1):556–567

Roell GW, Zha J, Carr RR, Koffas MA, Fong SS, Tang YJ (2019) Engineering microbial consortia by division of labor. Microb Cell Factories 18(1):1–11

Rudresh D, Shivaprakash M, Prasad R (2005) Effect of combined application of Rhizobium, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aeritenium* L.). Appl Soil Ecol 28(2):139–146

Santoyo G, Gamalero E, Glick BR (2021a) Mycorrhizal-bacterial amelioration of plant abiotic and biotic stress. Front Sustain Food Syst 5:1–18

Santoyo G, Guzmán-Guzmán P, Parra-Cota FI, Santos-Villalobos SDL, Orozco-Mosqueda M, Glick BR (2021b) Plant growth stimulation by microbial consortia. Agronomy 11(2):1–24

Sarkar P, Chourasia R (2017) Bioconversion of organic solid wastes into biofortified compost using a microbial consortium. Int J Recycl Org Waste Agric 6(4):321–334

Sarma BK, Yadav SK, Singh S, Singh HB (2015) Microbial consortium-mediated plant defense against phytopathogens: Readdressing for enhancing efficacy. Soil Biol Biochem 87:25–33

Schoonbeek H-J, Raaijmakers JM, De Waard MA (2002) Fungal ABC transporters and microbial interactions in natural environments. Mol Plant-Microbe Interact 15(11):1165–1172

Schouten A, Maksimova O, Cuesta-Arenas Y, Van Den Berg G, Raaijmakers JM (2008) Involvement of the ABC transporter BcAtrB and the laccase BcLCC2 in defence of *Botrytis cinerea* against the broad-spectrum antibiotic 2, 4-diacetylphloroglucinol. Environ Microbiol 10(5): 1145–1157

Sharma CK, Vishnoi VK, Dubey R, Maheshwari DK (2018) A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen *Macrophomina phaseolina* in mung bean. Rhizosphere 5:71–75

Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43

Siddiqui ZA, Akhtar MS (2009) Effect of plant growth promoting rhizobacteria, nematode parasitic fungi and root-nodule bacterium on root-knot nematodes *Meloidogyne javanica* and growth of chickpea. Biocontrol Sci Tech 19(5):511–521

Sieberer BJ, Chabaud M, Timmers AC, Monin A, Fournier J, Barker DG (2009) A nuclear-targeted cameleon demonstrates intranuclear Ca^{2+} spiking in *Medicago truncatula* root hairs in response to rhizobial nodulation factors. Plant Physiol 151(3):1197–1206

Singh A, Sarma BK, Upadhyay RS, Singh HB (2013) Compatible rhizosphere microbes mediated alleviation of biotic stress in chickpea through enhanced antioxidant and phenylpropanoid activities. Microbiol Res 168(1):33–40

Singh BP, Rateb ME, Rodriguez-Couto S, Polizeli M, Li W-J (2019) Editorial: Microbial secondary metabolites: Recent developments and technological challenges. Front Microbiol 10(914)

Srivastava R, Khalid A, Singh US, Sharma AK (2010) Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biol Control 53(1):24–31

Stockwell V, Johnson K, Sugar D, Loper J (2011) Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. Phytopathology 101(1):113–123

Sun R, Guo X, Wang D, Chu H (2015) Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. Appl Soil Ecol 95:171–178

Toyota K, Watanabe T (2013) Recent trends in microbial inoculants in agriculture. Microbes Environ 28(4):403–404

Vacheron J, Desbrosses G, Bouffaud M-L, Touraine B, Moënne-Loccoz Y, Muller D et al (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4:1–19

Verma JP, Jaiswal DK, Sagar R (2014) Pesticide relevance and their microbial degradation: A-state-of-art. Rev Environ Sci Biotechnol 13(4):429–466

Wade WN, Vasdinnyei R, Deak T, Beuchat LR (2003) Proteolytic yeasts isolated from raw, ripe tomatoes and metabiotic association of *Geotrichum candidum* with *Salmonella*. Int J Food Microbiol 86(1-2):101–111

Walker V, Couillerot O, Von Felten A, Bellvert F, Jansa J, Maurhofer M et al (2012) Variation of secondary metabolite levels in maize seedling roots induced by inoculation with *Azospirillum*, *Pseudomonas* and *Glomus consortium* under field conditions. Plant Soil 356(1):151–163

Woo SL, Pepe O (2018) Microbial consortia: promising probiotics as plant biostimulants for sustainable agriculture. Front Plant Sci 9:1–6

Xu X, Robinson J, Jeger M, Jeffries P (2010) Using combinations of biocontrol agents to control Botrytis cinerea on strawberry leaves under fluctuating temperatures. Biocontrol Sci Tech 20(4): 359–373

Zaidi A, Khan MS, Musarrat J (2017) Microbes for legume improvement. Springer, Berlin

Zegeye ED, Santhanam A, Gorfu D, Tessera M, Kassa B (2011) Biocontrol activity of Trichoderma viride and *Pseudomonas fluorescens* against *Phytophthora infestans* under greenhouse conditions. J Agri Technol 7(6):1589–1602

Chapter 12
Consort Interactions of the Root Endophytes *Serendipita* spp. (Sebacinales, Agaricomycetes, Basidiomycota) with Crop Plants

Nader Rokni, Flemming Ekelund, and M. Hossein Borhan

Abstract Various microorganisms may be formulated and applied to enhance plant growth and vigor, e.g., the symbiotic fungal root endophytes *Serendipita indicia* and *S. vermifera* (Serendipitaceae, Agaricomycetes, Basidiomycota), which interact with a broad range of host plants. These symbionts have a wide range of beneficial effects on plants, such as induction of resistance to fungal pathogens and different insects/pests as well as overall unspecific growth-promoting effects. Unlike arbuscular mycorrhiza (AM) fungi, *Serendipita* spp. can grow axenically on synthetic media, which presents a potential for producing quantities of active inoculum for large-scale formulation and field use. This makes the *Serendipita*-based products promising candidates for application in sustainable agriculture. Fungal endophytes support crops in different crucial ways. However, the endophytes are not always beneficial to the plants, as the symbiosis may range from mutualism to antagonism. The host–endophytic relation presents an intricate balance between plant defenses and fungal virulence. Thus, the application of high concentrations of *S. indica* inoculum may lead to severe infection and strong negative effects on the plant. In this review, we address the importance of "inoculum quantity" as an often-overlooked factor in the study of plant–endophyte interactions. We also highlight the benefits of the endophyte-mediated interaction of plants and *Serendipita* spp. singly or in aggregates with other plant growth-promoting microorganisms. Finally, we discuss the potential advantages of using carrier-based formulations of the bioinoculants, especially for large-scale commercial seed production.

N. Rokni (✉)
Department of Science, Jundi-Shapur University of Technology, Dezful, Iran
e-mail: naderrokni@jsu.ac.ir

F. Ekelund
Department of Biology, University of Copenhagen, Copenhagen, Denmark

M. H. Borhan
Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_12

Keywords Biopesticides · Biofertilizers · Bioinoculants · Commercialization · Multipartite symbioses · Sustainability

12.1 Introduction

Plant–soil–microbiome interactions are essential determinants of plant health and thus soil fertility (Jeffries et al. 2003). Arbuscular mycorrhiza is the most frequent mutualism on earth and a classic example of a reciprocally beneficial symbiosis. However, several plant species, including major crops and weeds, seem to be nonmycorrhizal, e.g., Proteaceae, Chenopodiaceae, Caryophyllaceae, and Brassicaceae (Brundrett 2009; Lambers and Teste 2013). These plants may associate with various fungal endophytes with growth promotion properties. The genus *Serendipita* (*Serendipitaceae*, Sebacinales, Agaricomycetes, Basidiomycota) interacts with plant roots in various ways, including endophytic mycorrhiza-like associations (Weiss et al. 2016). The endophytic members include *Serendipita vermifera* (Warcup and Talbot 1967), *S. indica* (Varma et al. 1999) (formerly, *Sebacina vermifera* and *Piriformospora indica*, respectively), *S. williamsii* (formerly, *Piriformospora williamsi*) (Basiewicz et al. 2012), and *S. herbamans* (Riess et al. 2014). These species are axenically cultivable on synthetic media and show a broad spectrum interaction, i.e., endophytism and associations resembling orchid-, ericoid-, and ecto-mycorrhiza with broad host plants (Weiss et al. 2011; Selosse et al. 2007; Basiewicz et al. 2012). Serependita species function as plant biofertilizers in nutrient-deficient soils, protect plants against pathogens, insects, and heavy metals, and may modulate plant development, i.e., cause early flowering, enhance seed production, and stimulate the production of active ingredients in medicinal plants and promote hardening of tissue in cultivated plants (Pham et al. 2004a, b, c). In particular, *S. indica* has, since its discovery in 1998, been studied intensively because of its potential beneficial effects (Oelmüller et al. 2009). *S. indica* colonizes the root cortex and forms inter- and intracellular hyphae and often forms dense hyphal coils or branched structures in the cortical cells. In nutrient-deficient soils, *S. indica* enhances plant growth, overall biomass, and yield. Thus, it may protect against biotic stress (Weiss et al. 2011; Varma et al. 2012), including infection by fungal leaf pathogens (Serfling et al. 2007), and abiotic stress such as drought, temperature, or salt stress (Waller et al. 2005). Consortia of *S. indica* with other bio-fertilizers have been shown to promote plant fitness (Anith et al. 2011; Jafari et al. 2018; Kesh and Yadav 2016), making it a promising candidate for application in sustainable horticulture and agriculture (Gill et al. 2016; Kumar et al. 2009; Serfling et al. 2007; Varma et al. 2001; Waller et al. 2005; Yadav et al. 2010). Indeed, mass production of *S. indica* with different types of carriers has successfully demonstrated improved plant growth and protection against soil-borne fungal diseases in field trials (Bajaj et al. 2014; Mishra et al. 2014).

S. vermifera colonization enhances seed germination and biomass production of various plant species (Ghimire et al. 2009; Ghimire and Craven 2011; Barazani et al. 2005; Baldi et al. 2008). *S. vermifera* also confers resistance to toxins and heavy metal ions and may hence be used for phytoremediation because it accumulates heavy metals and prevents their uptake into plants (Yadav et al. 2010). Moreover, it promotes shoot and root growth, lateral root development, tolerance to salt, and drought stress (Baldi et al. 2008; Fakhro et al. 2010; Jogawat et al. 2013; Sun et al. 2010; Waller et al. 2005; Yaghoubian et al. 2014). However, plants may not always benefit from the interaction with *Serendipita*.

Schulz et al. (1999) described the plant–endophyte interaction as a balanced competition where the fungal virulence and plant defenses are in a state of equilibrium. This equilibrium can change from mutualism to antagonism through an imbalance in the environmental and ecological factors, including the predisposition of host tissue, the colonization density, environmental conditions, competition with other microorganisms, and biotic/abiotic stresses (Petrini 1991; Stone et al. 2004; Bodles et al. 2006; Sieber 2007). Insufficient understanding of the complex relationships of plant-bioinoculant, particularly in terms of the required inoculum concentration, has led to ambiguous results. Results from greenhouse and field studies are inconsistent (Varma et al. 1999) and differences between methods for the establishment of symbiosis systems further blur the results. Here, we review the effects of the endophytic *Serendipita* on crop fitness, biotic/abiotic stress tolerance, and mineral uptake, with a special focus on the inoculation methods and inoculum concentration.

12.2 Inoculum and Root Colonization

12.2.1 Measures of Assessing Mycorrhization

Serendipita mimics the AM fungi lifestyle. We argue that almost all the essential items of their methodology have been retrieved from their predecessor, the AM fungi. However, this approach has a major drawback, which is the lack of research-based evidence for the utilized inoculum type, source, density, and/or inoculation method. We investigated the literature of AM fungi with regard to the inoculum and inoculation methodologies. Based on our search of the literature traditionally, infectivity and efficacy are the parameters introduced for assessing the interaction between plant and AM fungi. Efficacy is defined as the effectiveness of a fungus in promoting plant growth. It is a more complicated phenomenon to model because it is affected by several factors. However, it can be empirically measured by the growth-promoting effects of mutualism. Infectivity, which is defined as the colonization ability of the inoculum, is directly determined by using mathematical models. One of the parameters that have been extensively used for calculating infectivity is the "percent root colonization" (PRC). PRC is the measure of the incidence of fungal structures in plant roots. Thus, it demonstrates the colonization condition of the root pieces (Buwalda et al. 1984; Sanders and Sheikh 1983; Walker and Smith 1984). Our survey also shows that optimal inoculum density has been considered an important key factor for functional plant root and soil colonization with AM fungi (Abbott and Robson 1982; Menge 1983; Haas and Krikun 1985).

12.2.2 PRC Vs. Inoculum Density

Studies with AM fungi have correlated the root colonization ability of the AM fungi and the consequent PRC with the number of established entry points, which in turn is a function of the inoculum densities. The plant growth-promoting effects of the AM fungi were also attributed to inoculum density in early studies (Carling et al. 1979; Smith and Smith 1981; Haas and Krikun 1985). Even though the first studies of AM inoculation demonstrated that the effectiveness of mutualism for promoting plant performance is determined by the optimal inoculum density rather than the amount of PRC (Kapoor et al. 2002; Toussaint 2007; Das et al. 2012), the importance of inoculum density was neglected in later research. Instead, later research favored the easy calculable PRC measure to establish a link between the efficacy of symbiosis and the extent of root colonization. The PRC measure has also been extensively used for the evaluation of the colonization pattern of plant roots with *Serendipitaceae*. In the next section, we have focused on how this legacy has transferred and affected the studies with *Serendipitaceae*.

12.2.3 Inoculum Quantity and the Outcome of the Interaction

Currently, there is no defined method for quantifying the fungal biomass in the *Serendipita* colonized roots, and this seems to have been neglected in nearly all the *Serenidipta* research (e.g., Varma et al. 1999; Sahay and Varma 1999). Some studies do not even describe the method of inoculation and/or inoculation concentration (del Barrio-Duque et al. 2019; Su et al. 2017; Peškan-Berghöfer et al. 2004; Rai and Varma 2005; Rathod et al. 2011). Kaldorf et al. (2005) emphasized the importance of using an optimized concentration of inoculum and a well-defined inoculation method since culture conditions may drastically alter the behavior of *S. indica*. They reported the growth-promoting activity of the fungus on 6-day-old pre-rooted *Populus* plantlets inoculated with mycelial plugs altered to severe growth inhibition and rooting blockage following the incubation of the explants in the presence of *S. indica* grown in woody plant medium. They suggested that prolonged incubation would stimulate the fungus to not only colonize and spread on the surface of the aerial parts (stem and leaves) but also to invade the cortical tissues inter- and intracellularly. Thus, depending on the cultural conditions, the interaction between plants and fungi could be either mutualistic or antagonistic.

In a recent experiment, we used real-time quantitative PCR (qPCR) to assess the correlation between inoculum concentration and colonization density of *S. indica* in oil rapeseed (*Brassica napus*) (Abin et al. 2021). Seeds were inoculated with fungus inoculum at five different concentrations (1–10% w/w basis). In line with Haas and Krikun (1985), the experiments showed that varying inoculum density could significantly affect root colonization severity. Meanwhile, even tenfold increase in the inoculation concentration did not affect the PRC. We obtained the same results in another recent study on the tripartite interactions between *S. indica*, *Trichoderma simmonsi*, and bell pepper (*Solanum capsicum*), in which PRC remained in a constant range of 55% in response to varying inoculum concentrations (1–15% w/w) across all the treatments. The results showed that seed inoculation with lower *S. indica* inoculum densities (1–3% w/w) resulted in a significant increase in the vegetative growth of the plant in both the greenhouse and field conditions. Surprisingly, higher concentrations of the *S. indica* inoculum either resulted in significant negative or less positive effects on plant growth in comparison to lower densities (Rokni et al. 2021). Fakhro et al. (2010) also showed a deleterious shift in the beneficial effects of *S. indica* inoculum on hydroponically grown tomato plants by an increase in the inoculum concentration from 3×10^5 cfu/mL to 9×10^5 cfu/mL. They also found no correlation between the PRC measure (10%–50%) and the shoot fresh weight of *S. inidca* colonized tomato plants. Thus, based on the evidence reviewed, the PRC should not be considered a valid analytical technique to correlate the inoculum concentrations to the efficiency of the plant–*Serendipita* interaction or density of root colonization. Accordingly, a standardization of optimal inoculum concentration should be included in any endophyte *S. indica* host combination research as a key factor for fine-tuning the interaction.

12.2.4 Inoculum Types and Sources

Different inoculum types may facilitate interaction between *Serendipita* spp. and plants; these include fungal plugs (5 mm), spores (chlamydospores), and biomass (mycelium and spores) from liquid or solid media (Table 12.1). Both the inoculum type and source may affect the performance of the plant–*Serendipita* symbiotic system (Andrade-Linares et al. 2013); e.g., *S. indica* inoculum type (biomass or spore) affected the extent of fungal spread in the tomato roots, and the levels of root colonization were inversely correlated with the plant performance (Andrade-Linares et al. 2013). Thus, when spores were used, the fungal spread in tomato root was higher than using fungal biomass as inoculum. To the present day, few studies incorporated the aspects of inoculum (i.e., source, type, and quantity) in the evaluation of plant–*Serendipta* interactions.

More trials with the incorporation of different concentrations of inoculum and more appropriate methods of assessing root colonization densities (see Sect. 12.2.3) are required to determine the exact impact of the variables (i.e., inoculum source, type, quantity, etc.) on plant–*Serendipita* interactions.

Table 12.1 Biostimulant effects of *Serendipitaceae* applications on agronomical and physiological aspects of crops

Plant species	Inoculation type	Inoculation quantity	Cultivation (conditions)	Beneficial effects	References
Maize, poplar, parsley, and some other plants seeds	Wet mycelium	1 g/100 g substrate (expanded clay)	Pot culture, agar plates	Promotion of plant growth and biomass production	Varma et al. (1999)
Tobacco, and tissue culture raised plantlets	Layer of live inoculum of *S. indica* mixed in a small amount of sterile soil	1% (w/v) of soil	Pot culture	Positive effect as a biohardening tool for tissue culture raised plantlets	Sahay and Varma et al. (1999)
The medicinal plants *Spilanthes calva* and *Withania somnifera seeds*	Fungal mycelium	1% w/w of seed	Nurseries and subsequent transferring to the field	Significant increase in growth and yield of both plant species	Rai et al. (2001)
Arabidopsis thaliana seedliings	Fungal plug Fungal mycelium	– 1% (w/v) of soil	Agar plates Pot culture	Promotion of plant growth	Peškan-Berghöfer et al. (2004)
Populus cuttings 1. Plantlets with roots 2. Explants (without root)	Mycelial plugs (5 mm diam.)	? Explants placed close to 10-day-old colonies from mycelial plugs	Agar plates	1. An increase in root biomass 2. Complete blocking of root production and severely inhibited plant growth	Kaldorf et al. (2005)
Nicotiana attenuate seeds	*S. indica* mycelium *S. vermifera* mycelium	Axenic mycelia of the fungi (seeds on agar plates of Gamborg's B5 medium previously inoculated with and incubated in the dark at 26 ° C for 10 days)	Pot culture	Seed inoculation with either fungus species stimulated seed germination, increased growth, and resistance to attack from *(Manduca sexta)* larvae	Barazani et al. (2005)
Adhatoda vasica (a medicinal plant) cuttings	Fungal mycelium	?	Glass bottles containing the cuttings of the plant Pot culture (containing cuttings with pre-inoculated roots)	Rapid proliferation of roots and improved growth of the plant	Rai and Varma 2005

Tobacco and *Arabidopsis* seedlings	Mycelium	Fungal plugs (pre-rooted tobacco and *Arabidopsis* seedlings on agar plates)	Agar plates Pot culture	Growth promotion due to co-regulated stimulation of enzymes involved in nitrate and starch metabolisms	Sherameti et al. (2005)
Barley	Mycelium and spores	2 g of fungal biomass/300 g substrate (expanded clay) 4 g/300 g of substrate	Hydroponic condition Pot culture	Enhanced yield, inducing resistance to fungal diseases and tolerance to salt stress	Waller et al. (2005)
Pelargonium (*Pelargonium* × *hortorum*), Poinsettia (*Euphorbia pulcherrima*), and *Petunia* (*Petunia hybrida*) cuttings	*S. indica* Mycelium and spores	2 g/1 L substrate	Pot culture 15 cuttings/pot	Revealed a diverse response from enhancing the number and length of the adventitious roots in pelargonium and poinsettia to no rooting response in *Petunia* plants	Druege et al. (2007)
Wheat seeds	Mycelium and spores	100 mL of mycelial suspensions containing ≈15 g fresh weight of *P. indica* A mycelial suspension of *S. indica* of 3000–6000 liter/ha	Pot culture Field condition	Induction of systemic resistance or priming of the host plant against *Pseudocercosporella herpotrichoides* and *Blumeria graminis f.* sp. *Tritici* under field conditions	Serfling et al. (2007)
Linum album Germinated internode sections	*S. indca or* *S. vermifera*	Different concentrations (0.5, 1, 2.5, 5, and 7.5 g/L)	Suspension cultures 2 g/L callus on dry cell weight basis	Increased plant biomass and enhanced production of anticancer compounds (podophyllotoxins) in suspension cultures	Baldi et al. (2008)
Barley (Hordeum vulgare) salt-sensitive cultivar Ingrid Seeds	*S. indica* Mycelium and spores	Two-day-old germinating seeds immersed in *S. indica* homogenate (2 g/L)	Pot cultures	Increased plant growth and revealed the possible role of antioxidants in both inherited and endophyte-mediated plant tolerance to	Baltruschat et al. (2008)

(continued)

Table 12.1 (continued)

Plant species	Inoculation type	Inoculation quantity	Cultivation (conditions)	Beneficial effects	References
				salinity. Elevated the activities of antioxidant enzymes in barley roots under salt stress conditions by upregulation of the antioxidative system	
Switchgrass (*Panicum virgatum*) seedlings	*S. vermifera*	Immersing seedlings roots in the homogenized fungal mycelial suspension for 3 h	5 seedlings/pot	Enhanced plant height, root length, and biomass production	Ghimire et al. (2009)
Barley *(Hordeum vulgare)* Seeds or seedlings	*S. indica* Mycelium and spores or *G. mosseae* and *F. graminearum*	– 2 g of crushed mycelium/300 g substrate for 4 weeks – Dipping 3-day-old seedling root spore suspension (5×10^4 spores/mL) for 5 min 10% w/v soil 10^6 spores/plant	Pot cultures (6 plantlets/6 L pots)	*G. mosseae* Increased total phosphate contents of host plant roots and shoots *S. indica* No significant effect on the phosphate supply Enhanced grain yield Suppressed *F. graminearum* root rot disease	Achatz et al. (2010)
Tomato seeds	Spore and mycelium	3×10^5 cfu/mL 9×10^5 cfu/mL	Hydroponic	Positive Negative	Fakhro et al. (2010)
Chinese cabbage seedlings	Mycelium and spore	Fungal plug	Pot culture	Enhanced root and shoot biomass and drought tolerance by activating the antioxidant enzyme systems	Sun et al. (2010)

Fennel (*Foeniculum vulgare*) germlings	*S. indica* Mycelium and spores or *S. vermifera* Mycelium	1% w/v of soil 1% w/v	Pot culture	Increased oil yield, dry weight of the green tissue and roots by both bioioculants	Kari Dolatabadi et al. (2011)
Soybean (*Glycine max*) seeds	*S. indica* *G. mosseae* *G. intraradices*	10 g soil culture 10 g soil culture 10 g soil culture/pot	Pot culture Diameter of 41.5 cm	Increased percentage of colonization, height, and drought Stress tolerance capacity of soybean plants by *G. mosseae* followed by *G. intraradices* and *S. indica*	Rathod et al. (2011)
Rice (*Oryza sativa*) seedlings	*S. vermifera* Mycelium	Five fungal plugs (5 mm diameter)/pot	Pot cultures Three seddlings/pot	Promoted rice performance under salinity stress. Increased tolerance index and P, N, and Zn uptake under slightly and moderate salinity levels	Pirdashti et al. (2012)
Indian pennywort (*Centella asiatica*) seedlings	*S. indica* biomass in PD broth	100 mg/mL fresh weight of *S. indica* in a 1:1 ratio of MS and PD broth	In vitro culture	Rapid enhancement of root and shoot biomass and increased synthesis of secondary metabolite production (asiaticosides, about twofold) of host plant	Satheesan et al. (2012)
Brassicaceae family (*Brassica oleracea*, *B. napus*, *B. nigra*, *Lepidium sativum*, *Descurainia sophia*, and *Matthiola incana*) Germlings	*S. indica* Mycelium and spores or *S. vermifera* Mycelium	1 g/2 seelings/pot (24 h after potting) 1 g/2 seelings/pot (24 h after potting)	Pot culture	Increased fitness, bigger leaves, more side shoots and roots by both fungi Different degrees of stimulation depends on the involved bioinoculant and the plant species	Kari Dolatabadi and Goltapeh (2013)

(continued)

Table 12.1 (continued)

Plant species	Inoculation type	Inoculation quantity	Cultivation (conditions)	Beneficial effects	References
Tomato (6-week-old tomato plantlets)	Spores Mycelium and spore	– 5 × 10^5 spores/mL – 10 g biomass/250 mL substrate	Hydroponic system (fertilized for tomato growth or with low N or 0% P)	The outcome of interaction, attributed to: 1. The type and amount of inoculum being used 2. The time point of inoculation 3. The nutrient conditions (P and N) in the environment	Andrade-Linares et al. (2013)
Arabidopsis thaliana and *Lotus japonicus*	*S. indica* or *P. williamsii* Mycelium and spores	5 × 10^5 spores/mL culture (1/2 strength) MS medium 1 g fresh biomas/100 g substrate (soil)	In vitro Pot culture	Increased stem height on low nutrient (NPK) soil. Independency of *S. indica* colonization pathway to AM-related plant common symbiosis genes	Banhara et al. (2015)
Brassica napus	*S. indica*	?	Pot culture field	Significant increase in agronomic parameters (plant biomass, lodging-resistance, early bolting and flowering, oil yield and quality) Reduced harmful metaboloite (erucic acid and glucosinolates contents), and increased accumulation of macro- and micro-elements	Su et al. (2017)

12.2.5 Inoculum Quantity and Nutritional Conditions of the Substrate

It was documented that inoculum overdose causes *S. indica* to revert from the beneficial plant growth-promoting effects it provides under optimal nutritional conditions to detrimental in nutrient-deficient substrates (Andrade-Linares et al. 2013). Inoculum overdose has even more drastic effects on biomass reduction of plants grown in low nitrogen. Thus, the presence of nitrogen at the optimal level in the growth substrate was reported as critical for *S. indica* induction of its growth promotional effects. At optimized inoculum concentration (3×10^5 cfu/mL) with normal N- and P-deprived conditions, the fungus displayed a significant potential for growth promotion (Andrade-Linares et al. 2013). Such findings are not surprising, since, in the definition of mycorrhizal dependency, Gerdemann (1975) connects the degree of plant well-being to both its mycorrhizal and soil fertility conditions and suggests how unsuitable quantity of inoculum and imbalanced nutritional conditions of the substrate may negatively affect plant–*Serendipita* interaction.

12.2.6 Inconsistent Choice of Inoculum Quantity

Another problem is the many different methods of inoculation, which makes the comparison of different studies difficult. For example, Dolatabadi et al. (2011) added 1% w/v (mycelium/soil) of *S. indica* or *S. vermifera* to pots of fennel (*Foeniculum vulgare*), whereas, Dolatabadi and Goltapeh (2013) used 1 g of crushed mycelium for *Brassica* seedlings. Satheesan et al. (2012) used 100 mg/mL fresh weight of *S. indica* to inoculate Asiatic pennywort (*Centella asiatica*,) and Sahay and Varma (1999) spread a layer of soil mixed with the inoculum of *S. indica*, over pots of tobacco plantlets. Lack of reproducibility may be the shared problem of such methodologies in which the concentrations of the inoculum were not specified by authors. Considering the impact of the inoculum quantity on the outcome of the interactions, designing reliable procedure(s) for quantitative measurements of inoculum concentration would lead to reproducible methodologies and facilitate to focus *Serpendita* research on other critical factors that may impact the interactions with plants.

12.3 Inoculation Methods

To establish contact between plant and *Serendipta,* several techniques have been used. These methods include fresh/dry weight (g) of the fungal biomass to weight or volume (mL) of plant seeds or growth substrate, dipping seeds/roots/reproductive organs in homogenized fungal biomass, placing fungal plugs in growth substrate, placing seeds on the pre-grown culture of the inoculants, and using colony-forming unit (CFU) enumeration [spores, fungal biomass (spore(s), mycelial fragments or spore(s) attached to mycelia)] (Table 12.1).

12.3.1 Inoculation Based on Weight/Volume Ratio

Inoculation based on the weight/weight or weight/volume measures is the most common method. For example, Varma et al. (1999) used 1 g of fresh fungal mycelium mixed with 100 g substrate (expanded clay) for inoculation of seedlings or plantlets of maize, poplar, parsley, and some other plants, which reportedly enhanced the growth and biomass production of the plants. Rai et al. (2001) used a homogenized culture of *S. indica* in sterile water to inoculate *Spilanthes calva* and *Withania somnifera* at two phenologic stages of plant growth. They inoculated seeds at 1% w/w and reinoculated the juvenile roots with 50 mg/mL mycelia (fresh weight) and found a significant increase in the growth and yield of both plant species.

Peškan-Berghöfer et al. (2004) mixed the fungus at 1% w/v with soil to study the association of *S. indica* with *Arabidopsis thaliana* roots. The inoculum was fungal mycelium obtained from liquid culture, but the inoculation quantity was not specified. Peškan-Berghöfer et al. (2004) reported a positive effect of the fungus during the whole lifetime of the plant (faster growth, enhanced vegetation, earlier flowering, earlier seed ripening, and higher seed yield). Rai and Varma (2005) used 5 g of homogenized mycelium of *S. indica* to inoculate the pre-rooted cuttings of *Adhatoda vasica* in glass bottles and found a rapid proliferation of roots and improved growth of the plant due to this association. Druege et al. (2007) used 2 g of homogenized *S. indica* biomass/L substrate to assess the fungal potential for stimulation of adventitious root formation in *Pelargonium*, *Poinsettia*, and *Petunia* cuttings. Except for Petunia, the formation of adventitious roots for the other plants was enhanced in the presence of *S. indica.* Waller et al. (2005) used 2 g of *S. indica* mycelium/300 g substrate (expanded clay) before sowing barley (*Hordeum vulgare*) seeds under hydroponic conditions and 4 g/300 g of the substrate for yield evaluations in a greenhouse before transplantation to outdoor conditions. They found enhanced tolerance of the *S. indica* inoculated plants to Fusariose and powdery mildew *Blumeria graminis* f.sp. *hordei* and mild salt stress and ascribed this as the induction of systemic resistance. Serfling et al. (2007) used various inoculation methods and concentrations of *S. indica* inoculum to examine its biocontrol potential for preventing the fungal disease of wheat under greenhouse and field conditions. These authors used suspensions of 15 g fresh weight of *S. indica* biomass/100 mL for inoculations of substratum immediately before sowing the seeds or 7 days after sowing to evaluate the germination rate of the wheat seeds and infection assays of the plantlets encountering the pathogen in the presence of *S. indica*, respectively, and a twofold water-diluted suspension from an initial 220 g of *S. indica* biomass/1.5 L liquid medium under field conditions. Serfling et al. (2007) found a significant reduction in the severity of leaf, stem base, and root pathogens due to the induction of systemic resistance in the endophyte-inoculated plants. Thus, *Serendipita* inoculation may affect plants positively, but valid documentation of quantification and reasoning behind the choice of inoculum concentration is missing.

12.3.1.1 Mycelial Plugs as the Source of Inoculum

Many experiments used mycelial plugs of *Serendipita* spp. as inoculum. However, the numbers, depth in the substrate, distance from host tissue, and quantity of the inoculum do not comply with a defined protocol. For example, Sherameti et al. (2005) placed fungal plugs (5 mm diam) at a distance of 1 cm of 10-day-old pre-rooted tobacco and *Arabidopsis* seedlings on top of a modified Melin-Norkrans (MMN) 1/10 (very low N, P, and carbohydrate) medium. They found stimulation of plant growth, which was attributed to the fungal stimulation of nitrate and starch metabolism. Sun et al. (2010) used one mycelial plug/seedling to inoculate 5-day-old seedlings in glass jars containing 1/2 Murashige and Skoog (MS) nutrient medium (Murashige and Skoog, 1962) to study the drought tolerance in Chinese cabbage in the presence of *S. indica.* They reported enhancing effects of the fungus on seedling growth, rooting, and drought tolerance, which they attributed to increased plant tolerance to the fungus-mediated suppression of oxidative stress under water stress. In a similar study of plant performance under salt stress, Pirdashti et al. 2012 used five mycelial plugs (5 mm) of *S. vermifera* grown on Kaefer medium (Käfer 1977) per three rice seedlings (*Oryza sativa*) in a pot culture condition. They reported *S. vermifera* to significantly increase plant salt tolerance, nutrient uptake, and yield under light to moderate salinity.

12.3.1.2 Other Inoculation Methods

In some reported research, the inoculum concentration is too imprecise to enable replication of the experiment. For example, Banhara et al. (2015) used 1 mL of either *S. indica* or *S. williamsii* chlamydospore suspensions containing 5×10^5 spores/mL to inoculate *Arabidopsis thaliana* or *Lotus japonicus* seedlings without explaining the distribution rate of the inocula per seedling. Barazani et al. (2005, 2007) and Ghimire et al. (2009) only named the plant name or its phenologic stage of association with *Srerendipita* spp. but provided no data on the quantity of inoculum.

12.3.2 Improving the Reproducibility of the Inoculation Technique

Although some data about the quantity of the inoculation, such as the weight/volume ratio of inoculum to plant material, could be found in the methodologies, quantification, however, is still not clear in most of these reports. The CFU enumeration is a more reliable method in terms of quantification, but it still has its drawbacks. *S. indica* produces chlamydospores that are not deciduous. It means that the spores are barely detached from the mycelia. The mycelia are functional, the same as the spores, and are known as the source of inoculum. They also share a greater part of the biomass, especially when produced in liquid culture. However, the source of inconsistency is that a CFU, as a unit of inoculation, could be a spore, mycelial fragment, or spore(s) attached to mycelia. Therefore, to resolve this challenge, we suggest using a combination of the above-mentioned techniques. Thus, to ensure the reproducibility of results and large-scale applications, we suggest using the fresh weight ratio of the fungal biomass as inoculum with a specified CFU and dry-to-fresh weight ratio (Rokni et al. 2021).

12.4 Multipartner Symbioses

Several naturally occurring examples of the evolutionary persistent mutualism between plants/animals and microorganisms exist, e.g., leguminous plants and rhizobium (Werner et al. 2015), plants–AM fungi (Hart et al. 2013), and insect–bacteria relations (Douglas 2016). These involve a single plant or animal species that interact simultaneously with several fungi/bacteria. Multipartite symbioses are well known in mammalian digestive systems and have been described in insects, such as bark beetles, aphids, termites, and fungus-farming ants (Hardoim et al. 2015). Multipartite symbioses represent dynamic communities, where spatial, temporal, and genetic variations in the community may affect host fitness (Roe et al. 2011). Despite their success in the laboratory, most of the mutualism models that involve a single partner fail to thrive in natural habitats. Evolutionary persistence mutualisms involve a single plant interacting simultaneously with many fungal or bacterial partners. A dynamic network of multi-species symbioses would guarantee its competence in the face of diverse soil microbial activities (Hardoim et al. 2015).

12.4.1 Consortium of **S. indica** *and/or* **S. vermifera** *with Other Microorganisms*

For many crops, researchers have demonstrated that the beneficial effects of *S. indica* and *S. vermifera* are more pronounced when co-cultured with other plant growth-promoting fungi and bacteria. Our survey showed that *Trichoderma* and AM species are the most common fungi in which their cooperation with *Serendipitaceae* has been examined for improving agricultural systems. Table 12.2 shows the integrated effects of *Serendipita* spp., plant growth-promoting microbes, and host plants.

Table 12.2 Biostimulant effects of *Serendipitaceae* in consortia with other plant growth promotion microbes on agronomical and physiological aspects of crops and the respected carrier-based formulations

Plant species/part	Inoculum/inoculation		Carrier	Cultivation (conditions)	Beneficial effects	References
	Type	Quantity				
Flax *Linum album* cell suspensions	*P. indica* and *S. vermifera*	Different concentrations (0.5, 1, 2.5, 5, and 7.5 g/L on dry cell weight basis)	–	Cell suspensions	Significant enhancement of the anti-cancer lignans a 20% increase in biomass compared to the control cultures	Baldi et al. (2008)
Chickpea (*Cicer arietinum*) seeds	*S. indica* (Mycelium and spore) *Pseudomonas striata*	2 g/100 mL distilled water 6.2×10^6 cfu/mL	–	Pot culture	Synergistic effect on population buildup of *P. striata* and plant dry biomass with respect to their single inoculation No influence on P uptake by single or combined inoculation of two species	Meena et al. (2010)
Tomato seeds	*S. indica* (mycelium and spore) Fluorescent Pseudomonad strains R62 and R81	10 mL (9.5 g/L dry cell weight of *S. indica*) 10 mL (2×10^{11} CFU/mL) (0.5 g of bioinoculant carrier formulations/50 g seeds)	80 g talcum or vermiculite	Pot culture Filed study	Improved productivity of tomato plants by talcum based formulation of pseudomonad strains (R62 and R81) and *S. indica*	Sarma et al. (2011)
Chickpea (*Cicer arietinum*) seeds	*S. indica* (mycelim and spores) *P. lentimorbus* Cell suspension	2 g/23 cm diameter pots 10^9 CFU/mL	–	Pot culture	*S. indica* ranked last compared to *P. lentimorbus* as the most efficient one in terms of promoting plant height, dry weight, and root nodulation followed by consortium	Nautiyal et al. (2010)
Green gram (*Phaseolus aureus*) seeds	*S. indica* Mycelium and spore AM fungi (Soil along with	1 g of hyphal mass/100 g soil 400 spores/100 g soils along with hyphae and infected root segments	–	Pot culture	Enhanced biomass production of dual inoculated plants with AM fungi and *Rhizobium* Ineffectiveness of *P. indica* on the	Ray and Valsalakumar (2010)

(continued)

Table 12.2 (continued)

Plant species/ part	Inoculum/inoculation		Carrier	Cultivation (conditions)	Beneficial effects	References
	Type	Quantity				
	spores, hyphae, and root segments) *Rhizobium* Isolated from *Phaseolus multiflorus*	10 mL of 10^9 CFU/mL to100 g soil			green gram in enhancing biomass production	
Black pepper (*Piper nigrum*)	*S. indica* Mycelium and spore *T. harzianum* Mycelium and spore	1% (w/w) of soil 1% (w/w) of soil	–	Pot culture	Increased root colonization and beneficial effects on the growth of the plant by the root endophyte *S. indica* by sequential application of the *S. indica* at the hardening stage followed by *T. harzianum* during transplanting into a soil-sand mixture	Anith et al. (2011)
Two varieties of soybean (Glycine max) Seeds	*S. indica* *G. mosseae* *G. intraradices*	**Not defined** 10 g soil culture 10 g soil culture	–	Pot culture	The cultivar-based response over the effectiveness of *G. mosseae* or *G. intraradices*, for enhancing drought tolerance with *S. indica*, the least effective in either case	Rathod et al. (2011)
Lentil (*Lens culinaris*) Seeds	*S. indica* and *S. vermifera* mycelia *T. viride* and *T. harzianum*	*1* g of crushed 10^6 CFU g^{-1}	–	Pot culture	Maximum plant height and minimum disease severity were observed with *S. vermifera* + *T. harzianum*	Dolatabadi et al. (2011)
Basil (*Ocimum basilicum*) Germinated seeds	*S. indica* spores *T. tomentosum* spores	5×10^5 spore/mL 1×10^7 spore/mL	–	Pot culture	The positive effect of simultaneous use of two fungi along with copper nitrate on basil growth and yield improvement	Abdollahi et al. (2015)

Artemisia annua seeds	*S. indica* *A. chroococcum*	2 g of fungal mycelium per 100 g of soil 10^5–10^6 CFU/mL	–	Pot culture	Increased productivity of the plant over the interaction with the consortium of the bioinoculants	Arora et al. (2016)
Rice (*Oryza sativa*) seeds Seedlings Seeds and seedlings	*S. indica* biomass *T. virens* biomass	1 × 10^9 CFU/mL 1 × 10^9 CFU/mL	–	Pot culture	The positive effects of using both microorganisms and P were more pronounced than using P alone	Mohammadi et al. (2018)
Tomato Germinated seeds	*S. indica* Mycelia and spores *Mycolicibacterium* isolates	– Bacterial cells (5 × 10^7 CFU/mL) -*S. indica* spores + mycelia (5 × 10^5 CFU/mL) – Mixture of bacterial cells (5 × 10^7 CFU/mL) and *S. indica* (5 × 10^5 CFU/mL) Immersion for 30 min in bioinoculant(s)	–	Pot culture	– Detrimental effects of Bacillaceae, Enterobacteraceae, and Burkholderiaceae and stimulatory effects of Rhizobiaceae strains on *S. indica* growth – Introduction of the Mycolicibacterium strains as the most effective in consortia with *S. indica* biocontrol of the root rot diseases and enhancement of plant growth	del Barrio-Duque et al. (2019)
Rice (*Oryza sativa*) 7-day-old seedlings		*S. indica* 5 × 10^5 spore/mL *Azotobacter vinelandii* O.D. 0.4 at 600 nm	–	Pot culture	Enhanced plant biomass and chlorophyl content due to sequential inoculation of *A. vinelandii* strain on seventh day after *S. indica* inoculation in rice as compared to the non-inoculated and to singly inoculated plants	Dabral et al. (2020)
Bell pepper (*Capsicum annum*) Seeds	*S. indica* biomass *T. simmonsii* spores	1% w/w 1 × 10^6 spore/mL	–	Pot culture Field	– No effects on seed germination or germination earliness – Significant increase in the vegetative growth and yield of the plant in the greenhouse and field conditions	Rokni et al. (2021)

12.4.1.1 Consortium with Trichoderma spp.

Baldi et al. (2008) examined the impact of the combined use of both *S. indica* and *S. vermfera* on living cells of flax (*Linum album)*. They reported that the interaction caused a 20% enhancement of anticancer lignans in cell suspension cultures. Anith et al. (2011) evaluated the compatibility of *Trichoderma harzianum* and *S. indica* on tissue-cultured black pepper (*Piper nigrum*) plantlets. Besides individual use of each bioinoculant, they examined a simultaneous application of the fungal agents or application of *S. indica* followed by *T. harzianum* or vice versa. They reported a negative growth response to the consortium of the bio-inoculants in comparison to when a sequential inoculation with *S. indica* and *T. harzianum* (30 days later) increased the plant growth and root colonization. Enhanced plant biomass and chlorophyll content due to sequential inoculation of *Azotobacter vinelandii* on the seventh day after *S. indica* inoculation in rice was also reported by Dabral et al. (2020) as compared to the noninoculated and singly inoculated plants. Dolatabadi et al. (2011) studied the antagonistic effects of *S. indica*, *S. vermifera*, *T. viride*, and *T. harzianum* solely or in combination on *Fusarium* wilt in lentils (*Lens culinaris*). All of the individual or multipartite amendments were efficient; however, consortia of *S. vermifera* and *T. harzianum* further increased plant growth and reduced the disease severity.

12.4.1.2 Consortia with Bacteria and AM Fungi

Arora et al. (2016) studied the association of *Artemisia annua* that produces artemisinin, a potent antimalarial compound, and *S. indica* and the nitrogen-fixing bacterium *Azotobacter chroococcum*, either singly and/or in combination. They reported a significant improvement in plant growth and contents of phosphorus, nitrogen, and artemisinin due to a dual biological consortium. Sarma et al. (2011) showed that the tripartite association of *S. indica*, fluorescent pseudomonads, and tomato plants improved the plant growth, yield, and biocontrol of *Fusarium* wilt disease under both greenhouse and field conditions. By contrast, in a study by Nautiyal et al. (2010) to enhance nodulation in the rhizosphere of chickpea (*Cicer arietinum*) in the presence of *Paenibacillus lentimorbus*, *S. indica*, in the presence of native rhizobial population (they didn’t use any special rhizobial strain), was less effective in promoting plant growth and root nodulation than the most efficient *P. lentimorbus* and the consortium of the endophytes. The same results were obtained by Ray and Valsalakumar (2010) to examine the influences of individual use or consortium of *S. indica*, AM fungi, and *Rhizobium* on the growth and yield of green gram (*Phaseolus aureus*). *S. indica* was the least effective in improving plant biomass after *Glomus microcarpum* and the most effective consortium of *Rhizobium* and *G. mosseae.* Rathod et al. (2011) evaluated the effects of *S. indica*, *Glomus mosseae*, and *G. intraradices* for enhancing drought stress tolerance in soybean. The results showed a cultivar-based response over the effectiveness of *G. mosseae* or

G. intraradices, but in either case, *S. indica* was the least effective. Achatz et al. (2010) compared the influences of *S. indica* and *G. mosseae* on barley plant growth under various nutrient regimes and for biocontrol of *Fusarium* wilt. Their results suggested an increase in barley yield and biomass due to the biostimulation of the plant by either of the bioinoculants. Achatz et al. (2010) attributed the yield enhancement of the plant to increased root and tiller formation, as a result of increased uptake of P and N in the presence of *S. indica*. However, *S. indica* appeared significantly more effective than *G. mosseae* in protecting the plant from root rot disease. These authors also reported that *S. indica* only increased the photosynthesis rate of the host at low light, in comparison to increased photosynthesis by AM fungi at both low and saturated light conditions (Mathur and Vyas 1995; Caravaca et al. 2003).

12.4.2 Antagonisms/Synergisms in Multicomponent Systems

In a comprehensive study, Varma et al. (2013) assessed the inhibitory/stimulatory effects of rhizobacteria on *S. indica* growth by using both in vitro (confrontation assays on nutrient agar plates) and in vivo (barley seedlings) methods. Enhancement of the barley root system has already been shown in association with *S. indica* (Baltruschat et al. 2008; Achatz et al. 2010). While a majority of the experiments involving rhizobacteria showed a neutral interaction with *S. indica* on solid agar medium, most of the well-known plant growth-promoting rhizobacteria (PGPRs) such as *Pseudomonas* strains, *Burkholderia cepacia*, *Gluconacetobacter* sp., *Bacillus amyloliquefaciens*, and *Streptomyces lividans* inhibited fungal growth. This growth inhibition that was exhibited by a significant reduction in growth radius, hyphal lysis, and loss of chlamydospore production ability was attributed to the production of inhibitory lipopeptides and bacillomycin metabolites by *Ps. Fluorescence* and *Burkholderia*. *Cepacia,* respectively. Interestingly, *P. putida* was the only strain that stimulated the growth of *S. indica*. Under in vivo conditions, the selected rhizobacteria also showed different stimulatory or inhibitory impacts on *S. indica* root enhancement of barley seedlings where *Azospirillum brasilense* were neutral, *Serratia liquefaciens* inhibited, and *Pseudomonas putida* prompted the stimulation of root growth by *S. indica*. However, experimental evidence suggests that an in vitro antagonism to *S. indica* can alter to beneficial under in vivo conditions. An example is the study of tripartite interaction between *T. harzainum*, *S. indica*, and black pepper (*Piper nigrum*) by Anith et al. (2011) where, in contrast to a complete disintegration of *S. indica* mycelium in the interacting zone by *T. harzianum* in the dual culture assay, combined inoculation of the bioinoculants significantly enhanced the plant growth. Thus, interactions of the bioinoculants in a multipartner biological system could be more complex to be estimated in a simple dual culture assay. Therefore, we suggest that even plant growth-promoting biological agents that display neutral or negative interaction under in vitro conditions should be examined for their response under natural conditions. Meena et al. (2010) analyzed the combined effects of *S. indica* and Tn5-lacZ-tagged phosphate-solubilizing bacterium *P. striata* on chickpea (*Cicer arietinum*). They used the blended fresh biomass of the fungus (2 g/100 mL sterile distilled water) and/or concentrations of 6.2×10^6 CFU/mL of the bacterium for seed inoculation. Meena et al. (2010) showed the synergistic effect of two microorganisms resulted in the population buildup of *P. striata* and an increase of plant dry biomass, but P uptake was not significantly influenced by any single or combined treatments. Recently, the impact of endophytic bacterial strains was investigated on the growth of *S. indica* under in vitro conditions by del Barrio-Duque et al. (2019). Among the examined isolate, *Bacillus*, *Enterobacter*, and *Burkholderia* had negative effects, while in contrast, *Mycolicibacterium*, *Rhizobium*, and *Paenibacillus* stimulated the growth of *S. indica.* Also, the bioprotection potential of *Mycolicibacterium* strains as the best stimulant in consortium with *S. indica* against *Rhizoctonia solani* and *Fusarium oxysporum* showed the effect of strain-dependency on boosting the plant growth and the disease control in a consortium. Thus, in some cases, *S. indica* or bacteria alone were more effective than their dual combinations. Molecular studies related the beneficial effects of the *Mycolicibacterium* to the genes involved in vitamin and nitrogen metabolisms.

12.5 Development of Carrier-Based Formulation

Biofertilizers/biopesticides are highly valuable in sustainable agriculture, as they improve soil fertility, crop protection, and plant productivity (Wardle 2006; Sharma et al. 2011). However, their successful functioning depends highly on their persistence in the soil (Berruti et al. 2016). Bioformulation describes specifically formulated products, which are easier to use and are more persistent in the soil (Mishra and Arora 2016). Carrier selection and preparation is an essential step for the formulation of beneficial rhizospheric microorganisms as commercial bioinoculants (Malik et al. 2005; Tripathi et al. 2015). Carriers could be classified as (1) simple sticky materials to improve the stickiness, stability, and dispersal abilities of liquid inoculants (Singleton et al. 2002; Bashan et al. 2014), (2) plant waste carriers, such as peat materials, and coir dust, composts of various origins and compositions, sugarcane filter mud, bagasse, soils mixed with various organic amendments, and vermiculite, (3) inorganic and partly organic carries such as talcum powder, vermiculite, clay, coal, and biochar, and (4) polymeric carriers including alginate, agar, pectin, chitosan, bean gum, and several proprietary polymers (Bashan et al. 2016). These synthetic formulations offer substantial advantages over plant waste materials including a much longer shelf life, appropriate survival in the field, and improved performance of plants in general (Bashan 1998; John et al. 2011; Bashan et al. 2014). Encapsulation is one of the currently experimentally applied methods in the field of agricultural technology. It underlies entrapping live microorganisms into a polymeric matrix while maintaining their active forms, capacity, and viability for as long as possible. This technology has been used for immobilizing and co-immobilizing (more inoculants) of plant growth-promoting microorganisms (bacteria, rhizobacteria, and AM fungi) (Bashan et al. 2002, 2006; Vassilev et al. 2001; Morsy 2015). The main purpose of encapsulation is to provide physical protection of applied inoculant(s) under stressful environmental conditions, microbial competitors, and during the gradual release of the inoculant(s) to boost the inoculant root colonization potential following the gradual degradation of the polymer by soil natural microbiome.

12.5.1 Carrier-Based Formulations of **S. indica** *and* **S. vermifera**

Tripathi et al. (2015) evaluated powder formulation of four different carrier-based formulations of *S. indica* viz., talcum powder, clay, sawdust, and bio boost (organic supplement) on the growth of *Phaseolus vulgaris* plants under greenhouse conditions. The study aimed at the development of a commercial product with a longer shelf-life and improved biological activity. Tripathi et al. suggested a 5% formulation (w/w) of talcum powder with 10^8 CFU/L of *S. indica* inoculum, the most stable at 30 °C, effective for a storage period of 6 months, and effective for enhancing the growth parameters of the plant. Table 12.2 shows the effect of carrier-based formulations of *Serendipitaceae* on the performance of different plant hosts. The development of protocols to support vegetative growth and spore production is an essential step for large-scale production of bioinoculants and commercialization. This can be achieved by using optimized culture media along with the proper operating conditions. Using a glucose deprivation strategy in a bioreactor, Kumar et al. (2011a, b) developed an economic method based on a modified Kaefer medium for *S. indica*, which resulted in maximum biomass and spore yield during growth and the subsequent sporulation phase. Variables studied were the medium composition, culture conditions, required time, and biomass productivity, which reportedly caused an enhancement of 100% in overall biomass productivity (9.25×10^7 spores/mL) and a 70% reduction in required time in comparison to the original Kaefer medium. Osman et al. (2020) also recommended a sucrose-supplemented Vj medium that is based on commercially available vegetable juice for large-scale production of *S. indica* inoculum. This medium was described as being less complex and laborious than the Hill and Kafer (2001) complex medium and also superior for the production of biomass and spore in both liquid and agar states, respectively. Varma et al. (2013) developed a powder formulation of *S. indica* by using magnesium sulfate as a carrier with the registered trademark, Rootonic. The compound was standardized at 2% (w/w) with a CFU of 10^9/g and a moisture content of 20%. A user guide for seed treatment of a large number of plants was also listed for commercialization purposes ranging from 50 g/h for plants like tomato, capsicum, cabbage, and cauliflower to 2000 g/h for potato, wheat, and sugarcane. Sarma et al. (2011) used an inorganic carrier-based consortium formulation of two fluorescent pseudomonad strains and

S. indica based on talcum powder to test the plant growth promotion of tomato plants under greenhouse and field conditions. They reported a considerable increase in tomato plant growth both in the glasshouse, field, and bioprotection of the plants against wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* under glasshouse conditions. Overall, the shelf life and field efficacy are the most crucial factors that need to be considered in developing carrier-based formulations of bioinoculants and commercialization purposes. As described before, the development of efficient formulations should rely on experiment-based validation of the optimal inoculum quantity.

12.6 Conclusions

It is estimated that the world population will increase by 1.5 billion in the next 50 years (UN estimates). To meet the nutritional needs of the growing population, agriculture will face challenges such as intensive use of nitrogen (N)- and phosphate (P)-based fertilizers, depletion of soil and water resources (Den Herder et al. 2010), globalization, and climate change (Hubbard et al. 2015). Therefore, microbial-based biostimulants are promising tools that can complement crop nutrition (P uptake and N fixation) and strengthen the innate immune system of plants. Several factors, viz., time of infection, stage of crop development, and genetic variability of the host, have been known to affect the occurrence of interspecific interactions (disease or symbiosis). However, contrary to what is common for plant pathogens, the impact of the inoculum quantity on symbiotic systems and its attribution to endophytic behavior (i.e., percentage of root colonization and/or growth response of plant) have not been well addressed in the current literature. It seems that methodologies for *Serendipita* are adapted from AM fungi. Meanwhile, we can hardly find a reference-based methodology for AM fungi with regard to the evaluation of the efficiency of inoculum concentration on plant performance. This has caused the researchers to use unspecified or diverse quantities of inoculation concentrations without providing supporting data to their choice of inoculum size. The initial infection of the white button mushroom (*Agaricus bisporus*) with dry bubble disease caused by *Lecanicillium fungicola* is among the good examples that reveal the influence of inoculum quantity on the pathogenicity and the disease severity. It was shown that the appearance of the disease symptoms and severity could be attributed to concentrations of the inoculum on the mushroom growth bed. The fluctuations in disease symptoms ranged from no symptoms with spraying 10^3 conidia/m^2 mushroom casing soil to up to 80% increase of the disease incidence and severity in successive flushes (application) by using 10^6 conidia/m^2 and total loss of mushroom yield at a concentration of 10^8 conidia/m^2 mushroom casing soil (Mamoun and Olivier 1995; Mills et al. 2000). The drastic effects of the inoculum concentration on disease occurrence were also documented by Rokni et al. (2019) where the resistance of highly tolerant wild strains of button mushroom remained unaffected by spreading conidia at a concentration of 10^6/m^2 growth bed but was broken down when inoculum concentration was increased to 10^8 conidia/m^2 mushroom casing soil. Our recent study on the tripartite interactions of *S. indica*, *T. simmonsii*, and bell pepper (*Capsicum annum*) showed an indirect correlation between inoculum ratios and the positive impact of the fungus on growth parameters (Rokni and Goltapeh 2019). In an experiment to test the effects of the inoculum of *S. indica* against *Phytophthora capsici*, the causal agent of the stem and fruit blight, we observed the same trend in the increase of the disease indices with the application of a higher concentration of *S. indica*. Surprisingly, high concentrations of the *S. indica* inoculum not only did not contribute to the disease suppression but also caused the pathogen to synergistically be more damaging than the *P. capsici* itself (Rokni et al. 2021). The negative impact of using large amounts of *S. indica* inoculum on plant performance has been documented in other research (Kaldorf et al. 2005; Andrade-Linares et al. 2013; Fakhro et al. 2010). However, further studies are required to elucidate the implications of inoculum levels on the outcome of interaction in a symbiotic system and the extent of root colonization. As a standard method, currently, the ratio of root colonization is determined by root staining protocol (Phillips and Hayman 1970) and microscopy. In our recent study (Rokni et al. 2021), we didn't observe any significant difference in the colonization ratio of the bell pepper roots inoculated at different (1–20%) w/w of seeds with *S. indica*. Although all the treatments were in the same range of colonization ratios (56%), the concentrations of the fungal biomass (mycelia and spores) per root piece were directly relevant and drastically increased upon using higher inoculum concentrations. Studies with AM fungi showed that greater infectivity or higher inoculum concentrations would result in a larger proportion of roots being mycorrhizal (Daniels et al. 1981; Sanders and Sheikh 1983; Walker and Smith 1984), but the extent of root colonization was not necessarily correlated with the effects of the symbionts on plant performance (Kapoor et al. 2002; Toussaint 2007; Das et al. 2012). Earlier on, Mosse (1972) noted that the "percent root colonization" did not always correlate with the effectiveness of the fungus in growth promotion. Thus, the root colonization ratio does not seem to be a reliable parameter to evaluate the advantages of mycorrhization.

This may explain the reason that using large amounts of *S. indica* inoculum shifted the interaction from mutualistic to antagonistic in the referenced studies (Kaldorf et al. 2005; Andrade-Linares et al. 2013; Fakhro et al. 2010). Therefore, in addition to tracking the root colonization condition by the conventional root staining method, applying molecular tools for specific monitoring of the bioinoculants inside the host roots is recommended (Abin et al. 2021). This approach is practical for both the detection and quantification of fungal bodies, especially in planning to work with multipartner symbiotic systems and/or for those taxa like *S. vermifera* that do not produce distinguished reproductive organs. The commercial application requires the development of carrier-based formulations of bioinoculants. The main purpose of encapsulation is to provide physical protection of applied inoculant(s) to stressful environmental conditions, microbial competitors, and to a gradual release of the inoculant(s). It will boost the root colonization potential as a result of the gradual degradation of the polymer by the soil's natural microbiome.

As agricultural production is facing severe challenges due to climate change with extreme weather events and emerging pathogens, consumers demand more sustainable production systems. Here we propose that in comparison with previous works that relied on a single or few partners, (1) more research should be devoted to further insight into the microbial behavior in a multipartner network and the ruling relationships among the multitude of microorganisms that have evolutionarily persisted in diverse soil microbial communities. (2) A greater focus should be placed on establishing a network of symbioses in which the presence of different complementary microorganisms would guarantee persistence and efficiency when exposed to diverse natural soil microbial communities. This should provide mutualisms with a continued capacity of persistence, encountering diverse soil microbiota and abiotic environmental conditions, that would guarantee the sustainability of crop functional traits in the agroecosystem, and (3) more emphasis should be on the development of a beneficial seed-applied microbial network, which successfully acts in diverse natural habitats. Thus, the optimal seed treatment and coating protocols should be developed to enhance seed germination, growth of crop plants, and tolerance to biotic/abiotic stress.

Compliance with Ethical Standards *Conflict of interest*: The authors declare that they have no competing interests.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of funding: This research did not receive any specific funding.

Data availability statement: Data sharing is not applicable as no new data were generated or analyzed during this study.

References

Abbott LK, Robson AD (1982) The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust J Agric Res 33(2):389–408

Abdollahi L, Yaghoubian Y, Alavi SM (2015) Effect of *Piriformospora indica* and *Trichoderma tomentosum* fungi on basil (*Ocimum basilicum* L.) growth under copper nitrate levels. J Soil Manag Sustain Product 5(1):113–127

Abin N, Rokni N, Shafeinia AR, Borhan MH (2021) Quantification of endophyte Serendipita indica in Brassica napus roots by qPCR. Crop Pasture Sci 72(12):985–993. https://doi.org/10.1071/CP21265

Achatz B, von Rüden S, Andrade D, Neumann E, Pons-Kühnemann J, Kogel KH, Franken P, Waller F (2010) Root colonization by *Piriformospora indica* enhances grain yield in barley under diverse nutrient regimes by accelerating plant development. Plant Soil 333(1–2):59–70

Andrade-Linares DR, Müller A, Fakhro A, Schwarz D, Franken P (2013) Impact of Piriformospora indica on tomato. In: Piriformospora indica. Springer, Berlin, pp 107–117

Anith KN, Faseela KM, Archana PA, Prathapan KD (2011) Compatibility of Piriformospora indica and Trichoderma harzianum as dual inoculants in black pepper (Piper nigrum L.). Symbiosis 55(1):11–17

Arora M, Saxena P, Choudhary DK, Abdin MZ, Varma A (2016) Dual symbiosis between *Piriformospora indica* and *Azotobacter chroococcum* enhances the artemisinin content in Artemisia annua L. World J Microbiol Biotechnol 32(2):19

Bajaj R, Agarwal A, Rajpal K, Asthana S, Kumar R, Prasad R, Kharkwal AC, Sherameti I, Oelmüller R, Varma A (2014) Co-cultivation of Curcuma longa with *Piriformospora indica* enhances the yield and active ingredients. Am J Curr Microbiol 2(1):6–17

Baldi A, Jain A, Gupta N, Srivastava AK, Bisaria VS (2008) Co-culture of arbuscular mycorrhiza-like fungi (Pir*i*formospora *indica* and *Sebacina vermifera*) with plant cells of *Linum* album for enhanced production of podophyllotoxins: a first report. Biotechnol Lett 30(9):1671

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schäfer P, Schwarczinger I, Zuccaro A (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytol 180(2):501–510

Banhara A, Ding Y, Kühner R, Zuccaro A, Parniske M (2015) Colonization of root cells and plant growth promotion by Piriformospora indica occurs independently of plant common symbiosis genes. Front Plant Sci 6:667

Barazani O, Benderoth M, Groten K, Kuhlemeier C, Baldwin IT (2005) *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. Oecologia 146(2):234–243

Barazani O, von Dahl CC, Baldwin IT (2007) *Sebacina vermifera* promotes the growth and fitness of *Nicotiana attenuata* by inhibiting ethylene signaling. Plant Physiol 144(2):1223–1232

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16(4):729–770

Bashan Y, Hernandez JP, Leyva LA, Bacilio M (2002) Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol Fertil Soils 35(5):359–368

Bashan Y, Bustillos JJ, Leyva LA, Hernandez JP, Bacilio M (2006) Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by Azospirillum brasilense. Biol Fertil Soils 42(4):279–285

Bashan Y, de Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378(1–2):1–33

Bashan Y, de Bashan LE, Prabhu SR (2016) Superior polymeric formulations and emerging innovative products of bacterial inoculants for sustainable agriculture and the environment. In: Agriculturally important microorganisms. Springer, Singapore, pp 15–46

Basiewicz M, Weiss M, Kogel KH, Langen G, Zorn H, Zuccaro A (2012) Molecular and phenotypic characterization of *Sebacina vermifera* strains associated with orchids, and the description of *Piriformospora williamsii* sp. nov. Fungal Biol 116(2):204–213

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol 6:1559

Bodles WJA, Fossdal C-G, Woodward S (2006) Multiplex real-time PCR detection of pathogen colonization in the bark and wood of *Picea sitchensis* clones differing in resistance to *Heterobasidion annosum*. Tree Physiol 26:775–782

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320(1–2):37–77

Buwalda JG, Stribley DP, Tinker PB (1984) The development of endomycorrhizal root systems V. the detailed pattern of development of infection and the control of infection level by host in young leek plants. New Phytol 96(3):411–427

Caravaca F, Diaz E, Barea JM, Azcón-Aguilar C, Roldan A (2003) Photosynthetic and transpiration rates of *Olea europaea* subsp. *sylvestris* and *Rhamnus lycioides* as affected by water deficit and mycorrhiza. Biol Plant 46(4):637

Carling DE, Brown M, Brown RA (1979) Colonization rates and growth responses of soybean plants infected by vesicular–arbuscular mycorrhizal fungi. Can J Bot 57(17):1769–1772

Dabral S, Saxena SC, Choudhary DK, Bandyopadhyay P, Sahoo RK, Tuteja N, Nath M (2020) Synergistic inoculation of Azotobacter vinelandii and Serendipita indica augmented rice growth. Symbiosis 81:139–148

Daniels BA, McCool PM, Menge JA (1981) Comparative inoculum potential of spores of six vesicular-arbuscular mycorrhizal fungi. New Phytol 89(3):385–391

Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A (2012) The root endophyte fungus Piriformospora indica leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. Plant Signal Behav 7(1):103–112

del Barrio-Duque A, Ley J, Samad A, Antonielli L, Sessitsch A, Compant S (2019) Beneficial endophytic bacteria-Serendipita indica interaction for crop enhancement and resistance to phytopathogens. Front Microbiol 10:2888

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15(11):600–607

Dolatabadi HK, Goltapeh EM (2013) Effect of inoculation with Piriformospora indica and Sebacina vermifera on growth of selected Brassicaceae plants under greenhouse conditions. J Hortic Res 21(2):115–124

Dolatabadi HK, Goltapeh EM, Jaimand K, Rohani N, Varma A (2011) Effects of *Piriformospora indica* and *Sebacina vermifera* on growth and yield of essential oil in fennel (*Foeniculum vulgare*) under greenhouse conditions. J Basic Microbiol 51(1):33–39

Douglas AE (2016) How multi-partner endosymbioses function. Nat Rev Microbiol 14(12):731

Druege U, Baltruschat H, Franken P (2007) *Piriformospora indica* promotes adventitious root formation in cuttings. Sci Hortic 112(4):422–426

Fakhro A, Andrade-Linares DR, von Bargen S, Bandte M, Büttner C, Grosch R, Schwarz D, Franken P (2010) Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. Mycorrhiza 20(3):191–200

Gerdemann JW (1975) Vesicular-arbuscular mycorrhizae. Dev Funct Roots 576:591

Ghimire SR, Craven KD (2011) Enhancement of switchgrass (*Panicum virgatum* L.) biomass production under drought conditions by the ectomycorrhizal fungus *Sebacina vermifera*. Appl Environ Microbiol 77(19):7063–7067. https://doi.org/10.1128/AEM.05225-11

Ghimire SR, Charlton ND, Craven KD (2009) The mycorrhizal fungus, *Sebacina vermifera*, enhances seed germination and biomass production in switchgrass (*Panicum virgatum* L). Bioenergy Res 2(1–2):51–58

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:332

Haas JH, Krikun J (1985) Efficacy of endomycorrhizal-fungus isolates and inoculum quantities required for growth response. New Phytol 100(4):613–621

Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79(3):293–320

Hart MM, Forsythe J, Oshowski B, Bücking H, Jansa J, Kiers ET (2013) Hiding in a crowd—does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis? Symbiosis 59(1):47–56

Hill TW, Kafer E (2001) Improved protocols for Aspergillus minimal medium: trace element and minimal medium salt stock solutions. Fungal Genetics Rep 48(1):20–21

Hubbard A, Lewis CM, Yoshida K, Ramirez-Gonzalez RH, de Vallavieille-Pope C, Thomas J, Kamoun S, Bayles R, Uauy C, Saunders DG (2015) Field pathogenomics reveals the emergence of a diverse wheat yellow rust population. Genome Biol 16(1):23

Jafari M, Yari M, Ghabooli M, Sepehri M, Ghasemi E, Jonker A (2018) Inoculation and co-inoculation of alfalfa seedlings with root growth promoting microorganisms (*Piriformospora indica*, *Glomus intraradices* and *Sinorhizobium meliloti*) affect molecular structures, nutrient profiles and availability of hay for ruminants. Anim Nutr 4(1):90–99

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37(1):1–16

Jogawat A, Saha S, Bakshi M, Dayaman V, Kumar M, Dua M, Varma A, Oelmüller R, Tuteja N, Johri AK (2013) Piriformospora indica rescues growth diminution of rice seedlings during high salt stress. Plant Signal Behav 8(10):e26891

John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D (2011) Bio-encapsulation of microbial cells for targeted agricultural delivery. Crit Rev Biotechnol 31(3):211–226

Käfer E (1977) Meiotic and mitotic recombination in Aspergillus and its chromosomal aberrations. Adv Genet 19:33–131

Kaldorf M, Koch B, Rexer KH, Kost G, Varma A (2005) Patterns of interaction between Populus Esch5 and Piriformospora indica: a transition from mutualism to antagonism. Plant Biol 7(02): 210–218

Kapoor R, Giri B, Mukerji KG (2002) *Glomus macrocarpum*: a potential bioinoculant to improve essential oil quality and concentration in Dill (Anethum graveolens L.) and Carum (Trachyspermum ammi (Linn.) Sprague). World J Microbiol Biotechnol 18(5):459–463

Kesh H, Yadav AS (2016) Synergetic effect of *Rhizobium* sp. and *Piriformospora indica* on bioenhancing activity, symbiotic parameters and grain yield in pigeon pea (*Cajanus cajan* L.). applied. Biol Res 18(3):239–245

Kumar M, Yadav V, Tuteja N, Johri AK (2009) Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. Microbiology 155(3):780–790

Kumar V, Sahai V, Bisaria VS (2011a) High-density spore production of *Piriformospora indica*, a plant growth-promoting endophyte, by optimization of nutritional and cultural parameters. Bioresour Technol 102(3):3169–3175

Kumar M, Yadav V, Kumar H, Sharma R, Singh A, Tuteja N, Johri AK (2011b) *Piriformospora indica* enhances plant growth by transferring phosphate. Plant Signal Behav 6(5):723–725

Lambers H, Teste FP (2013) Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game. Plant Cell Environ 36(11):1911–1915

Malik KA, Hafeez FY, Mirza MS, Hameed S, Rasul G, Bilal R (2005) Rhizospheric plant—microbe interactions for sustainable Griculture. In: Biological nitrogen fixation, sustainable agriculture and the environment. Springer, Dordrecht, pp 257–260

Mamoun M, Olivier JM (1995) Receptivity of cloned hazels to artificial ectomycorrhizal infection by Tuber melanosporum and symbiotic competitors. Mycorrhiza 6(1):15–19

Mathur N, Vyas A (1995) I. Influence of VA mycorrhizae on net photosynthesis and transpiration of Ziziphus mauritiana. J Plant Physiol 147(3–4):328–330

Meena KK, Mesapogu S, Kumar M, Yandigeri MS, Singh G, Saxena AK (2010) Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate-solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea. Biol Fertil Soils 46(2):169–174

Menge JA (1983) Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture. Can J Bot 61(3):1015–1024

Mills PR, Fermor T, Muthumeenakshi S, Lincoln S (2000) Cell wall degrading enzymes produced by verticillium spp. and their relationship to infection in *Agaricus bisporus*. Mushroom Sci 15: 601–605

Mishra J, Arora NK (2016) Bioformulations for plant growth promotion and combating phytopathogens: a sustainable approach. In: Bioformulations: for sustainable agriculture. Springer, New Delhi, pp 3–33

Mishra M, Prasad R, Varma A (2014) Rootonic with bio-zinc to accelerate *Pennisetum glaucum* seed germination and plant growth. Int J Plant Anim Environ Sci 4:552–561

Mohammadi KF, Pirdashti H, Yaghoubian Y (2018) Inoculation with *Trichoderma virens* and *Piriformospora indica* for improving the morphological and physiological traits related to grain yield of rice under different rates of phosphorus fertilizer. J Crop Ecophysiol 11(44):857–874

Morsy M (2015) Microbial symbionts: a potential bio-boom. J Investig Genomics 2:00015

Mosse B (1972) The influence of soil type and Endogone strain on the growth of mycorrhizal plants in phosphate deficient soils. Rev Ecol Sol 9:529–537

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15(3):473–497

Nautiyal CS, Chauhan PS, DasGupta SM, Seem K, Varma A, Staddon WJ (2010) Tripartite interactions among *Paenibacillus lentimorbus* NRRL B-30488, *Piriformospora indica* DSM 11827, and *Cicer arietinum* L. World J Microbiol Biotechnol 26(8):1393–1399

Oelmüller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis 49(1):1–17

Osman M, Stigloher C, Mueller MJ, Waller F (2020) An improved growth medium for enhanced inoculum production of the plant growth-promoting fungus *Serendipita indica*. Plant Methods 16(1):1–7

Peškan-Berghöfer T, Shahollari B, Giong PH, Hehl S, Markert C, Blanke V, Kost G, Varma A, Oelmüller R (2004) Association of *Piriformospora indica* with Arabidopsis thaliana roots represents a novel system to study beneficial plant-microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. Physiol Plant 122(4):465–477

Petrini O (1991) Fungal endophytes of tree leaves. In: Microbial ecology of leaves. Springer, New York, NY, pp 179–197

Pham GH, Singh A, Malla R, Kumari R, Prasad R, Sachdev M, Luis P, Kaldorf M, Tatjana P, Harrmann S, Hehl S, Declerck S, Buscot F, Oelmuller R, Rexer KH, Kost G, Varma A (2004a) Interaction of *P. indica* with other microorganisms and plants. In: Varma A, Abbott L, Werner D, Hampp R (eds) Plant surface microbiology. Springer, Berlin Heidelberg/New York, pp 237–265

Pham GH, Kumari R, Singh A, Sachdev M, Prasad R, Kaldorf M, Buscot F, Oelmuller R, Tatjana P, Weiβ M, Hampp R, Varma A (2004b) Axenic cultures of *Piriformospora indica*. In: Varma A, Abbott L, Werner D, Hampp R (eds) Plant surface microbiology. Springer, Berlin Heidelberg/New York, pp 593–616

Pham GH, Srivastava A, Saxena AK, Pareek A, Varma A (2004c) Protocol to understand the interaction between rhizobacteria and symbiotic fungus: *Piriformospora indica*. In: Podila G, Varma A (eds) Basic research and applications: mycorrhizae, Microbiology series, vol 1. IK International, New York, pp 425–450

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55(1):158–161

Pirdashti H, Yaghoubian Y, Goltapeh E, Hosseini S (2012) Effect of mycorrhiza-like endophyte (Sebacina vermifera) on growth, yield and nutrition of rice (Oryza sativa L.) under salt stress. J Agric Technol 8:1651–1661

Rai M, Varma A (2005) Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. Electron J Biotechnol 8(1):1–6

Rai M, Acharya D, Singh A, Varma A (2001) Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by Piriformospora indica in a field trial. Mycorrhiza 11(3):123–128

Rathod DP, Brestic M, Shao HB (2011) Chlorophyll a fluorescence determines the drought resistance capabilities in two varieties of mycorrhized and non-mycorrhized Glycine max Linn

Ray JG, Valsalakumar N (2010) Arbuscular mycorrhizal fungi and *Piriformospora indica* individually and in combination with rhizobium on green gram. J Plant Nutr 33(2):285–298

Riess K, Oberwinkler F, Bauer R, Garnica S (2014) Communities of endophytic Sebacinales associated with roots of herbaceous plants in agricultural and grassland ecosystems are dominated by *Serendipita herbamans* sp. nov. PLoS One 9(4):e94676

Roe AD, Rice AV, Coltman DW, Cooke JE, Sperling FA (2011) Comparative phylogeography, genetic differentiation and contrasting reproductive modes in three fungal symbionts of a multipartite bark beetle symbiosis. Mol Ecol 20(3):584–600

Rokni N, Goltapeh EM (2019) Tolerance to dry bubble disease (*Lecanicillium fungicola*) in Iranian wild germplasm of button mushroom (*Agaricus bisporus*). Mycoscience 60(2):125–131

Rokni N, Alizadeh HS, Bazgir E, Darvishnia M, Mirzaei-Najafgholi H (2021) The tripartite consortium of *Serendipita indica*, *Trichoderma simmonsii*, and bell pepper (*Capsicum annum*). Biol Control 158:104608

Sahay NS, Varma A (1999) *Piriformospora indica*: a new biological hardening tool for micropropagated plants. FEMS Microbiol Lett 181(2):297–302

Sanders FE, Sheikh NA (1983) The development of vesicular-arbuscular mycorrhizal infection in plant root systems. In: Tree root systems and their mycorrhizas. Springer, Dordrecht, pp 223–246

Sarma MVRK, Kumar V, Saharan K, Srivastava R, Sharma AK, Prakash A, Sahai V, Bisaria VS (2011) Application of inorganic carrier-based formulations of fluorescent pseudomonads and *Piriformospora indica* on tomato plants and evaluation of their efficacy. J Appl Microbiol 111(2):456–466

Satheesan J, Narayanan AK, Sakunthala M (2012) Induction of root colonization by Piriformospora indica leads to enhanced asiaticoside production in Centella asiatica. Mycorrhiza 22(3):195–202

Schulz B, Römmert AK, Dammann U, Aust HJ, Strack D (1999) The endophyte-host interaction: a balanced antagonism? Mycol Res 103(10):1275–1283

Selosse MA, Setaro S, Glatard F, Richard F, Urcelay C, Weiss M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. New Phytol 174(4):864–878

Serfling A, Wirsel SG, Lind V, Deising HB (2007) Performance of the biocontrol fungus *Piriformospora indica* on wheat under greenhouse and field conditions. Phytopathology 97(4):523–531

Sharma S, Kumar V, Tripathi RB (2011) Isolation of phosphate solubilizing microorganism (PSMs) from soil. J Microbiol Biotechnol Res 1(2):90–95

Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmüller R (2005) The endophytic fungus Piriformospora indica stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. J Biol Chem 280(28):26241–26247

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biol Rev 21(2–3): 75–89

Singleton P, Keyser H, Sande E (2002) Development and evaluation of liquid inoculants. Inoculants and nitrogen fixation of legumes in Vietnam. ACIAR Proceedings 109e, Canberra, pp. 52–66

Smith FA, Smith SE (1981) Mycorrhizal infection and growth of *Trifolium subterraneum*: use of sterilized soil as a control treatment. New Phytol 88(2):299–309

Stone JK, Polishook JD, White JF (2004) Endophytic fungi, p. 241–270.In G. M. Mueller, G. F. bills, and M. S. Foster (ed.), biodiversity of fungi. Elsevier, Amsterdam, Netherlands function of *NPR1*. Plant Cell Physiol 49(11):1747–1751

Su ZZ, Wang T, Shrivastava N, Chen YY, Liu X, Sun C, Yin Y, Gao QK, Lou BG (2017) *Piriformospora indica* promotes growth, seed yield and quality of Brassica napus L. Microbiol Res 199:29–39

Sun W, Li K, Sirois E (2010) Simulated elliptical bioprosthetic valve deformation: implications for asymmetric transcatheter valve deployment. J Biomech 43(16):3085–3090

Toussaint JP (2007) Investigating physiological changes in the aerial parts of AM plants: what do we know and where should we be heading? Mycorrhiza 17(4):349–353

Tripathi S, Das A, Chandra A, Varma A (2015) Development of carrier-based formulation of root endophyte *Piriformospora indica* and its evaluation on Phaseolus vulgaris L. World J Microbiol Biotechnol 31(2):337–344

Varma A, Verma S, Sahay N, Bütehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Appl Environ Microbiol 65(6):2741–2744

Varma A, Singh A, Sudha Sahay N, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Blechert O, Rexer KH, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica*: a cultivable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) Mycota IX. Springer, Germany, pp 123–150

Varma A, Bakshi M, Lou B, Hartmann A, Oelmueller R (2012) *Piriformospora indica*: a novel plant growth-promoting mycorrhizal fungus. Agric Res 1(2):117–131

Varma A, Fekete A, Srivastava A, Saxena AK, Frommberger M, Li D, Gschwendter S, Sherameti I, Oelmueller R, Schmitt-Kopplin P, Tripathi S (2013) Inhibitory interactions of rhizobacteria with the symbiotic fungus *Piriformospora indica*. In: Piriformospora indica. Springer, Berlin, Heidelberg, pp 201–219

Vassilev N, Vassileva M, Azcon R, Medina A (2001) Application of free and ca-alginate-entrapped *Glomus deserticola* and Yarowia lipolytica in a soil–plant system. J Biotechnol 91(2–3): 237–242

Walker P, Smith S (1984) Stroop interference based on the synaesthetic qualities of auditory pitch. Perception 13(1):75–81

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, von Wettstein D, Franken P (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A 102(38):13386–13391

Warcup JH, Talbot PHB (1967) Perfect states of Rhizoctonias associated with orchids. New Phytol 66(4):631–641

Wardle DA (2006) The influence of biotic interactions on soil biodiversity. Ecol Lett 9(7):870–886

Werner GD, Cornwell WK, Cornelissen JH, Kiers ET (2015) Evolutionary signals of symbiotic persistence in the legume–rhizobia mutualism. Proc Natl Acad Sci 112(33):10262–10269

Weiss M, Sýkorová Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D (2011) Sebacinales everywhere: previously overlooked ubiquitous fungal endophytes. PLoS One 6(2):e16793

Weiß M, Waller F, Zuccaro A, Selosse MA (2016) Sebacinales–one thousand and one interactions with land plants. New Phytol 211(1):20–40

Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. J Biol Chem 285(34):26532–26544

Yaghoubian Y, Goltapeh EM, Pirdashti H, Esfandiari E, Feiziasl V, Dolatabadi HK, Varma A, Hassim MH (2014) Effect of Glomus mosseae and *Piriformospora indica* on growth and antioxidant defense responses of wheat plants under drought stress. Agric Res 3(3):239–245

Chapter 13
Applications of Microbial Consortia and Microbiome Interactions for Augmenting Sustainable Agrobiology

Vidya Niranjan, Anagha S. Setlur, Sinosh Skariyachan, and K. Chandrashekar

Abstract The microbiome of the soil plays a critical role in plant nutrition, growth, and crop yield. Comprehending the entire soil microbiome is necessary for illustrating the complex interactions that transpire among the diverse microorganisms existing in soil and in the rhizosphere of the plants. Currently, modern technology has paved the way for such extensive research via metagenomic studies. With the fast-spreading omics studies such as metagenomics, transcriptomics, metabolomics, and proteomics, intricate analysis of the interactions of the microbiome with that of plant nutrition is gained. Soil metagenomics aid in decoding the role of soil bacteria in the nutrition of plants and in turn the management of the overall soil environment and its inherent microbial communities. Moreover, applications of next-generation sequencing technologies in agrobiology promise novel solutions for sustainable crop production via innovative approaches. Dominance of chemical pesticides and fertilizers in the agricultural sector disrupts not only the soil microbiome but human health as well. Thus, one major alternative approach is the design and development of microbial consortia via carefully analyzed bioformulations that boost plant productivity by working in synergy with each other. Therefore, with these vigilantly formulated consortia, an augmentation in the agricultural output can be obtained, bolstering growth, productivity, and soil and plant health. Additionally, it reduces the adverse effects on humans, plants, and animals as the natural microbiome of the soil is maintained. With this background, the current chapter aims to provide comprehensive perspectives on developing and using various types of microbial consortia for maintaining a sustainable and balanced agrobiology.

V. Niranjan · A. S. Setlur · K. Chandrashekar
Department of Biotechnology, R V College of Engineering, Bengaluru, India
e-mail: vidya.n@rvce.edu.in; anaghass@rvce.edu.in

S. Skariyachan (✉)
Department of Microbiology, St. Pius X College, Kasaragod, Kerala, India
e-mail: sinoshmicro@stpius.ac.in

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_13

Keywords Microbiome · Soil metagenomics · Plant nutrition · Next generation sequencing · Microbial consortia · Sustainability

13.1 Introduction

The association between plants and their surroundings is very intricate and complicated. For several centuries, comprehending their relationship has been the emphasis of much research. The prime focus has been on the plants rather than on the beneficial interactions existing between the plants and the soil microbes. Due to the close relationship between microbes of the soil and plants, they can survive and co-exist well. The interactions between the soil microbes themselves are as critical as plant–microbe associations as the influence of beneficial synergistic effects of soil microbes on the growth of plants is a major topic of research. With the immense capability of influencing the diversity, composition, productivity, and survival of plant communities, soil microbes greatly impact the overall complexity of the ecosystem (Chaparro et al. 2012). Furthermore, with advancements in technology and research, improvements to the soil microbiome are being made that can further enhance the overall productivity and fertility of the plants. Some examples include adding useful microbes to the already existing microbiome that can aid in improving the nutrient uptake by the plants (Kirankumar et al. 2010), suppressing disease occurrences, and conferring abiotic stress resistance (De Vleesschauwer and Höfte 2009; Selvakumar et al. 2012) and enhancing plant growth (Cummings 2009). However, it is now necessary to steer away from simplistic plant–microbe interactions and focus more on boosting plant sustainability by considering all factors responsible for augmenting agrobiology. Thus, an understanding of the overall microbiome interactions, types of microbiome and factors influencing its survival, and the role of the microbiome in sustainable agriculture are essential.

13.2 Overview of the Soil Microbiome

The microbiome of the soil is described as the microorganisms that are collectively found in the soil, including bacteria, archaea, fungi, viruses, protists, and other microbial eukaryotes (Fierer 2017). The microbial communities of the soil are considered highly diverse primarily because of the heterogeneity of the soil environmental conditions. An extensive range of microbial habitats can be found that comprise unique microbes involved in different functions. These soil microbes have a direct or indirect impact on the plants, their nutrient uptake, growth, fertility, and diversity. Since the soil is equipped with a diversity of microorganisms, recent advancements in soil agrobiology aid in enhancing the understanding of the soil environmental changes and in turn crop production.

The soil microbes have vital roles to play in the upkeep of fertility, carbon sequestration in the soil, and the cycling of nutrients. The health of plants, animals, and humans is also influenced by the microbiome of the soil. For more than a century, the magnitude of the microbiome of soil has been widely recognized (Waksman 1927), and as such, research describing the type of microorganisms that exist in the soil, their impact on the fertility of the soil, and their metabolic potentialities are well established. The usefulness of soil microorganisms goes back to the discovery of antibiotics, understanding the distinct metabolic pathways such as oxidation of ammonia and nitrogen fixation, which mainly stemmed from the microbiome of the soil. Furthermore, the abiotic environment of the soil is also greatly heterogeneous, inundated with either water-filled or air-filled pores, which can function as effective hotspots for the growth of diverse microorganisms (Jansson and Hofmockel 2020). This environment is also highly dynamic when combined with the influence of plants, the fauna of soil, and alterations in various factors such as temperature, moisture, redox states, etc. However, with the changing climatic conditions, the consequences on the soil microbiome are also high. Therefore, a better comprehension of the microbiome of the soil, its types, and the influence of external and internal factors on the microbiome is extremely vital.

13.2.1 Major Types of Soil Microbiome

The soil microbiome is very vast and diverse. However, some common microbes exist in soils of all types. Generally, soil microbes can be categorized into bacteria, fungi, actinomycetes, protozoa, and algae. Each of these microbe groups has specified characters that influence its functions in the soil and toward the plants (Islam et al. 2021).

Bacteria: As the most abundant group of microbes in the soil, bacteria and archaea are responsible for carrying out several vital processes, nitrogen fixation being the most important one (Warembourg 1993). Soil bacteria are responsible for colonizing the minerals and aid in inducing the breakdown of these minerals. Furthermore, soil bacteria are considered tremendously versatile (Falkowski et al. 2008). *Pseudomonas* are responsible for metabolizing several fertilizers and chemicals. *Pseudomonas aeruginosa* can survive both aerobically and anerobically by exploiting nitrates as its electron acceptors. *Clostridium,* being anerobic in nature, can grow without oxygen. Additionally, *Nitrobacter* obtains its energy by the conversion of nitrite to nitrates. This versatility of the soil bacteria aids in several biochemical pathways that are beneficial to both bacteria and plants.

Actinomycetes: These are a class of bacteria that have similar characteristics to fungi, such as branching of the mycelium, formation of conidia and aerial mycelium, and their growth as pellets/clumps in liquid cultures (Sharma et al. 2014). A noteworthy feature of actinomycetes is their ability to produce various antibiotics. Some examples of antibiotics produced by actinomycetes include neomycin, tetracycline, streptomycin, and erythromycin, all of which are utilized for the treatment of several infections caused by bacteria.

Fungi: These microbes are vital food sources for larger organisms and are known to have useful symbiotic associations with other microbes for augmenting the health of the soil. Just as how environmental factors influence the growth of bacteria and actinomycetes, fungi are also impacted by external factors. The growth of fungi can occur in soils that are dry and acidic and have good moisture and oxygen content (Mueller et al. 2013).

Algae: These are microbes that make their food and nutrients via photosynthesis. Typically, they live on the surface of the soil where appropriate temperature and moisture are found. Algae also have a role to play in nitrogen fixation. Generally, algae can be classified into three types: Chlorophyceae (which have chlorophyll in them and are green in color), Cyanophyceae (contain chlorophyll and other pigments that make them blue-green in color), and Bacillariaceae (contain chlorophyll and other pigments making them brown in color) (Chapman 1973).

Protozoa: These are eukaryotes that reproduce sexually and are categorized as amoebae, flagellates, and ciliates (Clarholm et al. 2007). Protozoa participate in the nutrient cycles such as carbon cycle, sulfur cycle, nitrogen cycle, phosphorus cycle, and water cycle. The amount of participation depends on the distribution of protozoa in the soil and other external factors. Thus, although there are several types of microbes existing in the soil, their survival is dependent on many factors.

13.2.2 Factors Influencing the Growth, Survival, and Diversity of Soil Microbiome

Several internal and external factors influence the growth and survival of the soil microbiome. These factors also impact the microbial diversity of the soil. Soils enable the existence of varied microbial communities with 10,000–50,000 species living in 1 g of soil (Schloss and Handelsman 2006). Several fungal and bacterial communities are known to be associated with soils having a certain amount of nitrogen content (Frey et al. 2004), phosphorus content (Faoro et al. 2010), and varying textures (Girvan et al. 2003). Furthermore, the pH of the soil also influences the growth, survival, and diversity of the soil microbiome (Rousk et al. 2010). Evidence suggests that the pH of the soil has the most effect on the bacterial soil communities (Fierer and Jackson 2006). Previous studies have identified a robust correlation between the pH of the soil and the diversity of the bacteria, making this factor a driver of the composition of the bacterial community in the soil (Rousk et al. 2010). Furthermore, it is also postulated that this strong association between soil pH and bacteria is due to the sensitivity of the bacterial cells to pH since bacteria comparatively show a slimmer pH growth tolerance (Rousk et al. 2010). Other studies highlight the importance of factors such as altitude and cation ratios such as calcium (Ca^{2+}), aluminum (Al^{3+}), and magnesium (Mg^{2+}) (Faoro et al. 2010).

Additionally, other important factors that influence the soil microbiome include the availability of nitrogen, temperature, soil organic carbon content, and redox status (Fierer 2017). A recent study evidenced the influence of temperature on the survival and growth of different bacterial and archaeal communities and their response to varying temperatures. These were identified as “bioindicators,” which were beneficial for distinguishing between the direct and indirect effects that soil warming has on the microbiome (Oliverio et al. 2017). Another study tested the idea of indicator taxa by using a fertilization experiment, and it was found that the abundance of the major taxa reduced with the fertilization of nitrogen. This study also reported that the fertilization of nitrogen was the major driver of changes in the bacterial community (Cederlund et al. 2014). Another important factor that influences the growth and survival of the microbial communities in the soil is redox fluctuation. The high amplitude redox fluctuations are considered a strong driver on the physiological and phylogenetic composition of the soil bacteria. Redox fluctuations are also known to promote the mechanisms of redox tolerance and metabolic plasticity (Pett-Ridge and Firestone 2005). Another study demonstrated the importance of soil organic carbon (SOC) content wherein, from 290,000 sequences that were obtained from pyrosequencing, the factor that most elucidated the differences in the soil microbial communities was SOC (Sul et al. 2013). SOC is also known to enhance the structure of the soil and its fertility.

Other important factors include soil oxygen quantity, soil moisture, availability of phosphorus, the texture of the soil, and its structure. Thus, it is evident that several factors ultimately influence the growth, survival, and diversity of the soil microbial community. These factors show synergistic effects allowing harmony between the soil, plants, and microbes.

13.2.3 Overview of the Role of the Microbiome in Sustainable Agriculture

Research dedicated to soil microbiome is now primarily focused on improving understanding of the microbial communities for enhancing agriculture management. This can be easily achieved by the addition of specific microorganisms to the soil, managing the soil content to promote the growth of these microbes, diminishing soil erosion, and accelerating soil remediation (Chiquoine et al. 2016; Wood et al. 2021). Despite there being several ways of enhancing productivity and agricultural sustainability, managing the microbiome of the soil is one of the best methods. Since there is no “ideal” soil microbial community, the existence of diverse microbes influenced by internal and external factors implies a highly complex and intricate ecosystem. Generally, the microbiome plays a vital role in the following areas in boosting sustainable agriculture:

- Maintenance of relationship between plant–microbe–soil by transfer of nutrients
- Inducing suppression of soil diseases
- Improving soil health biologically, chemically, and physically
- Soil texture enhancement

- Establishment of new, healthy symbiotic relationships between microbiome and plants
- Low-input, high productivity, and better sustainability

13.3 Rhizosphere and Its Importance in Plant Systems

The narrow region of the soil that surrounds the roots and is directly impacted by the soil microbes and secretions of the root is called the rhizosphere. The microorganisms that inhabit the plants are generally classified into two types: epiphytes (located on the plant surface) and endophytes (located within the plant tissues). Phyllospheric microbes are those that colonize the surface of the leaves, and rhizospheric microbes exist within the soil close to the roots (Vishwakarma et al. 2020). Among these, the rhizosphere is considered the most dynamic that critically impacts the status of plant nutrition and in turn plant growth (Bakker 2013; Lakshmanan et al. 2014). Soil underground system encompasses primary roots, root hairs, and other lateral root growths, which interact with innumerable microbial communities in the rhizosphere, thus considerably affecting the stages of plant growth (Bandyopadhyay et al. 2017).

Specifically, the rhizosphere is the zone where crucial and complicated interactions take place among the soil, its diverse microbial community, and the plants. Rhizosphere includes three main zones: the endorhizosphere, where the endodermis and the cortex microbiome are included; the rhizoplane, which is directly adjacent to the root, including the mucilage and epidermis; and the ectorhizosphere, which extends into the bulk of the soil from the rhizoplane (Meena et al. 2017). The biochemical interactions that trigger the exchange of molecules between the soil microbes and the plants occur due to the competition between the microbes for nutrients and water. At times, their synergistic relationship with the plants aid in improving the sustainability of agriculture. Some of the specific microbiomes that exist near the plant roots are considered part of intricate ecosystems. These microbial communities vary depending on the type of soil, the pattern of land use, the genotype of the host, and the species of the plants.

Furthermore, it is also understood that the exudates of the roots act as substrates and as signaling molecules that are essential for interactions between the plant and the microbes in the rhizosphere (Kour et al. 2019; Mendes et al. 2013). Thus, understanding the importance of the rhizosphere in plant systems enables researchers to manipulate the soil microbiomes and develop strategies to manage and enhance agricultural production in a sustainable way. Overcoming the challenge of rhizosphere biodiversity conservation during this process of enhancing agricultural productivity is also important to counteract the effects of overexploitation of the soil microbial communities.

13.3.1 Major Types of Interactions in the Soil

For the soil to function sustainably, several major interactions occur in the rhizosphere among the components of the soil ecosystem. These include microbe–microbe interactions, plant–microbe interactions, root–root interactions, etc.

13.3.1.1 Plant–Microbiome Interactions

The identity of the species of the plants greatly impacts the diversity of the soil microbiome, especially those that live near the plant. Therefore, the microbes present within the soil influence plant development and growth (Jones et al. 2019). These soil microbes engage in the release of several beneficial compounds in the rhizosphere that is used for uptake by the plants. Such compounds allow the regulation of the transcriptome of the plants. Additionally, the plants also produce hormones such as gibberellins, cytokinins, auxins, etc., that inadvertently benefit the microbes residing close to the plants.

Moreover, the interactions between the plants and microbiome occur in two ways: via root exudates and the influence of external conditions such as climate and soil conditions. The roots of the plants secrete exudates and other phytochemicals that engage the microbial populations in developing niches. Some of these metabolites filter out the unwanted strains of microbes present in the niches, while some other metabolites enable the co-existence of microbial populations in the same niches. This aids in the secretion of molecules required for the growth of other microbes. Conversely, the beneficial bacteria present in the soil promote the growth of plants by several mechanisms, including the production of phytohormones such as indole acetic acid, antibiotics to protect against harmful pathogens, and secreted effectors. Furthermore, they also chelate the nutrients and make them available for transport to the plants. For instance, the siderophore–Fe transporter transports iron (Vishwakarma et al. 2020). Examples of plant–microbe interactions include the secretion of citric acids from the roots of *Cucumis sativus,* which influences the attraction of *Bacillus amyloliquefaciens* and release of fumaric acid from roots of *Musa* sp., which attracts *Bacillus subtilis* toward the roots leading to biofilm formation (Zhang et al. 2014a, b). Another example is the symbiotic relationship between legumes and rhizobia.

13.3.1.2 Root–Root Interactions

Due to the existence of various plants in the same kind of soil environment, a competition for the necessary resources between the root systems that overlap occurs. This co-existence is known to be due to the differences in rooting patterns of the plant species (Berendse 1982). However, this theory only substantiates the competitive interactions that occur under the ground in the rhizosphere.

Co-existence also assists in demonstrating interactions that are both facilitative and competitive between the overlapping roots. Secretion of signaling molecules such as allelochemicals and root exudates aids in the communication between the roots of the neighboring plants. Allelopathy is a common process of communication where the phytotoxins such as catechins are secreted by the plants. Catechins can mediate both intraspecific and interspecific associations by preventing the growth of adjoining plants, thereby allowing augmented nutrient availability and diminished competition (Mommer et al. 2016). Moreover, other allelochemicals such as volatile inorganic compounds (VOCs) mediate the rhizospheric signaling via the networks of mycorrhizae existing among plants, intensifying their transmission. Examples of root–root interactions include improved productivity of *Oryza sativa* when grown in pairs due to the increase in mixture productivity in crops (Montazeaud et al. 2018).

13.3.1.3 Microbe–Microbe Interactions

Interactions between two microbial species in the soil also influence the growth of plants. For instance, the association between specific rhizobacteria and mycorrhizal fungi encourages symbioses of mycorrhizae with the plant host. This includes both ecto- and endo-mycorrhizal interactions (Artursson et al. 2006; Battini et al. 2017). These helper bacteria aid in several functions, including enhancing the receptivity of the root to mycorrhiza, increasing the conduciveness of the soil to the fungus, boosting the survival of the mycelium, and promoting germination of the fungal spores (Frey-Klett et al. 2007). Additionally, some bacterial endosymbionts of fungi that are root-associated impact the plant host. *Serendipita indica*'s bacterial endosymbiont grows devoid of its fungal host and enhances plant growth. It also improves plant resistance to leaf pathogens, implying that bacterial symbionts partially mediate the growth of *S. indica* (Hassani et al. 2018).

Another example of microbe–microbe interaction in the soil is the production of oxalic acid by fungal root pathogen *Rhizoctonia solani*, which promotes the growth of specific bacterial families such as *Burkholderiaceae* and *Oxalobacteraceae* causing a shift in the bacterial communities and activation of bacterial stress responses (Hassani et al. 2018). Moreover, *Streptomyces* strains isolated from disease-suppressive soils secrete antifungals that trigger cell wall biosynthesis in the fungus *Fusarium oxysporum.* These pieces of evidence suggest that microbe–microbe interactions in the soil promote soil health, as well as plant growth (Fig.13.1).

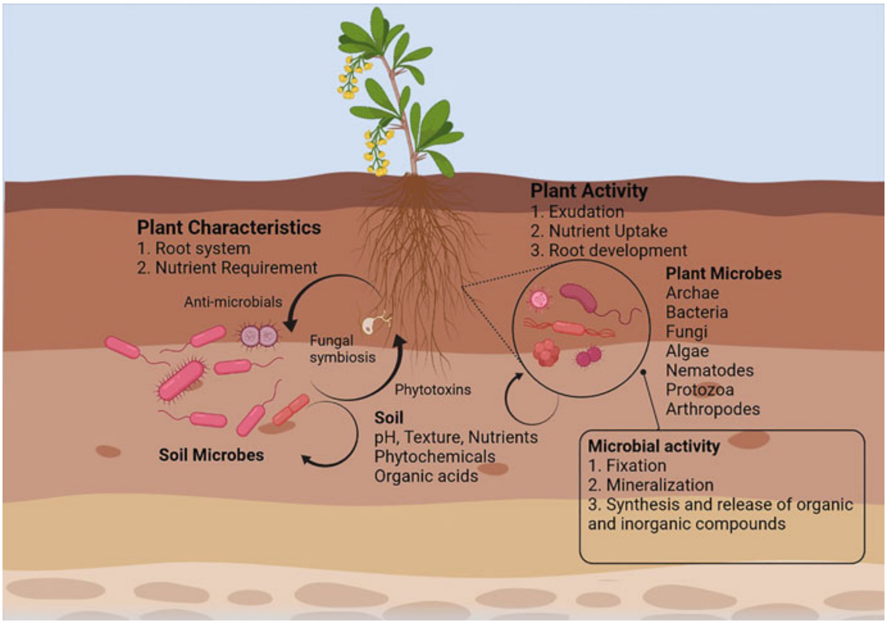


Fig. 13.1 Illustration summarizing the major interactions that occur in the soil. The plant–microbe interactions, root–root interactions, and the microbe–microbe interactions are elucidated. Plants have a root system and a requirement for nutrients. Their main activities include production of exudates from the roots, uptake of nutrients and the development of the roots. They interact with the soil microbes such as archaea, bacteria, fungi, algae, nematodes, protozoa, and arthropods. The soil microbes are responsible for the fixation, mineralization, and synthesis and release of organic and inorganic compounds. The soil microbes also aid in improving the soil texture. The interactions are also influenced by the soil environmental factors such as pH, temperature, redox and presence of other phytochemicals

13.4 Modern Technology Used in Sustainable Agriculture: Major Goals and Concepts

Advancements in technological interventions have opened-up several possibilities for exploring and researching the structures and functions of genes, proteins, and metabolites. Information and data generated based on these studies have now materialized as new scientific domains in research such as genomics, proteomics, transcriptomics, and metabolomics. Computational biology approaches comprise several bioinformatics concepts embedded with algorithms and models to interpret biological data (Upadhyay et al. 2021). The present scientific era stresses a lot on the importance of using in silico studies before in vitro and in vivo experiments since the use of complex algorithms and computational analysis has revolutionized research. The agriculture sector plays an important role in global sustainability, economy, and food security. Using modern computational biology and bioinformatics approaches to solve problems in the agriculture sector are now being comprehensively used.

These approaches have made it possible to analyze large amounts of data and screen desired targets of interest. Thus, some of the major goals of using modern technology in agriculture include the following:

- Assessing and analyzing large datasets
- Screening of desired targets for future experimental purposes
- Storage and easy manipulation of massive amounts of data
- Allowing the discovery of novel biological insights to create global perspectives from unified biological principles
- Potential to uncover relationships between lifeforms that were previously not considered

13.4.1 Data Science Concepts Involved in Agrobiology

13.4.1.1 Genomics and Metagenomics

Genomics plays a critical role in modern biological research wherein the nucleotide sequences of all the chromosomes of specific organisms can be mapped and genomic locations of different genes and their sequences can therefore be established. This process encompasses widespread analysis of the nucleic acids both in silico and in vitro. Thus, the application of this knowledge for the improvement of sustainability and crop productivity is called agriculture genomics. Sequencing of the genomes at affordable prices offers opportunities for targeted breeding of crops. Likewise, the massive cultured and uncultured diversity of microorganisms present in the soil that is being used in sustainable agriculture can be unlocked using metagenomics. Soil metagenomics includes DNA isolation and screening of clone libraries, which can provide a comprehensive valuation of several untapped genetic reservoirs of microbial communities. Additionally, metagenomic approaches have led to the identification of novel biomolecules and genes, comprehension of metabolic footprinting, and nutrient concentration studies (Gupta et al. 2018).

13.4.1.2 Proteomics and Transcriptomics

The study of the structure and function of a broad spectrum of proteins in organisms is called proteomics. The advent of proteomics enables easy identification of several proteins in living systems, especially for crops, since valuable information about the nutritional values and yield can be obtained. Proteomics in agriculture aids in understanding the plant responses to various abiotic stress. Comprehension of the DNA, mRNA, and protein levels in agricultural crops can also aid in simplifying plant breeding techniques Salekdeh and Komatsu 2007).

Likewise, the study of the RNA profiles at a given point within the organism is called transcriptomics. Recent advancements in transcriptomics studies assist researchers in characterizing the transcriptome to unravel the molecular basis that can help in the enhancement of crop productivity and diversity (Pandit et al. 2018). The use of transcriptomics helps in understanding the genes and their corresponding pathways that can respond to and counteract the biotic and abiotic environmental stresses.

13.4.1.3 Metabolomics

The comprehensive study of metabolites in a biological system is called metabolomics. This area of research is now being widely used for obtaining a better understanding of the complexity of biological systems via chemical compositions and relationships with plant physiology (Prasain 2016). In agriculture, metabolic content is associated with fruit maturation processes, crop developmental processes, resistance to environmental influences, adverse pathogen attacks, and stress responses. Thus, for the improvement of crop productivity, metabolomics plays a critical role via pathway analysis and subsequent biochemical network studies.

13.5 Scope of Data Sciences for the Analysis of Interactions in the Soil Microbiome

Currently, there is considerable evidence on the various types of soil microbiome interactions and how they affect and influence each other and, in turn, plant productivity. However, to expand the existing knowledge on plant–microbiome–soil interactions, there is a need to explore different approaches. “Omics” studies are an emerging area of data sciences, where an in-depth understanding of various unexplored avenues in agrobiology can be obtained. With the integration of several “omics” approaches, a thorough attempt can be made to solve several underlying issues to improve sustainability in agriculture. Therefore, the scope of using data sciences to analyze the soil microbiome interactions is as follows:

- **Tool for plant–microbe interactions:** Advancements in metagenomics and next-generation sequencing approaches have allowed examinations of the genetic diversity and related functions of a variety of microbes without any prior predispositions of competition among plants and microbes, manual cultivation, biotic/abiotic stresses, and parasitism (Oulas et al. 2015). This has enabled a deeper perception of microbial ecology.
- **Reconstructing organisms:** Genome-scale models (GEMs) of soil microorganisms can be reconstructed to further understand and perform pan-genome analyses (Fang et al. 2020).
- **Assessment of phylogenetic diversity:** Modern technology and data sciences enable critical evaluation of the microbial phylogenetic diversity and its

functional information, which keep altering due to varying agronomic practices and external environmental factors (Bertola et al. 2021).
- **Identification of molecular mechanisms:** Particular metabolites in the root exudates urge the microbiome selection and assembly. Integration of the multiomics data can further aid in the identification of specific molecular mechanisms that underlie the recruitment of the microbiome. For this purpose, the metabolite–metabolite interactions, metabolite–microbe interactions, and microbe–microbe interactions will be considered (Zancarini et al. 2021).
- **Identification of interactions in the synthetic communities:** In a synthetically formulated consortium, the microbes engage in several interactions such as commensal, competitive, mutualistic, or neutral. Since directly studying the interactions in natural systems is slightly challenging, understanding them via synthetic communities provides a much better approach to the prediction of metabolic interactions (Heinken et al. 2021).

13.5.1 Soil Metagenomics and Their Applications

Since soil contains a plethora of microorganisms, metagenomic approaches can aid in getting a more complete picture of the microbial communities in the soil to better comprehend their interactions with each other and with neighboring plants. High-throughput screening methods are utilized nowadays to overcome and understand the intricacy of the soil metagenome. Therefore, some of the general applications of soil metagenomic studies are as follows:

13.5.1.1 Soil Health

Elucidating the functional potential of the microbial communities in the soil can provide better insights into the health of the soil than other taxonomy-based approaches (Hozzein 2020). For instance, the phosphor content of the soil is essential in determining the proper functioning of the nutrient cycle and plant growth. If there is a deficiency of soil phosphorus, the secretion of acid phosphatase from the roots of the plant is increased to improve the remobilization and solubilization of phosphate, thereby allowing the plants to cope with phosphor deficiency stress (Dey et al. 2021).

13.5.1.2 Discovery of Antibiotics

Soil metagenomics presents a tremendous opportunity for the discovery of several antibiotics yet unknown to man. Previously, scientists have isolated novel antibiotics called turbomycin A and B from the metagenomic library of microbial DNA from soil (Gupta et al. 2018). Additionally, another study isolated a gene cluster that codes for indole-tryptoline-based compounds, which demonstrate good activity against tumor cell lines (Hozzein 2020).

13.5.1.3 Industrial Use

New classes of genes and enzymes can be identified using soil metagenomic data. For example, cellulase has been identified from a variety of soil environments, and using metagenomic approaches such as the construction of metagenomic libraries and screening of the biologically active clones, its function could further be explicated (Rondon et al. 2000). Likewise, another enzyme called xylanase has also been isolated from the compost soil metagenome (Verma et al. 2013).

13.5.1.4 Bioremediation

The microbes in the rhizosphere produce biosurfactants that play a major role in plant–microbe interaction. In agriculture, these biosurfactants are utilized for the elimination of plant pathogens and for augmenting the nutrient bioavailability for useful plant microbes. Therefore, soil metagenomic studies assist in tracing new microbial communities that produce such biosurfactants that are beneficial for bioremediation (Hozzein 2020).

13.5.1.5 Sustainable Agriculture

Since metagenomics is an advanced genomic tool that is commonly employed to comprehend the complete microbial diversity of the soil, modern molecular methods such as extraction of DNA, preparation of metagenomic libraries, and sequencing of environmental samples using high-throughput techniques can further be used at an advanced level. For augmenting sustainable agriculture, characterization and exploitation of plant growth-promoting bacteria are essential. In this regard, soil metagenomics substantially offers predominant information and genetic data and bridges the gaps existing genetic evolution of various unidentified microbes (Bakshi et al. 2020). An illustration summarizing the major areas covered in metagenomics that decipher the role of soil bacteria in the nutrition of plants is shown in Fig. 13.2.

13.6 Scope of Next-Generation Sequencing in Agrobiology

The introduction of next-generation sequencing (NGS) technologies has improved and revolutionized the field of agrobiology due to the integration and use of “omics” strategies. These modern approaches offer high efficiency in terms of distinct

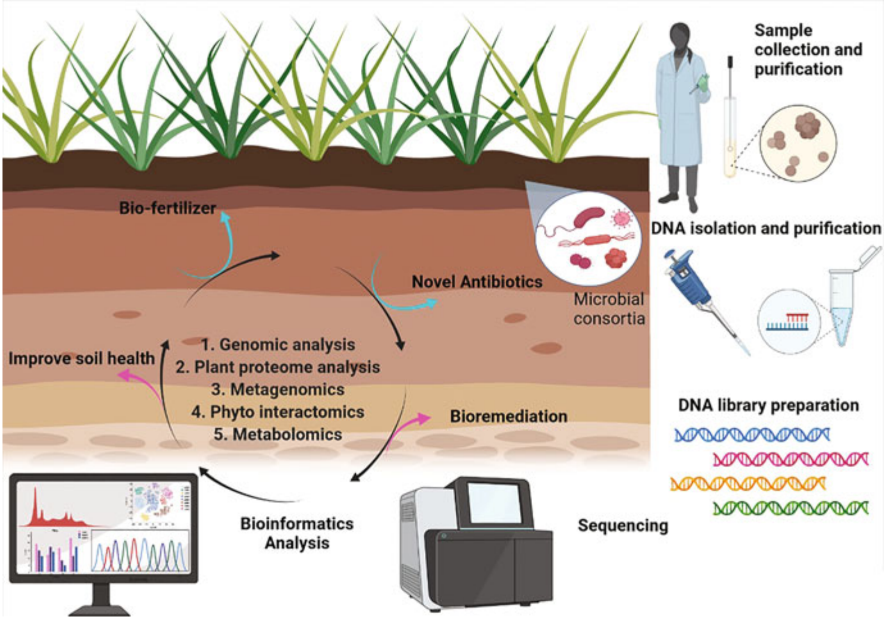


Fig. 13.2 Illustration summarizing the major areas in metagenomics that decipher the role of soil bacteria in the nutrition of plants. The bacteria of the soil, including natural consortia, take part in several functions such as acting as bio-fertilizer, producing novel antibiotics, playing a role in bioremediation and improvement of the overall soil health. Major areas in metagenomics such as genomic analysis, plant proteomic studies, metagenomics, phyto-interactomics, metabolomics, and transcriptomics play a vital role in analyzing these functions of the soil microbes. The soil microbes are generally collected and purified, their DNA is isolated, and their DNA library is prepared and sent for sequencing prior to performing bioinformatic analyses

resolution and experimental execution time. Thus, the novelties in the advent of NGS technologies and its scope in agrobiology are discussed here:

13.6.1 Single and Multiple Species Genomics in Agriculture

The samples for omics studies are generally derived from single or multiple individuals of a species or multiple species. In single-sample approaches, the functionality and organization of specific tissues, organs, and cells, for instance, the fruits, roots, etc., are examined to identify certain emergent factors such as shape and quality. Additionally, it also paves the way for comprehension of more complex traits such as suppression of plant diseases, yield, resistance to stresses, etc. (Barh et al. 2013; Van Emon 2016). Furthermore, using the metagenomics approaches, elucidation of the molecular components in a population having the same species helps determine the evolutionary routes that impact the variability in genetics. This in turn can largely contribute to the understanding of intricate quantitative traits through the discovery of novel genes (Semagn et al. 2010) and the effect of genetic variations on phenotypic plasticity.

13.6.2 Impact of NGS on Agrobiology

NGS of whole genomes/whole exomes from various plant species allows the definition of their organization and offers a starting point to understand its functionality, thereby permitting agriculture practice (Esposito et al. 2016). The knowledge that arises from the analysis of proteome, transcriptome, and metabolome is also necessary to better portray the content of genes, their protein structures, and functions. Explication of the gene complexity, their primary networks, and functions is also rudimentary before moving forward with crop breeding practices. The contribution of the "omics" approaches to agriculture via NGS technologies spans from gene identification and manipulation of specific genotypic/phenotypic traits to breeding by the marker-assisted selection of the variants (Zhang et al. 2014a, b; Iovene et al. 2004). Thus, agri-genomics finds innovative solutions to problems of sustainable productivity and provides insights into crop genetics for its better usage in agrobiology.

13.6.3 NGS and Omics Approaches

The primary method used for the profiling of microbial communities in the soil to elucidate their major functions involves sequencing by NGS on the samples to characterize the fragments of DNA of the metagenome. Metagenomics in soil agrobiology aids in depicting the complex interaction patterns occurring among the soil microbes and between the plant and soil microbes in the rhizosphere (Carbonetto et al. 2014; Mendes et al. 2014). Metagenomics also traces the taxonomic shifts and the functional redundancy of the soil microbes associated with changes in environmental conditions (Esposito et al. 2016). Metagenomics aid in translating the role of soil bacteria in plant nutrition and for understanding the mechanisms of plant stress responses (Lavecchia et al. 2015; Timmusk et al. 2014).

13.6.4 Revolution of Omics and Impact on Bioinformatics Research

The emergence of omics approaches influenced bioinformatics in terms of the collection of data, organization of data, integration of the data, and using various omics approaches and implementation of appropriate tools for mining data. The introduction of high-throughput technologies cemented the way for upgrading genome sequencing efforts of several models and nonmodel species of agricultural interest. These were then followed by transcriptomic data analysis for deciphering the transcriptional processes, defining cell functionalities in stress and physiological conditions. All these approaches necessitated the design of resources to distribute the amassed data to the whole of the scientific community, thereby bolstering the need for adequate pipelines for translating from raw information to value-added and integrative approaches (Mita et al. 2003; Christoffels et al. 2001; D'Agostino et al. 2005). Thus, with the advent of NGS and omics analysis, bioinformatics research has now taken a new turn and plays a key role in the field of sustainable agriculture.

13.7 Challenges of Chemical Pesticides and Fertilizers in Agrobiology

"Pesticide" is an overarching term that includes a wide array of compounds such as herbicides, insecticides, rodenticides, fungicides, molluscicides, rodenticides, nematicides, plant growth regulations, and several others (Aktar et al. 2009; Alewu and Nosiri 2011). "Fertilizers" are chemical substances, prepared synthetically, which are added to the soil to improve its fertility and in turn productivity (Savci 2012). Although there are several advantages of using chemical pesticides and fertilizers such as killing unwanted pests, improving crop productivity, protection of crops from losses and yield improvements, control of vector-borne diseases, food quality improvements, etc., long-term use of chemical pesticides and fertilizers poses a lot of challenges. The following are some of the challenges of the overuse of chemical pesticides and fertilizers in agriculture:

- **Directly affects humans:** The chemical pesticides travel through the food chain and affect humans directly or indirectly via the plants consumed as food (Rasheed et al. 2019).
- **Environmental impacts:** Long-term exposure to chemicals affects soil fertility and has adverse effects on microbial communities. Additionally, chemical pesticides and fertilizers can be toxic to several other organisms such as fish, insects, birds, and other nontargeted plants. Among all pesticides, insecticides are known to be more acutely toxic than others; however, herbicides also tend to pose a risk to non-target organisms (Aktar et al. 2009).
- **Contamination of food commodities:** Residues of chemical pesticides and fertilizers have previously been found in various foods and beverages such as water, wine, instant cooked meals, fruit juices, animal feeds, and other refreshments (Nag and Raikwar 2011; Witczak and Abdel-Gawad 2014; Chourasiya et al. 2015). Consumption of pesticides adversely affects humans and animals in the long run.

- **Contamination of water:** Soil water and groundwater contamination is another side effect of long-term exposure to chemical pesticides. The levels of pesticides identified in water have now amplified because of their excessive utilization in the agricultural domain (Saleh et al. 2020).
- **Impact on the fertility of soil:** Overuse of chemical pesticides and fertilizers can kill several beneficial soil microorganisms and disrupt the fertility of the soil, thereby causing an overall deleterious effect on the soil ecosystem (Boudh and Singh 2019).
- **Contamination of air:** Volatile pesticides drift into the air and contaminate it. It is established that almost 80–90% of pesticides, when applied, are volatilized after a few days of application (Majewski and Capel 1995).

Due to these drawbacks, there is a pressing need to use modern technology and explore alternative approaches for augmenting sustainable agriculture.

13.8 Alternative Approaches: Design and Development of Novel Microbial Consortia for Enhancing Plant Productivity

The presence of two or more bacterial or microbial species/groups that live symbiotically is called consortia. The applications of using microbial consortia have been widely explored in the scientific field. Advancements in the field of microbial consortia present a valuable alternative approach for broadening the scope and understanding of alternative approaches in agriculture. Systems biology allows a comprehensive understanding of various processes occurring in the cells along with their interactions, thereby offering insights into the design of microbial consortia. Recent years of research have explicated the workings of microorganisms in nature and how chronic use of chemical pesticides and fertilizers has diminished their capabilities to enhance the soil health and fitness of plants. Thus, designing microbial consortia that vigilantly assess the relationship between the residing microbiome and the inoculants will considerably augment plant growth (Ray et al. 2020).

13.8.1 Principles Involved in Formulating Microbial Consortia

The activity and survival of microorganisms in the soil face competition with innumerable other microbes that are naturally adapted to the soil. Therefore, along with having a compatible association with the host, a microbial inoculant must be able to consequently contend and exist along with other soil-adapted microbes and the abiotic conditions that exist in the soil (Finkel et al. 2017). Furthermore, it has also been stated that bacterial inoculants can persevere in the soil for up to 7 weeks;

however, it is still blurry if the same inoculum can offer growth benefits to the plants (Schreiter et al. 2014). Thus, this challenge can be overcome by inoculating the plants with microbial consortia in-lieu of single bacterial strains (Maiyappan et al. 2010; Nemergut et al. 2013). This implies that microbial diversity is vital for promoting sustainable agriculture (Cordero and Polz 2014).

Moreover, to augment the chances of plant growth promotion, prior knowledge of the existing indigenous microorganisms is essential. For instance, the interactions that occur among the microbes in a consortium also play a vital role in determining the performance of the said consortium. These interactions include the following:

- **Mutualism:** Corresponds to cross-feeding, where both the bacteria are mutually benefitted from each other via the interchange of metabolites that are useful for both partners.
- **Commensalism:** Where one member of the consortia benefits the survival of the other, while the other member is neither positively nor negatively affected.
- **Parasitism/predation:** One bacterium act as the predator to another bacterium in a consortium.
- **Competition:** Both members of the consortium compete with one another for survival. Both can be detrimental to one another.
- **Amensalism:** Where one member is detrimental to the other, while the other is neither positively nor negatively affected.
- **Neutralism:** Neither of the microbial members is affected positively nor negatively by the other's presence (Che and Men 2019).

Therefore, studies on the microbiome-based strategies driven by next-generation sequencing technology enable researchers to design and develop better microbial consortia for augmenting productivity and sustainability.

13.8.2 Methods for Formulating Microbial Consortia

Apart from using naturally available microbes to formulate a consortium, synthetic consortia can also be engineered using synthetic biology techniques. Additionally, the design of microbial consortia that depend on principles such as channeling substrates between different organisms, cross-feeding, or crosstalk provides novel opportunities for the design of "intelligent" consortia (Calvo et al. 2014; Vorholt et al. 2017; Herrera Paredes et al. 2018). To design novel microbial consortia, enhancing knowledge about how the microbial community works at the systems level is crucial. Additionally, the number of tools available for designing and constructing consortia at computational levels must be increased. Some methods used for designing synthetic consortia include the following:

- **Top-down approach (complex to simple):** In this method, the members of the consortium are the identified chief players from a particular complex microbial community. This is based on the multiomics analysis, commencing from macroscopic consortia to construing the principles of the system to interpreting the molecular mechanisms (Che and Men 2019; Jia et al. 2016). This approach offers more independence to naturally occurring microbes.

- **Bottom-up approach (simple to complex):** In this method, the members of the consortium are chosen from a pool of engineering microbes having the desired attributes. Depending on the complexity of the genetic elements, circuits, modules, and metabolic networks, engineering principles are used to design consortia having higher stability and efficiency (Jia et al. 2016; Che and Men 2019). A variety of synthetic biology tools are used for this purpose and are the most preferred method of designing consortia.

Moreover, after designing consortia, it is also studied as to whether the consortia must be grown as co-cultures or mixed cultures in synthetic systems.

13.8.3 General Applications and Recent Case Studies of Designed Microbial Consortia

Synthetic microbial consortia permit comprehensive interpretation of various physiological processes of cells and their diverse interactions, thereby providing illumination on the optimal design of the consortia. The designed consortia can be programmed via quorum sensing-based cell-cell communications, microbial ecosystems wired by bi-directional communications, and sender–receiver microbial communications (Song et al. 2014). These designed consortia find applications in various fields such as bio-computing, production of bio-energy and chemicals, human health and medicine, environmental sciences, and sustainable agriculture. The use of synthetic and computational biology tools and concepts provides a detailed understanding of the logistic engineering of complex consortia and naturally occurring consortia for use in novel purposes.

Previous studies have designed a consortium of *Bacillus megaterium* and *E. coli*, where the *B. megaterium* cells expressed a two-component AgrCA (accessory gene regulators), while the *E. coli* cells expressed the proteins AgrD and AgrB to help in the recognition of autoinducing peptides (Marchand and Collins 2013). Likewise, another study designed a methanogenesis consortium, where *Methanosaeta harundinacea* and *Geobacter metallireducens* interchanged electrons directly by pili that are electrically conductive (Rotaru et al. 2014). In the field of agriculture, synthetic microbial consortia comprised of species of *Clostridium* and *Enterococcus* vitiates the wheat straw into butanol and hydrogen in a two-step reaction (Valdez-Vazquez et al. 2015). A synthetic microbial consortium constructed using PGPBs and AMFs, enhances the content of the metabolites such as sugar, folic acid, ascorbic acid, vitamins, volatile compounds, glucosinolates, anthocyanins, etc., as well as the nutrients available to plants including calcium, sodium, phosphorous, magnesium, nitrogen, iron, zinc, copper and boron, thereby improving the nutrition value of plants (Torres et al. 2016; Avio et al. 2017). An illustration on the major steps

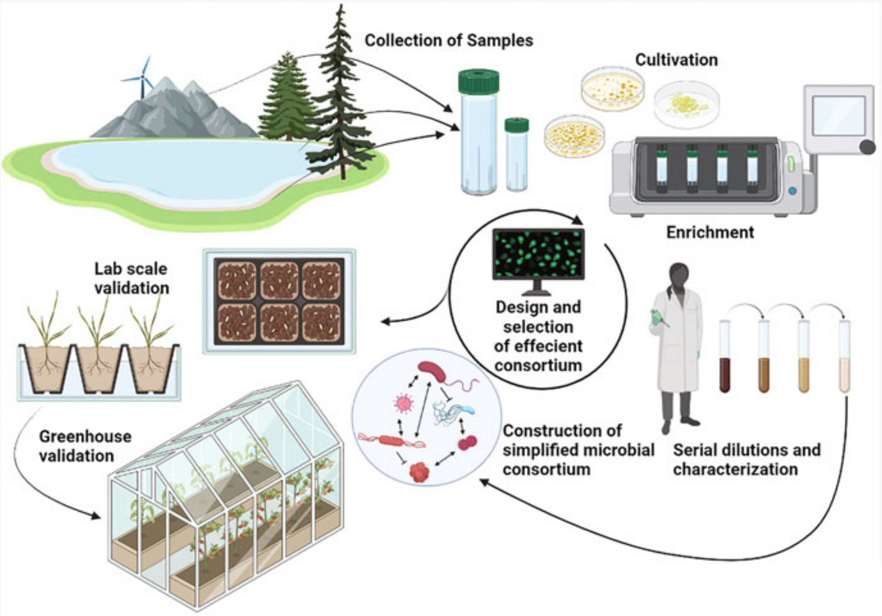


Fig. 13.3 Illustration summarizing the major steps in the design and development of novel consortia for enhancing plant productivity. The soil samples are first collected and the microbes in them are enriched and cultivated. Using serial dilutions, the pure isolates are characterized and simplified microbial consortia are constructed after thorough bioinformatic analysis and designing of efficient consortia. The constructed consortia are then validated in wet lab studies

involved in the design and development of novel consortia for enhancing plant productivity is given in Fig. 13.3.

13.9 Major Types of Microbial Consortia Responsible for Sustainable and Balanced Agrobiology

The acceptability of using microbial consortia rather than single microbes to boost sustainable agriculture has greatly increased. When the microbes are added together as a consortium, they exist symbiotically, while retaining their abilities to respond differently to biotic and abiotic environmental stresses. This is primarily due to their intrinsic useful interactions (Nuti and Giovannetti 2015). In general, the consortium uses its quorum-sensing signaling abilities via biochemical pathways that respond to the microbial density and nutrient gradient. The various types of microbial consortia responsible for sustainable and balanced agrobiology are discussed in what follows.

13.9.1 Bacterial Consortia and Their Interactions

This includes the plant growth-promoting rhizobacteria (PGPR) group of organisms such as *Arthrobacter*, *Pseudomonas*, *Klebsiella*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Serratia*, *Enterobacter*, and *Azospirillum* (Gehlot et al. 2021). These bacteria enhance the growth and development of plants via several processes (Jambon et al. 2018). Segregation of the pollutants of the soil is improved by the interaction of the aforesaid rhizobacteria with the plants (Eze et al. 2018). These PGPRs are entangled in several interesting roles such as the development of the plant, secretion of different plant hormones, solubilization of phosphates, and nitrogen fixation (Htwe et al. 2019). The bacterial communities communicate with each other in the consortia via quorum sensing (QS) signaling methods (Barriuso 2015).

During this process, gene expression and communication are regulated by molecules such as autoinducers and QS molecules. Some examples of QS molecules include autoinducer-2, autoinducing peptides, and acyl-homoserine lactones, which monitor and control biochemical mechanisms such as the formation of biofilms, sporulation, production of antibiotics, bacterial motility, and release of virulence factors (Fleitas Martínez et al. 2019). QS signaling is a regulatory response to identify the compounds by transcribing the specific genes of interest (Venturi and Keel 2016). This signaling involves defined pathogenic activities via adjustment of the microbes during stress conditions (Jiang et al. 2019). Furthermore, secretion of volatile organic compounds (VOCs) and nodulating factors (Nod) by the rhizobia aids in bacterial communication within the consortia (Hung et al. 2015). VOCs boost plan growth via acetoins, which can hinder gene expression in plants and activates plant pathogen resistances, thereby maintaining balanced agrobiology (Bennett et al. 2012).

13.9.2 Bacteria–Fungi Consortia and Their Interactions

There exists a close biophysical association and metabolic activities during bacteria–fungi co-occurrences that aid in the growth of both microbes. Interactions between the PGPRs and the arbuscular mycorrhizae fungi (AMF) enhance the development of the crops (Pathak et al. 2017). This consortium improves the concentration of nutrients in the soil and disseminates the soil microbiota. Thus, consortia of PGPR and AMF are considered potential alternatives to chemical fertilizers and as biocontrol agents for balanced and sustainable agriculture (Pathak et al. 2017).

Additionally, during the process of mycorrhization, the mycorrhizal aiding bacteria and the PGPRs have a symbiotic interaction with the roots of the mycorrhizae and fungi to help in nutrient uptake. For this purpose, the PGPRs can be either intra- or extracellular, and they release growth-promoting hormones to aid in plant growth (Zheng et al. 2018). Applications of PGPRs such as *Bacillus* sp., *Pseudomonas* sp., and AMF in consortia have proved to show an improvement in the growth of crops and in maintaining sustainable agrobiology (Philippot et al. 2013).

13.10 Merits and Demerits of Microbial Consortia-Based Approaches

The microbial consortia-based approaches despite having several applications in agriculture have certain advantages and disadvantages. Thus, the merits and demerits of using microbial consortia for sustainable agriculture are as follows:

13.10.1 Merits

- A mixed culture of microbial consortium can cross-feed the nutrients and regulate the surroundings to stimulate each other's growth and development (Shou et al. 2007).
- Higher productivity is observed when a consortium that works symbiotically is used than single strains.
- A complex set of multiple reactions are executed in a much quicker way than when single strains are inoculated.
- In a consortium, the microbial cultures producing different enzymes can degrade their substrates in different ways (Bhatia et al. 2015).
- Uncultured microorganisms can be utilized for co-culture techniques.
- Microbial consortia can also inhibit the growth and development of toxic and unfavorable microbes and control contamination.
- Boosts soil fertility and sustainability in agriculture.

13.10.2 Demerits

- Accurate development of consortia is essential since the properties and interactions of the microbes are different for individual strains.
- Unavailability of knowledge of the microbe functions, the metabolites it produces, and the nutrition it requires may affect the overall consortia.
- Challenging to detect the contaminating agent in consortia.
- Conservation of the consortia is difficult as different microbes have different rates of survival.
- Not all consortia can co-exist and survive for long periods in soil environments.

13.11 Computational Biology and Bioinformatics Tools and Resources for the Design and Formulation of Novel Microbial Consortia

The design and formulation of microbial consortia for sustainable agrobiology have been discussed previously. However, for this purpose, several computational biology algorithms and bioinformatics tools are utilized. For constraint-based modeling of consortia, dynamic and steady-state computational biology approaches are utilized, depending on their growth conditions. The following are some of the latest tools, algorithms, and resources for designing a microbial consortium:

13.11.1 Dynamic Modeling Tools

- **FLYCOP (Flexible sYnthetic Consortium Optimization):** A newly developed framework that works on metabolic engineering to design and model the consortium. This framework chooses the best configuration of the consortium to optimize a specific goal among multiple configurations and considers any temporal alterations into account (García-Jiménez et al. 2018).
- **BacArena:** This tool models microbial communities as aggregates of individuals with definite metabolism, which interact with each other via temporal and spatial means. This can also be used to hypothesize the cross-feeding mechanisms between the species in the consortia (Bauer et al. 2017).
- **COMETS (**Computation of Microbial Ecosystems in Time and Space): This tool predicts the rate of growth as per the concentration of spatial gradients. Commonly, this tool has been used to study the consortia of *E.coli*, *Salmonella enterica*, and *Methylobacterium extroquens* (Dukovski et al. 2020). A newer version called COMETS 2 employs newer biological modules and is compatible with Python and MATLAB interfaces.
- ***μ*bialSim:** This tool is a numerical simulator that predicts the time-course as activity and composition of the microbiomes that comprise countless species (Popp and Centler 2020). *μ*bialSim utilizes the COBRA toolbox (constraint-based reconstruction and analysis), and aside from modeling microbial communities, it can also be used to explore principles of microbial ecology.

13.11.2 Steady-State Modeling Tools

- **CarveMe:** This tool is primarily meant for designing and modeling single-species and microbial community models. This is a Python package that constructs a metabolic network of microbes from universal models (Machado et al. 2018) and is used to analyze the complex dynamics of consortia.

- **RedCom:** Another method whose main objective is to maximize the growth rate of the microbial community and predicts feasible ranges for the exchange of metabolites. This tool uses metaproteomic data to constrain the solution space for the microbial community models (Koch et al. 2019).
- **SteadyCom:** Used for steady-state modeling of microbial communities. The tool identifies the abundance of each of the species with the major function of improving community growth (Chan et al. 2017).
- **Microbe Modelling Toolbox:** MATLAB-based toolbox that is used to design, construct, and analyze the host–microbe interactions and the microbial communities. The tool uses metagenomic data and comprises certain built-in functions for analyses (Baldini et al. 2019).
- **MMinte:** It is a pipeline that utilizes the 16S rDNA sequence data to detect the reference genomes and enables comparison of the growth rates in microbial communities (Mendes-Soares et al. 2016).
- **OptCom:** Steady-state modeling tool that can be customized for every interaction type, such as parasitism, competition, mutualism, etc. This algorithm also aids in understanding the trade-offs between the community and individual fitness (Zomorrodi and Maranas 2012).

Other tools, databases, and software of importance include MetaQUAST, IDTAXA, COLEMNA, STIFDB, Plant Stress Protein Database, PASmiR, DroughtDB, Plant Micro RNA DB, EXPath, MicrobioLink, Metabarcoding, Calypso RAST server, ROCker, CLARK, SINA, etc. The functions, applications, and the references for all the tools and resources related to microbiome interactions and plant–microbe interactions that play a role in augmenting sustainable agrobiology are provided in Tables 13.1 and 13.2. An illustration depicting the overview of bioinformatics and data sciences in developing novel consortia for enhancing plant productivity is shown in Fig. 13.4.

13.12 Successful Applications of Data Sciences and Microbial Consortia-Based Approaches in Agrobiology

The use of data sciences and microbial consortia-based approaches has been discussed in detail in the prior sections. Thus, some important applications that have been previously reported for the successful use of data sciences and consortia are elucidated.

Previously, several studies have reported the use of compatible microbial consortia, either bacteria–fungi consortia or 2–3 bacteria in various combinations for the augmentation of crop resistance against stress and overall crop development (Jain et al. 2012; Singh et al. 2013; Patel et al. 2017). Additionally, it has also been stated in previous reports that uptake of nitrogen and phosphorous and defense against soil-borne plant pathogens were enhanced due to the use of *Rhizobium*, *Trichoderma*,

Table 13.1 Major databases and web-based tools available for the study of microbiome interactions for augmenting sustainable agrobiology

Tool/database name	Tool/database function	Application in sustainable agrobiology	Tool/database link	Reference
MetaQUAST	To evaluate assemblies that are related to metagenome	Metagenomic data obtained from microbial gene sequencing can be quality assessed with the help of this tool. This will aid in faster detection of sequence diversity, common sequences and sequences that are highly relative in nature	http://bioinf.spbau.ru/metaquast	Mikheenko et al. (2016)
IDTAXA	To accurately classify microbial sequences based on their taxonomies	R programming language can be used for maintaining the quality of the microbial sequences, designing oligos, sequence analysis, and identifying genes	http://decipher.codes/	Murali et al. (2018)
COLMENA	Group of native microorganisms that help in contributing to food security through the usage of agro-biotechnological potentials of soil	Collection of information that is related to soil-based and endophyte microorganisms	http://apps2.itson.edu.mx/colmena/	de los Santos-Villalobos et al. (2021)
Plant Stress Gene Database	Collection of gene information of plants that are subjected to various stress conditions with respect to 11 plant species	Stress-related genes across 11 different plant species are curated in this database, and its ortholog and paralog information are available	http://ccbb.jnu.ac.in/stressgenes/frontpage.html	Prabha et al. (2011)
PlantPRes Database	Collection of plant proteome database that are related to stress conditions. It comprises proteins whose count are more than 20,000	It has a comprehensive data about plant with various aspects such as stress types, plant type, protein name, developmental stage, and the protein accession numbers. This information can be used to filter out	http://www.proteome.ir/	Mousavi et al. (2016)

(continued)

Table 13.1 (continued)

Tool/database name	Tool/database function	Application in sustainable agrobiology	Tool/database link	Reference
		the proteins that are specific to plant species and further used for improving sustainable agriculture		
Plant Stress Protein Database (PSPDB)	Database that comprises plant stress proteins which are curated manually and submitted in the UniProt	There are about 2064 protein information which are related to different stress conditions along with other integrated tools such as BLAST, NJPLOT, NCBI, BLAST, and many more. These information can be used for harnessing sustainable agriculture	http://www.bioclues.org/pspdb/	Kumar et al. (2014)
STIFDB	Group of information that are related to stress-responsive transcription factors with respect to *Arabidopsis* species Arabidopsis stress-responsive transcription factor data	This database will help in analyzing stress-responsive genes especially with respect to the model plant organism *Arabidopsis thaliana*	http://caps.ncbs.res.in/stifdb2/	Shameer et al. (2009)
STIFDB2	An updated version of plant stress-responsive transcription factor database with additional stress signals, stress-responsive transcription factor binding sites and stress-responsive genes in *Arabidopsis* and rice	This is an updated version of the previous database which has information about stress-responsive genes in *Arabidopsis thaliana* and *Oryza sativa*	http://caps.ncbs.res.in/stifdb2/	Naika et al. (2013)
PASmiR	Database with information that is related to plant miRNA molecular regulation under different abiotic stress conditions	Comprehensive data about miRNA of about 30 different plant species under different abiotic stress conditions which can be used to study sustainable agrobiology	http://hi.ustc.edu.cn:8080/PASmiR and http://pcsb.ahau.edu.cn:8080/PASmiR	Zhang et al. (2013)

(continued)

Table 13.1 (continued)

Tool/database name	Tool/database function	Application in sustainable agrobiology	Tool/database link	Reference
DroughtDB	Database that comprises plant genes and their homologs that are with respect to stress conditions among nine different species	Abiotic stress condition such as drought stress genes with respective to nine different plant species can be obtained from this database for harnessing data for sustainable agribiology	http://pgsb.helmholtz-muenchen.de/droughtdb/	Alter et al. (2015)
Plant stress RNA-seq Nexus (PSRN)	Curated information of plant transcriptome data which are specially stress specific in nature	These RNA-seq information database which will help researchers support insights into breeding different crops aiding to sustainable agrobiology	http://syslab5.nchu.edu.tw/PSRN	Li et al. (2018)
Plant Micro RNA Database (PMRD)	Collation of information with respect to miRNA that are acquired from various sources such as public repositories, in-house generated databases, and data collected from recent literature	Database information about microRNAs of model organisms whose genomes are available with respect to its expression profile, secondary dimension structure, and their target genes	http://bioinformatics.cau.edu.cn/PMRD	Zhang et al. (2010)
EXPath	Metabolic pathway database with respect to plant and its comparative expression analysis	With up to 6 model plant organisms being analyzed in this study, the microarray data analysis obtained from various conditions can be used to study the effect on metabolic pathways	http://expath.itps.ncku.edu.tw/	Chien et al. (2015)
RiceSRTFDB	Collection of information with respect to rice and its transcription factors along with expression data, mutant information, and cis-regulatory	This information with respect to rice can be used to study the stress-responsive genes and its expression data along with other important mutant and	http://www.nipgr.res.in/RiceSRTFDB.html	Priya and Jain (2013)

(continued)

Table 13.1 (continued)

Tool/database name	Tool/database function	Application in sustainable agrobiology	Tool/database link	Reference
	elements in order to use it for functional analysis	cis-regulatory elements information which will further help in improving the agrobiology with respect to rice		
PlnTFDB	Consolidated database with plant transcription factor information	Curated information with respect to transcription factors and regulators in over 19 plant species	http://plntfdb.bio.uni-potsdam.de/	Riaño-Pachón et al. (2007)

and phosphate-solubilizing bacteria in a consortium (Rudresh et al. 2005). Furthermore, consortia of PGPRs, mycorrhizae, and endophytic bacteria have also been reported to successfully augment plant productivity and protection, thereby considerably reducing the dependence on chemical fertilizers (Perez et al. 2007). It is also established that AHLs (acyl homoserine lactones) are vital signaling molecules in bacteria (Mukherjee and Bassler 2019) and AHLs released by specific PGPRs in consortia such as *Serratia phymuthica* and *S. liquefaciens* have promoted the development of root and overall plant biomass. Other bacteria such as *Pantoea ananatis* and *Sinorhizobium fredii* have promoted biofilm formation in the roots of *Phaseolus vulgaris* (bean) and *Oryza sativa* (rice) (Pérez-Montaño et al. 2013).

Moreover, previous studies have reported culturing of *Ketogulonicigenium vulgare* and *Bacillus megaterium* for which a GCMS (gas chromatography-mass spectrometry) was employed for a metabolomics study for identifying the exchange of metabolites and interactions between the two species (Zhou et al. 2011). Recent studies have demonstrated the use of synthetic microbial consortia constructed after thorough omics data analyses to enhance the biocontrol activity against agricultural pathogens. In these studies, the consortia were constructed after selecting the microbes having different mechanisms of antagonistic activity to ensure consistent performance against multiple pathogens (Kong et al. 2018). Omics data of various soil microbes have also aided in the successful modeling of pesticide degradation, thereby augmenting sustainability (De Sousa et al. 2018). Previous studies have also established that omics tools equip researchers with information on the growth dynamics and metabolic fluxes in a specified consortium (Cobb et al. 2015). For instance, through complete proteomic and transcriptomic analysis, studies have understood the synergistic growth of *Dehalococcoides ethenogenes* with *Desulfovibrio vulgaris* and *Methanobacterium congolense* (Men et al. 2012).

Table 13.2 Major bioinformatics software (a) and resources (b) available for various aspects of plant microbe interactions used in sustainable agrobiology studies

(a)

Software/ Resource	Function	Application to study sustainable agrobiology	Website link	Reference
MicrobioLink	To study microbiome–host interactions and its functional effects through an integrative computational pipeline	Plant–microbiome interaction can be studied through computational approach for sustainable agrobiology	https://github.com/korcsmarosgroup/HMIpipeline	Andrighetti et al. (2020)
NetCooperate	A tool which is based on network aids in microbe–microbe and host–microbe cooperation	It can be applied to calculate the biosynthetic support score between plant microbiome species especially in nutritional requirements	http://elbo.gs.washington.edu/software_netcooperate.html	Levy et al. (2015)
Metabarcoding	A tool to study as well as investigate the microbiome in order to design the strategies that will protect plants	Important tool which can be used to strategize sustainable agrobiology by investigating various microbiome involved	–	Abdelfattah et al. (2018)
Calypso	A web-server which is user-friendly can be used for extracting and to visualize the interactions between microbiome and environment	This online software can be used to the study the taxonomic data obtained from 16 s rDNA datasets of microbial community that are involved in forming consortia	http://cgenome.net/calypso/	Zakrzewski et al. (2017)
RAST Server	A server which can be automatically exploits the metagenome through phylogenetic and functional analysis	This server can be used to the study the microbial communities relationship status in order to help achieve sustainable agrobiology	http://metagenomics.nmpdr.org/	Meyer et al. (2008)
ROCker	A tool to detect and quantify data that are related to short-read metagenomics of target genes with	With the help of the scores that are obtained from this tool, the short read metagenomics data of the microbes in	http://enve-omics.ce.gatech.edu/rocker/	Orellana et al. (2017)

(continued)

Table 13.2 (continued)

(a)

Software/ Resource	Function	Application to study sustainable agrobiology	Website link	Reference
	the help of sliding-window bitscores	the microbial community can be analyzed		
CLARK	Rapid and precise tool to classify genomic and metagenomic sequences with the help of *k*-mers which are discriminative in nature	Important resource which can be used to study the microbial sequences classification system which is more precise, rapid, and different	http://clark.cs.ucr.edu/	Ounit et al. (2015)
CheckM	Tool to evaluate the microbial genome quality which was obtained from single cells, metagenomes, and isolates	Useful tool to identify the sequenced genomes of the microbial communities which are complete in nature or contaminated with other species sequences through interpreting genomic characteristics, GC content, and alignment with a reference genome	http://ecogenomics.github.io/CheckM/	Parks et al. (2015)
Gramene	Resource to unite the pathway data as well as comparative genomics used for plant research	This particular resource can be applied for studying comparative functional genomics in plant species which are produced in large quantities and model crops	http://www.gramene.org/	Tello-Ruiz et al. (2018)
Plant Reactome	Database which houses information related to plant pathway which is manually curated and open access	It can be applied to study the pathway, regulation of transcription process, pathways that are involved in development and metabolism which also includes interaction between microbes	http://plantreactome.gramene.org/	Naithani et al. (2016)
Unipept	Interface which is command line	It can be applied for studying the	http://unipept.ugent.be/clidocs	Mesuere et al. (2018)

(continued)

Table 13.2 (continued)

(a)

Software/ Resource	Function	Application to study sustainable agrobiology	Website link	Reference
	based can be used for high-throughput analysis with respect to meta proteomics data	shotgun metagenomics data which is assigned to taxon. The results are visualized in the form of frequency table related to taxonomy		
SINA	Precise analysis of ribosomal RNA genes using high-throughput multiple sequence alignment	Important tool to study the quality of the sequenced rRNA from bacteria communities and also to interpret the alignment between them. This can aid in studying the relationship between microbial species in consortia	http://www.arb-silva.de/aligner	Pruesse et al. (2012)
Nonpareil 3	Fast assessment of metagenomic coverage and sequence diversity	This is can be applied for studying metagenomic data coverage in the microbiome communities for sustainable agrobiology	http://enve-omics.ce.gatech.edu/nonpareil	Rodriguez-R et al. (2018)
VSEARCH	A multipurpose tool which is used for studying metagenomics	It can be applied for various metagenomics data analysis since it performs optimal global alignment for shorter reads	https://github.com/torognes/vsearch	Rognes et al. (2016)

(b)

Company	Principle	Platforms	Application	Reference
Pacific Biosciences	Real-time DNA. Single-molecule sequencing by synthesis	PacBio RS	All these platforms can be used for sequencing plant and microbiome genomes for studying interactions between them. Also, to study the genes that are responsible for sustainable agrobiology	Knief (2014)
Life Technologies	Semiconductor-based sequencing by synthesis	Ion proton Ion PGM		
Illumina				

(continued)

Table 13.2 (continued)

(b)				
Company	Principle	Platforms	Application	Reference
	Reversible terminator sequencing by synthesis	Illumina HiSeq2500 Illumina HiSeq X ten Illumina MiSeq		
Roche	Pyrosequencing	454 GS junior titanium 454 FLX titanium 454 FLX+		
Life Technologies	Sequencing by ligation	SOLiD 5500xl W		
Cross-kingdom RNA interference (ck-RNAi)	sRNAs & mRNAs sequencing, alignment, and validation	Illumina		Zanini et al. (2018)

13.13 Future Perspectives

Despite a few demerits in the use of microbial consortia and omics technologies in sustainable agriculture, with flourishing developments, high-quality genomes are now available for diverse species. The re-sequencing of several species' genome-wide allows easy detection of core, pan, and accessory genomes which inflates our current knowledge on crop improvement. Omics studies nowadays are not just limited to genomics and proteomics, but also epigenomics, epi-transcriptomics, secretomics, and agrigenomics. Since omics data is now being generated under various environmental conditions, it is now reasonable to generate omics data for any species at affordable prices. Omics-based knowledge hastens the cloning of QTL (Quantitative trait loci) and enhances the resolution of the mapping to the gene level (Li and Yan 2020). Currently, in the era of massive omics data, an amalgamation of various omics approaches and data will facilitate high-throughput and swift identification of various genes simultaneously, essentially altering the present research paradigm from analysis of single-gene to network or pathway scrutiny. In the future, as part of maintaining and balancing sustainability in agriculture, omics data can be used in combination with novel gene-editing techniques to create and produce crops via a three-step road map (Fernie and Yan 2019), contributing to a revolutionary farming system, with least adverse effects on humans and animals. Thus, the knowledge attained through omics data and utilization of synthetically designed microbial consortia paves the way for similar such studies in the future, creating a balanced and augmented sustainable agriculture.

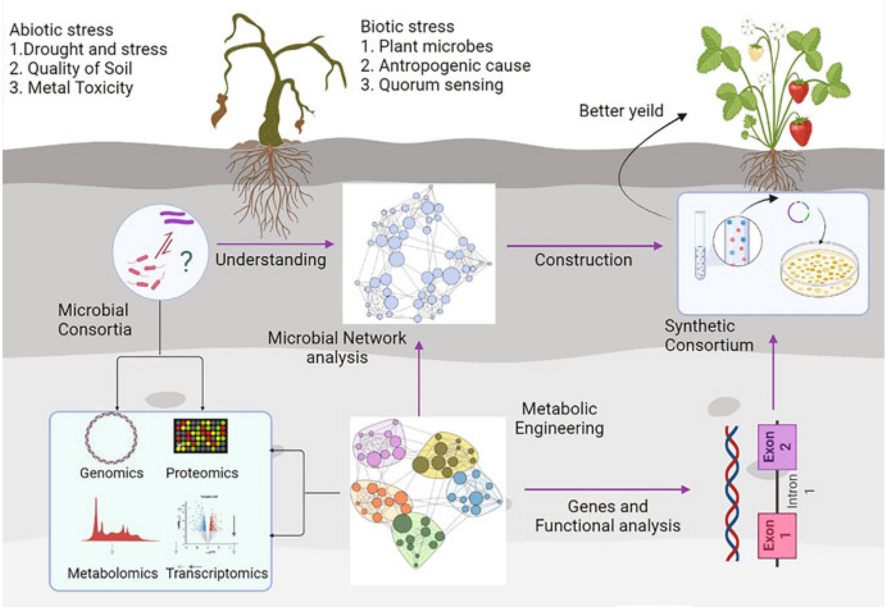


Fig. 13.4 Illustration depicting overview of bioinformatics and data sciences in developing novel consortia for enhancing plant productivity. Plants undergo several biotic (plant microbes, anthropogenic causes, quorum sensing) and abiotic (drought, salinity, metal toxicity) stresses. Thus, in order to enhance the yield, a microbial consortium can be constructed via metabolic engineering techniques after a thorough comprehension of genomics, proteomics, metabolomics, and transcriptomics approaches. Microbial network analysis can provide an understanding of the constructed synthetic consortium that can lead to better yield and productivity of the crops

13.14 Concluding Remarks

Research on soil microbiome is fixated on leveraging comprehension of the microbiome to augment management strategies for attaining and improving sustainability in agrobiology. This is primarily achieved by amending the soil ecosystem by adding specific microorganisms in various combinations as consortia to promote the growth of the plant, to suppress the diseases of plants, to improve the fertility of the soil, and to ameliorate the bioremediation of heavy metals in contaminated soils, due to the many disadvantages that chronic use of chemical fertilizers and pesticides pose. Therefore, for this purpose, the current chapter focused more on understanding various interactions in the soil microbiome such as plant–microbiome, root–root interactions, and microbe–microbe interactions, and different tools and techniques used for formulating microbial consortia. Studies have suggested that a microbial consortium designed and formulated after prior research into the strains performs better in augmenting sustainability than single microbial strains. Furthermore, with the advent of modern technology such as next-generation sequencing techniques, concepts such as genomics, metagenomics, proteomics, metabolomics, and transcriptomics have now paved the way for finding long-term solutions to the problems in agriculture sustainability. "Omics" approaches have high relevance and scope as high-throughput strategies aid in storing and analyzing a huge amount of agricultural data. Additionally, integrative "omics" strategies have also proven to be much better in formulating better microbial consortia, which reduces any adverse effects on humans. Thus, the strategies suggested in the current chapter aim to augment sustainable and balanced agriculture.

Acknowledgments The authors would like to thank Mr. Akshay Uttarkar, RV College of Engineering, Bangalore, for his support during the preparation of the figures.

Conflict of Interest The authors have declared no conflict of interest.

References

Abdelfattah A, Malacrino A, Wisniewski M, Cacciola SO, Schena L (2018) Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. Biol Control 120:1–10

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1):1

Alewu B, Nosiri C (2011) Pesticides and human health. Pesticides in the Modern World–Effects of Pesticides Exposure InTech, 231–250

Alter S, Bader KC, Spannagl M, Wang Y, Bauer E, Schön CC, Mayer KF (2015) DroughtDB: an expert-curated compilation of plant drought stress genes and their homologs in nine species. Database:2015

Andrighetti T, Bohar B, Lemke N, Sudhakar P, Korcsmaros T (2020) MicrobioLink: an integrated computational pipeline to infer functional effects of microbiome–host interactions. Cell 9(5): 1278

Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8(1):1–10

Avio L, Sbrana C, Giovannetti M, Frassinetti S (2017) Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties. Sci Hortic 224: 265–271

Bakker P (2013) a HM, Berendsen, RL, Doornbos, RF, Wintermans, PC a and Pieterse. CMJ:165

Bakshi A, Moin M, Madhav MS (2020) Metagenomics in agriculture: state-of-the-art. In: Metagenomics: techniques, applications, challenges and opportunities. Springer, Singapore, pp 167–187

Baldini F, Heinken A, Heirendt L, Magnusdottir S, Fleming RM, Thiele I (2019) The microbiome modeling toolbox: from microbial interactions to personalized microbial communities. Bioinformatics 35(13):2332–2334

Bandyopadhyay P, Bhuyan SK, Yadava PK, Varma A, Tuteja N (2017) Emergence of plant and rhizospheric microbiota as stable interactomes. Protoplasma 254(2):617–626

Barh D, Zambare V, Azevedo V (2013) Omics: applications in biomedical, agricultural, and environmental sciences. CRC Press

Barriuso J (2015) Quorum sensing mechanisms in fungi. Aims Microbiol 1(1):37–47

Battini F, Grønlund M, Agnolucci M, Giovannetti M, Jakobsen I (2017) Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. Sci Rep 7(1):1–11

Bauer E, Zimmermann J, Baldini F, Thiele I, Kaleta C (2017) BacArena: individual-based metabolic modeling of heterogeneous microbes in complex communities. PLoS Comput Biol 13(5): e1005544

Bennett JW, Hung R, Lee S, Padhi S (2012) 18 fungal and bacterial volatile organic compounds: an overview and their role as ecological signaling agents. Fungal Assoc:373–393

Berendse F (1982) Competition between plant populations with different rooting depths. Oecologia 53(1):50–55

Bertola M, Ferrarini A, Visioli G (2021) Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by-omics approaches: a perspective for the environment, food quality and human safety. Microorganisms 9(7):1400

Bhatia SK, Yi DH, Kim YH, Kim HJ, Seo HM, Lee JH et al (2015) Development of semi-synthetic microbial consortia of Streptomyces coelicolor for increased production of biodiesel (fatty acid methyl esters). Fuel 159:189–196

Boudh S, Singh JS (2019) Pesticide contamination: environmental problems and remediation strategies. In Emerging and eco-friendly approaches for waste management. Springer, Singapore, pp 245–269

Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383(1): 3–41

Carbonetto B, Rascovan N, Álvarez R, Mentaberry A, Vázquez MP (2014) Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in Argentine Pampas. PLoS One 9(6):e99949

Cederlund H, Wessén E, Enwall K, Jones CM, Juhanson J, Pell M et al (2014) Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. Appl Soil Ecol 84:62–68

Chan SHJ, Simons MN, Maranas CD (2017) SteadyCom: predicting microbial abundances while ensuring community stability. PLoS Comput Biol 13(5):e1005539

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48(5):489–499

Chapman DJ (1973) The algae. Springer

Che S, Men Y (2019) Synthetic microbial consortia for biosynthesis and biodegradation: promises and challenges. J Ind Microbiol Biotechnol 46(9-10):1343–1358

Chien CH, Chow CN, Wu NY, Chiang-Hsieh YF, Hou PF, Chang WC (2015) EXPath: a database of comparative expression analysis inferring metabolic pathways for plants. BMC Genomics 16(2):1–10

Chiquoine LP, Abella SR, Bowker MA (2016) Rapidly restoring biological soil crusts and ecosystem functions in a severely disturbed desert ecosystem. Ecol Appl 26(4):1260–1272

Chourasiya S, Khillare PS, Jyethi DS (2015) Health risk assessment of organochlorine pesticide exposure through dietary intake of vegetables grown in the periurban sites of Delhi, India. Environ Sci Pollut Res 22(8):5793–5806

Christoffels A, Gelder AV, Greyling G, Miller R, Hide T, Hide W (2001) STACK: sequence tag alignment and consensus knowledgebase. Nucleic Acids Res 29(1):234–238

Clarholm M, Bonkowski M, Griffiths B, Van Elsas JD, Jansson JK, Trevors JT (2007) Protozoa and other protista in soil. Modern soil microbiology. Marcel Dekker, Amsterdam, pp 147–175

Cordero OX, Polz MF (2014) Explaining microbial genomic diversity in light of evolutionary ecology. Nat Rev Microbiol 12(4):263–273

Cummings SP (2009) The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. Environ Biotechnol 5: 43–50

D'Agostino N, Aversano M, Chiusano ML (2005) ParPEST: a pipeline for EST data analysis based on parallel computing. BMC Bioinformatics 6(4):1–9

de los Santos-Villalobos S, Díaz-Rodríguez AM, Ávila-Mascareño MF, Martínez-Vidales AD, Parra-Cota FI (2021) COLMENA: a culture collection of native microorganisms for harnessing the agro-biotechnological potential in soils and contributing to food security. Diversity 13(8): 337

De Sousa CS, Hassan SS, Pinto AC, Silva WM, De Almeida SS, Soares SDC et al (2018) Microbial omics: applications in biotechnology. In Omics technologies and bio-engineering. Academic Press, pp 3–20

De Vleesschauwer D, Höfte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51: 223–281

Dey G, Banerjee P, Sharma RK, Maity JP, Etesami H, Shaw AK et al (2021) Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria—A review. Agronomy 11(8):1552

Dukovski I, Bajić D, Chacón JM, Quintin M, Vila JC, Sulheim S, et al. (2020) Computation of microbial ecosystems in time and space (COMETS): an open source collaborative platform for modeling ecosystems metabolism. arXiv preprint arXiv:2009.01734

Esposito A, Colantuono C, Ruggieri V, Chiusano ML (2016) Bioinformatics for agriculture in the next-generation sequencing era. Chem Biol Technol Agric 3(1):1–12

Eze CN, Odoh CK, Eze EA, Orjiakor PI, Enemuor SC, Okobo UJ (2018) Chromium (III) and its effects on soil microbial activities and phytoremediation potentials of Arachis hypogea and Vigna unguiculata. Afr J Biotechnol 17(38):1207–1214

Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. Science 320(5879):1034–1039

Fang X, Lloyd CJ, Palsson BO (2020) Reconstructing organisms in silico: genome-scale models and their emerging applications. Nat Rev Microbiol 18(12):731–743

Faoro H, Alves AC, Souza EM, Rigo LU, Cruz LM, Al-Janabi SM et al (2010) Influence of soil characteristics on the diversity of bacteria in the southern Brazilian Atlantic Forest. Appl Environ Microbiol 76(14):4744–4749

Fernie AR, Yan J (2019) De novo domestication: an alternative route toward new crops for the future. Mol Plant 12(5):615–631

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15(10):579–590

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci 103(3):626–631

Finkel OM, Castrillo G, Paredes SH, González IS, Dangl JL (2017) Understanding and exploiting plant beneficial microbes. Curr Opin Plant Biol 38:155–163

Fleitas Martínez O, Rigueiras PO, Pires ADS, Porto WF, Silva ON, de la Fuente-Nunez C, Franco OL (2019) Interference with quorum-sensing signal biosynthesis as a promising therapeutic strategy against multidrug-resistant pathogens. Front Cell Infect Microbiol 8:444

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. For Ecol Manag 196(1):159–171

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176(1):22–36

García-Jiménez B, García JL, Nogales J (2018) FLYCOP: metabolic modeling-based analysis and engineering microbial communities. Bioinformatics 34(17):i954–i963

Gehlot P, Pareek N, Vivekanand V (2021) Development of biofertilizers and microbial consortium an approach to sustainable agriculture practices. In Plant, soil and microbes in tropical ecosystems. Springer, Singapore, pp 315–348

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol 69(3):1800–1809

Gupta N, Vats S, Bhargava P (2018) Sustainable agriculture: role of metagenomics and metabolomics in exploring the soil microbiota. In silico approach for sustainable agriculture. Springer, Singapore, pp 183–199

Hassani MA, Durán P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6(1):1–17

Heinken A, Basile A, Thiele I (2021) Advances in constraint-based modelling of microbial communities. Curr Opin Syst Biol 27:100346

Herrera Paredes S, Gao T, Law TF, Finkel OM, Mucyn T, Teixeira PJPL et al (2018) Design of synthetic bacterial communities for predictable plant phenotypes. PLoS Biol 16(2):e2003962

Hozzein WN (ed) (2020) Metagenomics: basics, methods and applications. BoD–Books on Demand

Htwe AZ, Moh SM, Soe KM, Moe K, Yamakawa T (2019) Effects of biofertilizer produced from Bradyrhizobium and Streptomyces griseoflavus on plant growth, nodulation, nitrogen fixation, nutrient uptake, and seed yield of mung bean, cowpea, and soybean. Agronomy 9(2):77

Hung R, Lee S, Bennett JW (2015) Fungal volatile organic compounds and their role in ecosystems. Appl Microbiol Biotechnol 99(8):3395–3405

Iovene M, Barone A, Frusciante L, Monti L, Carputo D (2004) Selection for aneuploid potato hybrids combining a low wild genome content and resistance traits from Solanum commersonii. Theor Appl Genet 109(6):1139–1146

Islam W, Saqib HSA, Adnan M, Wang Z, Tayyab M, Huang Z, Chen HY (2021) Differential response of soil microbial and animal communities along the chronosequence of Cunninghamia lanceolata at different soil depth levels in subtropical forest ecosystem. J Adv Res 38:41–54

Jain A, Singh S, Kumar Sarma B, Bahadur Singh H (2012) Microbial consortium–mediated reprogramming of defence network in pea to enhance tolerance against sclerotinia sclerotiorum. J Appl Microbiol 112(3):537–550

Jambon I, Thijs S, Weyens N, Vangronsveld J (2018) Harnessing plant-bacteria-fungi interactions to improve plant growth and degradation of organic pollutants. J Plant Interact 13(1):119–130

Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18(1): 35–46

Jia X, Liu C, Song H, Ding M, Du J, Ma Q, Yuan Y (2016) Design, analysis and application of synthetic microbial consortia. Synth Syst Biotechnol 1(2):109–117

Jiang Q, Chen J, Yang C, Yin Y, Yao K (2019) Quorum sensing: a prospective therapeutic target for bacterial diseases. BioMed Res Int 2019:2015978

Jones P, Garcia BJ, Furches A, Tuskan GA, Jacobson D (2019) Plant host-associated mechanisms for microbial selection. Front Plant Sci 10:862

Kirankumar R, Jagadeesh KS, Krishnaraj PU, Patil MS (2010) Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tobacco mosaic virus pathogen. Karnataka J Agric Sci 21(2)

Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front Plant Sci 5:216

Koch S, Kohrs F, Lahmann P, Bissinger T, Wendschuh S, Benndorf D et al (2019) RedCom: a strategy for reduced metabolic modeling of complex microbial communities and its application for analyzing experimental datasets from anaerobic digestion. PLoS Comput Biol 15(2): e1006759

Kour D, Rana KL, Yadav N, Yadav AN, Kumar A, Meena VS et al (2019) Rhizospheric microbiomes: biodiversity, mechanisms of plant growth promotion, and biotechnological applications for sustainable agriculture. In Plant growth promoting rhizobacteria for agricultural sustainability. Springer, Singapore, pp 19–65

Kumar SA, Kumari PH, Sundararajan VS, Suravajhala P, Kanagasabai R, Kishor PK (2014) PSPDB: plant stress protein database. Plant Mol Biol Report 32(4):940–942

Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol 166(2):689–700

Lavecchia A, Curci M, Jangid K, Whitman WB, Ricciuti P, Pascazio S, Crecchio C (2015) Microbial 16S gene-based composition of a sorghum cropped rhizosphere soil under different fertilization managements. Biol Fertil Soils 51(6):661–672

Levy R, Carr R, Kreimer A, Freilich S, Borenstein E (2015) NetCooperate: a network-based tool for inferring host-microbe and microbe-microbe cooperation. BMC Bioinformatics 16(1):1–6

Li, Q., and Yan, J. (2020). Sustainable agriculture in the era of omics: knowledge-driven crop breeding

Li JR, Liu CC, Sun CH, Chen YT (2018) Plant stress RNA-seq nexus: a stress-specific transcriptome database in plant cells. BMC Genomics 19(1):1–8

Machado D, Andrejev S, Tramontano M, Patil KR (2018) Fast automated reconstruction of genome-scale metabolic models for microbial species and communities. Nucleic Acids Res 46(15):7542–7553

Majewski M, Capel P (1995) Pesticides in the atmosphere: distribution, tends, and governing factors. Volume one, pesticides in the hydrological system. Ann Arbor Press Ins 118:107–124

Marchand N, Collins CH (2013) Peptide-based communication system enables Escherichia coli to Bacillus megaterium interspecies signaling. Biotechnol Bioeng 110(11):3003–3012

Meena VS, Meena SK, Verma JP, Kumar A, Aeron A, Mishra PK et al (2017) Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. Ecol Eng 107:8–32

Men Y, Feil H, VerBerkmoes NC, Shah MB, Johnson DR, Lee PK et al (2012) Sustainable syntrophic growth of Dehalococcoides ethenogenes strain 195 with Desulfovibrio vulgaris Hildenborough and Methanobacterium congolense: global transcriptomic and proteomic analyses. ISME J 6(2):410–421

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37(5):634–663

Mendes LW, Kuramae EE, Navarrete AA, Van Veen JA, Tsai SM (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 8(8):1577–1587

Mendes-Soares H, Mundy M, Soares LM, Chia N (2016) MMinte: an application for predicting metabolic interactions among the microbial species in a community. BMC Bioinformatics 17(1):1–10

Mesuere B, Van der Jeugt F, Willems T, Naessens T, Devreese B, Martens L, Dawyndt P (2018) High-throughput metaproteomics data analysis with Unipept: a tutorial. J Proteome 171:11–22

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M et al (2008) The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9(1):1–8

Mikheenko A, Saveliev V, Gurevich A (2016) MetaQUAST: evaluation of metagenome assemblies. Bioinformatics 32(7):1088–1090

Mita K, Morimyo M, Okano K, Koike Y, Nohata J, Kawasaki H et al (2003) The construction of an EST database for Bombyx mori and its application. Proc Natl Acad Sci 100(24):14121–14126

Mommer L, Hinsinger P, Prigent-Combaret C, Visser EJ (2016) Advances in the rhizosphere: stretching the interface of life

Montazeaud G, Violle C, Fréville H, Luquet D, Ahmadi N, Courtois B et al (2018) Crop mixtures: does niche complementarity hold for belowground resources? An experimental test using rice genotypic pairs. Plant Soil 424(1):187–202

Mousavi SA, Pouya FM, Ghaffari MR, Mirzaei M, Ghaffari A, Alikhani M et al (2016) PlantPReS: a database for plant proteome response to stress. J Proteome 143:69–72

Mueller KE, Hobbie SE, Tilman D, Reich PB (2013) Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. Glob Chang Biol 19(4):1249–1261

Mukherjee S, Bassler BL (2019) Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol 17(6):371–382

Murali A, Bhargava A, Wright ES (2018) IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. Microbiome 6(1):1–14

Nag SK, Raikwar MK (2011) Persistent organochlorine pesticide residues in animal feed. Environ Monit Assess 174(1):327–335

Naika M, Shameer K, Mathew OK, Gowda R, Sowdhamini R (2013) STIFDB2: an updated version of plant stress-responsive transcription factor database with additional stress signals, stress-responsive transcription factor binding sites and stress-responsive genes in Arabidopsis and rice. Plant Cell Physiol 54(2):e8–e8

Naithani S, Preece J, D'Eustachio P, Gupta P, Amarasinghe V, Dharmawardhana PD et al (2016) Plant Reactome: a resource for plant pathways and comparative analysis. Nucleic Acids Res: gkw932

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF et al (2013) Patterns and processes of microbial community assembly. Microbiol Mol Biol Rev 77(3):342–356

Nuti M, Giovannetti G (2015) Borderline products between bio-fertilizers/bio-effectors and plant protectants: the role of microbial consortia. J Agric Sci Technol A 5:305–315

Oliverio AM, Bradford MA, Fierer N (2017) Identifying the microbial taxa that consistently respond to soil warming across time and space. Glob Chang Biol 23(5):2117–2129

Orellana LH, Rodriguez-R LM, Konstantinidis KT (2017) ROCker: accurate detection and quantification of target genes in short-read metagenomic data sets by modeling sliding-window bitscores. Nucleic Acids Res 45(3):e14–e14

Oulas A, Pavloudi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas G et al (2015) Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinformatics Biol Insights 9:BBI-S12462

Ounit R, Wanamaker S, Close TJ, Lonardi S (2015) CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics 16(1):1–13

Pandit AA, Shah RA, Husaini AM (2018) Transcriptomics: A time-efficient tool with wide applications in crop and animal biotechnology. J Pharmac Phytochem 7:1701–1704

Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25(7):1043–1055

Patel JS, Kharwar RN, Singh HB, Upadhyay RS, Sarma BK (2017) Trichoderma asperellum (T42) and Pseudomonas fluorescens (OKC)-enhances resistance of pea against Erysiphe pisi through enhanced ROS generation and lignifications. Front Microbiol 8:306

Pathak D, Lone R, Koul KK (2017) Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) Association in Potato (Solanum tuberosum L.): a brief review. Probiotics Plant Health:401–420

Perez E, Sulbaran M, Ball MM, Yarzabal LA (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem 39(11):2905–2914

Pérez-Montaño F, Jiménez-Guerrero I, Sánchez-Matamoros RC, López-Baena FJ, Ollero FJ, Rodríguez-Carvajal MA et al (2013) Rice and bean AHL-mimic quorum-sensing signals specifically interfere with the capacity to form biofilms by plant-associated bacteria. Res Microbiol 164(7):749–760

Pett-Ridge J, Firestone MK (2005) Redox fluctuation structures microbial communities in a wet tropical soil. Appl Environ Microbiol 71(11):6998–7007

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11(11):789–799

Popp D, Centler F (2020) μbialSim: constraint-based dynamic simulation of complex microbiomes. Front Bioeng Biotechnol 8:574

Prabha R, Ghosh I, Singh DP (2011) Plant stress gene database: a collection of plant genes responding to stress condition. ARPN J Sci Technol 1:28–31

Prasain J (ed) (2016) Metabolomics: fundamentals and applications. BoD–Books on Demand

Priya P, Jain M (2013) RiceSRTFDB: a database of rice transcription factors containing comprehensive expression, cis-regulatory element and mutant information to facilitate gene function analysis. Database 2013:bat027

Pruesse E, Peplies J, Glöckner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28(14):1823–1829

Rasheed T, Bilal M, Nabeel F, Adeel M, Iqbal HM (2019) Environmentally-related contaminants of high concern: potential sources and analytical modalities for detection, quantification, and treatment. Environ Int 122:52–66

Riaño-Pachón DM, Ruzicic S, Dreyer I, Mueller-Roeber B (2007) PlnTFDB: an integrative plant transcription factor database. BMC Bioinformatics 8(1):1–10

Rodriguez-R LM, Gunturu S, Tiedje JM, Cole JR, Konstantinidis KT (2018) Nonpareil 3: fast estimation of metagenomic coverage and sequence diversity. MSystems 3(3):e00039–e00018

Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584

Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR et al (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol 66(6):2541–2547

Rotaru AE, Shrestha PM, Liu F, Shrestha M, Shrestha D, Embree M et al (2014) A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. Energy Environ Sci 7(1):408–415

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4(10):1340–1351

Rudresh DL, Shivaprakash MK, Prasad RD (2005) Effect of combined application of rhizobium, phosphate solubilizing bacterium and Trichoderma spp. on growth, nutrient uptake and yield of chickpea (Cicer aritenium L.). Appl Soil Ecol 28(2):139–146

Saleh IA, Zouari N, Al-Ghouti MA (2020) Removal of pesticides from water and wastewater: chemical, physical and biological treatment approaches. Environ Technol Innov 19:101026

Salekdeh GH, Komatsu S (2007) Crop proteomics: aim at sustainable agriculture of tomorrow. Proteomics 7(16):2976–2996

Savci S (2012) Investigation of effect of chemical fertilizers on environment. Apcbee Procedia 1: 287–292

Schloss PD, Handelsman J (2006) Toward a census of bacteria in soil. PLoS Comput Biol 2(7):e92

Schreiter S, Sandmann M, Smalla K, Grosch R (2014) Soil type dependent rhizosphere competence and biocontrol of two bacterial inoculant strains and their effects on the rhizosphere microbial community of field-grown lettuce. PLoS One 9(8):e103726

Selvakumar G, Panneerselvam P, Ganeshamurthy AN (2012) Bacterial mediated alleviation of abiotic stress in crops. In Bacteria in agrobiology: stress management. Springer, Berlin, Heidelberg, pp 205–224

Semagn K, Bjørnstad Å, Xu Y (2010) The genetic dissection of quantitative traits in crops. Electron J Biotechnol 13(5):16–17

Shameer K, Ambika S, Varghese SM, Karaba N, Udayakumar M, Sowdhamini R (2009) STIFDB—Arabidopsis stress responsive transcription factor dataBase. Int J Plant Genomics 2009:583429

Sharma M, Dangi P, Choudhary M (2014) Actinomycetes: source, identification, and their applications. Int J Curr Microbiol App Sci 3(2):801–832

Shou W, Ram S, Vilar JM (2007) Synthetic cooperation in engineered yeast populations. Proc Natl Acad Sci 104(6):1877–1882

Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB (2013) Rhizosphere microbes facilitate redox homeostasis in Cicer arietinum against biotic stress. Ann Appl Biol 163(1):33–46

Song H, Ding MZ, Jia XQ, Ma Q, Yuan YJ (2014) Synthetic microbial consortia: from systematic analysis to construction and applications. Chem Soc Rev 43(20):6954–6981

Sul WJ, Asuming-Brempong S, Wang Q, Tourlousse DM, Penton CR, Deng Y et al (2013) Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon. Soil Biol Biochem 65:33–38

Tello-Ruiz MK, Naithani S, Stein JC, Gupta P, Campbell M, Olson A et al (2018) Gramene 2018: unifying comparative genomics and pathway resources for plant research. Nucleic Acids Res 46 (D1):D1181–D1189

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kännaste A, Behers L et al (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9(5):e96086

Torres N, Goicoechea N, Morales F, Antolín MC (2016) Berry quality and antioxidant properties in Vitis vinifera cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. Crop and pasture. Science 67(9):961–977

Upadhyay AK, Sowdhamini R, Patil VU (eds) (2021) Bioinformatics for agriculture: high-throughput approaches. Springer Nature

Valdez-Vazquez I, Pérez-Rangel M, Tapia A, Buitrón G, Molina C, Hernández G, Amaya-Delgado L (2015) Hydrogen and butanol production from native wheat straw by synthetic microbial consortia integrated by species of enterococcus and clostridium. Fuel 159:214–222

Van Emon JM (2016) The omics revolution in agricultural research. J Agric Food Chem 64(1): 36–44

Venturi V, Keel C (2016) Signaling in the rhizosphere. Trends Plant Sci 21(3):187–198

Verma D, Kawarabayasi Y, Miyazaki K, Satyanarayana T (2013) Cloning, expression and characteristics of a novel alkalistable and thermostable xylanase encoding gene (Mxyl) retrieved from compost-soil metagenome. PLoS One 8(1):e52459

Vishwakarma K, Kumar N, Shandilya C, Mohapatra S, Bhayana S, Varma A (2020) Revisiting plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture: a review. Front Microbiol 11:3195

Vorholt JA, Vogel C, Carlström CI, Müller DB (2017) Establishing causality: opportunities of synthetic communities for plant microbiome research. Cell Host Microbe 22(2):142–155

Waksman SA (1927) Principles of soil microbiology, 12-13, Baltimore. Williams and Wilkins Co. Md, London

Warembourg FR (1993) Nitrogen fixation in soil and plant systems. Nitrogen isotope techniques, 127–156

Witczak A, Abdel-Gawad H (2014) Assessment of health risk from organochlorine pesticides residues in high-fat spreadable foods produced in Poland. J Environ Sci Health B 49(12): 917–928

Wood J, Liu W, Tang C, Franks A (2021) Microorganisms in heavy metal bioremediation: strategies for applying microbial-community engineering to remediate soils

Zakrzewski M, Proietti C, Ellis JJ, Hasan S, Brion MJ, Berger B, Krause L (2017) Calypso: a user-friendly web-server for mining and visualizing microbiome–environment interactions. Bioinformatics 33(5):782–783

Zancarini A, Westerhuis JA, Smilde AK, Bouwmeester HJ (2021) Integration of omics data to unravel root microbiome recruitment. Curr Opin Biotechnol 70:255–261

Zanini S, Šečić E, Jelonek L, Kogel KH (2018) A bioinformatics pipeline for the analysis and target prediction of rna effectors in bidirectional communication during plant–microbe interactions. Front Plant Sci 9:1212

Zhang Z, Yu J, Li D, Zhang Z, Liu F, Zhou X et al (2010) PMRD: plant microRNA database. Nucleic Acids Res 38(suppl_1):D806–D813

Zhang S, Yue Y, Sheng L, Wu Y, Fan G, Li A et al (2013) PASmiR: a literature-curated database for miRNA molecular regulation in plant response to abiotic stress. BMC Plant Biol 13(1):1–8

Zhang N, Wang D, Liu Y, Li S, Shen Q, Zhang R (2014a) Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. Plant Soil 374(1):689–700

Zhang Z, Ober U, Erbe M, Zhang H, Gao N, He J et al (2014b) Improving the accuracy of whole genome prediction for complex traits using the results of genome wide association studies. PLoS One 9(3):e93017

Zheng W, Zeng S, Bais H, LaManna JM, Hussey DS, Jacobson DL, Jin Y (2018) Plant growth-promoting rhizobacteria (PGPR) reduce evaporation and increase soil water retention. Water Resour Res 54(5):3673–3687

Zhou J, Ma Q, Yi H, Wang L, Song H, Yuan YJ (2011) Metabolome profiling reveals metabolic cooperation between bacillus megaterium and Ketogulonicigenium vulgare during induced swarm motility. Appl Environ Microbiol 77(19):7023–7030

Zomorrodi AR, Maranas CD (2012) OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities. PLoS Comput Biol 8(2):e1002363

Part IV
Biofertilizer, Biocontrol Agents, and Crop Growth

Chapter 14
Effect of Microbial Consortium Vs. Perfected Chemical Fertilizers for Sustainable Crop Growth

Dinesh Kumar Maheshwari, Sandeep Kumar, Prashant Kumar, Sachin Kumar, and Shrivardhan Dheeman

Abstract Recruitment of microbial consortia having plant growth and health-promoting traits, instead of individual strains is an approach to raise the growth and development of the crop throughout the duration. The application of microbial consortia is proven to be as good as pure chemical fertilizers (e.g., urea, diammonium phosphate (DAP), muriate of potash (MoP), etc.) for raising the crop under abiotic stress conditions of soil salinity. Plant-associated microbial consortia mainly involved various genera and species of plant growth-promoting bacteria that exert beneficial effects on plants, it is constructed by two or more interacting beneficial bacteria synergistically or by additive, and mean and results are described. The multiple genera, both indigenous and nonindigenous, can perform various tasks in the stress ecosystems.

To understand overall performance, their interactions and beneficial effects are highlighted both on soil and plants. Finally, it is aimed to propose the use of different microbial consortia as well as strengthening research on different mixtures of microorganisms that facilitate adequate plant growth and yield. The overall success of beneficial bacterial consortium offers a safety device that reduces the excessive application of chemical fertilizers and could avoid economic loss by reducing the cost of agricultural practices.

Keywords Rhizobacteria · Fertilizers · Biofertilizers · Microbial Consortia · Crop production

---

D. K. Maheshwari (✉) · S. Kumar · P. Kumar · S. Kumar
Department of Botany and Microbiology, Faculty of Life Sciences, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India

S. Dheeman
Department of Biotechnology and Microbiology, School of Allied Health Sciences, MVN University, Palwal, Haryana, India

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_14

14.1 Introduction

Among 2,50,000–3,00,000 plant species, about 4% are used as edible plants by human beings. Just three crops (wheat, maize, and rice) contribute 60% of the calories and proteins required by humans from these crops. This leads to severe food security issues and causes food crises. As human populations continue to increase, the disturbance of the soil ecosystem to enhance productivity may place greater demand on supplying soil's essential nutrients. Therefore, it is essential to increase the understanding of soil's biological, physical, and chemical properties along with the soil–microbe–plant relationship to enhance productivity with available nutrient pools. The soil-native ability to supply sufficient nutrients continuously decreases and emerges as greater challenges for enhancing the productivity of crops and the quality of water, air, and fragile soil ecosystems. This relationship of soil–plant microbes, especially soil interaction, influences plant compounds accurately identifying the yield-limiting potential factors and growth and development, minimizing the influence of those to manage the enhancement productivity.

Ecologically, abiotic factors are the major causes behind lowering crop productivity. However, the adverse effects of biotic factors on declining productivity cannot be ruled out. Among abiotic constraints, the major one is soil salinity, which significantly reduces crop productivity (Zorb et al. 2019). The issue is the conversion of agronomically useful farmer's field soil into unproductive land. The United Nations Environment Program estimated that approximately 20% of agricultural land and 50% of cropland around the world is salt stressed. It is estimated that >1 billion hectares of land is salinized (Soltabayeva et al. 2021) and is a major factor limiting plant growth and productivity (Parida and Das 2005).

Salinity has majorly affected agricultural productivity due to the inhibition of photosynthesis, protein synthesis, and lipid metabolism in plants. About 1.5 million hectares of land are turning unfit for agricultural practices every year (Hossain 2019). Anthropogenic activities and excessive use of chemical fertilizers such as urea, diammonium sulfate, muriate of potash (MoP), gypsum, etc., accelerate the rate of salinization in arid and semi-arid regions. The rate of high salinization is due to poor agricultural practices, saline water irrigation, reduced precipitation and enhanced surface evaporation (due to climate change), and industrial pollution. In terms of economics, the annual cost of salt-induced land degradation in irrigated areas could be US$27.3 billion in terms of loss in crop production. Saline soil is clearly defined by Mishra et al. (2021), who stated if electrical conductivity (EC) is less than 4ds/m at 25 °C (pH 8.2–10.5), accumulation of mineral salts such as NaCl Na_2SO_4, $MgSO_4$, $NaHCO_3$, $CaSO_4$, and $CaCO_3$ occur in saline soil. India consumed about 500 Lakh Metric Tons (LMT) of chemical fertilizers per year. Urea is the most consumed fertilizer with around 300 LMT and there is a steady increase in the rate of consumption of urea, DAP, and NPKs in the current year, i.e., highest in the last six-year period from 2015 to 2020–2021 (Bishnoi et al. 2020; Singh 2020).

Plants comprise functional microbiota, each with the other, forming a network of species that is commonly called a "holobiont." These plant-associated microbial communities contain host-adapted microorganisms that impact plant fitness. Since the origin of microbes on earth is more than 3.5 billion years ago and plant lineages are 450 million years ago, the microbial origin suggests that microbe–microbe interactions are important in selective sculpting complex network in the soil, rhizosphere, and phyllosphere including internal tissues of plants. The evolutionary nature of microbe–microbe interaction mechanisms that favor the co-existence of highly diverse microbial consortia in or inside plant habitats is yet to be fully explored. It is a challenging task due to the complex ecological interactions occurring naturally and the different ways and means of their inheritance such as vertical transmission via seeds (Truyens et al. 2015) vs. horizontal requisition from the environment (Bulgarelli et al. 2013).

Most of the workers during the last decades were focused on the use of fertilizers and manures. Soil fertility is the status or the inherent capacity of the soil to supply nutrients to plants in adequate amounts in suitable proportions. On the other hand, soil productivity is the capacity of the soil to produce crops with a specific system of management and is expressed in terms of yields. All productive soils are fertile, but all fertile soils are not necessarily productive. To produce crops of economic value and to maintain the health of the soil without deterioration are most important. Modern farming, driven by economic constraints, is forced to use artificial fertilizers, often to the detriment of the soil's natural fertility (Rana and Rana 2011). Thus, there is a need for an integrated approach to plant nutrition and the sustainability of soil fertility and crop productivity.

Green agriculture is a system of cultivation with the help of an integrated nutrient supply. The integrated pest management (IPM) and integrated management (INM) systems of natural resources do exclude the use of minimum essential quantities of mineral fertilizers and chemical pesticides. Thus, biofertilizers can supplement chemical fertilizers judiciously to supplement sustainable crop production and protection (Maheshwari 2011). Plant growth promotion enhancement is virtually possible by using microbial inoculants along with allowing the concentration of chemical fertilizers and supplying for each farm from Green Revolution to Evergreen Revolution (Kesavan and Swaminathan 2020).

14.2 Chemicals Vs. Biologicals

The use of synthetic chemicals (fertilizers and pesticides) was observed to be the critical input for holding the production of pulses, millets, etc. (Ankineedu et al. 1983). The advanced agriculture practices using fertilizers increased crop productivity, but their improper use disorder made the soil sick. Nearly 2000 ha of land is becoming saline every year due to the imbalanced use of chemical fertilizers and unmanaged cropping systems. It is, therefore, considered that the application and additions of biofertilizers not only help proliferate beneficial microbes in soil but also provide residual effects on the subsequent crop in the field and contribute to the recycling and decomposition of organic matter (Deshwal et al. 2003; Kumar et al. 2006). In an intensive cropping system, supplementing soil nutrients using chemical fertilizer is considered inevitable for obtaining the optimum yield of crops. But it has been observed that continuous use of chemical fertilizers may adversely affect soil health and may lead to a negative impact on soil production (Paul and Savitri 2003).

Adverse consequences have occurred due to the excessive application of chemical fertilizers, pesticides, etc., on soil structure and health. Besides imparting groundwater pollution, and mismanagement of ecology and environment hence, adherences to the scientific principles of soil and plant health management to sustain the benefits of enhanced productivity over a long period are of utmost desirable. Thus, sustaining the benefits of natural resources and progressive enhancement of soil quality, biodiversity, and productivity are a few steps that may lead toward productivity enhancement. This can achieve higher productivity in perpetuity without accompanying ecological harm, water quality, biodiversity, atmosphere, and renewable energy sources (Kesavan and Swaminathan 2006; Maheshwari 2010; Maheshwari and Dheeman 2019; Maheshwari et al. 2015).

In literature, three terminologies are used to convey the same meaning: Integrated Plant Nutrition Systems (IPNS), Integrated Plant Nutrient Supply Systems (IPNS System), and Integrated Nutrient Management (INM). Although these terminologies may look the same, they convey somewhat different connotations, yet. IPNS means the supply of nutrients to the plants from various sources of nutrients such as nutrient reserves in the soil, (1) organic sources include amendment of farm yard manure, compost, green manure, crop residues, and other organic fertilizers, and (2) IPNS is a concept "which aims at the maintenance or adjustment of soil fertility and plant nutrient supply to an optimum level for sustaining the desired crop productivity through optimization of benefit from all possible sources of plant nutrients in an integrated manner" (Roy and Ange 1991).

Mineral Nutrient Management (MNM) is the key issue in sustainable soil fertility. Nitrogen, phosphorous, potassium, etc., are given in the form of chemical fertilizers for a high economic return through optimized crop yield. The basic concept underlying the integrated plant nutrient system is the maintenance and possible increase of soil fertility for sustaining enhanced crop productivity through judicious use of all plant nutrients, particularly inorganic (chemical) fertilizers (Duhoon et al. 2001). The balanced dose of fertilizers alone can increase by about 50% of the yield in rice (Muntasir et al. 2001). Babich and Stotzky (1983) developed the concept of an "Ecological Dose 50" (ED50), as a concentration of a toxicant that inhibits a microbe's ecological process by 50%. The concentration that kills 50% of the total population is termed sub-lethal (LC50). The use of sub-lethal doses of chemicals weakens the pathogen, and it becomes more effective against the parasite (Mukhopadhyay et al. 1992). A judicious combination of chemical fertilizer with an organic source of plant nutrients through composting green manuring or due to the application of suitable microorganisms in the form of biofertilizer of bioinoculants should be optimized for plant growth promotion and yield (Kumar et al. 2006).The combined ever-increasing application of biofertilizers and reduced dose of chemical fertilizers increased wheat yield, obtained from the single application of recommended doses of fertilizers (Stephens and Rask 2000). Saraf and Sood (2002) raised the pesticide-adoptive mutants of rhizobial strains and observed PGP activities to use them as bioinoculants. Reasonable results were achieved from the fertilized field using fertilizers/adaptive indigenous strains (Vargas et al. 2000).

INM seems to be essential not only for the enhancement of crop productivity but also for the maintenance and possibly improvement of soil fertility. The management of C and N requirements through the active intervention of microbial inoculants derive three benefits: (i) reduction in the cost of cultivation, (ii) enhanced use efficiency judiciously in the field, and (iii) improved quality of environment and ecology. Various workers (Idris 2003; Ayala and Rao 2002; Kumar et al. 2006) observed various cropping systems, which exhibited positive interaction of the integrated use of fertilizers with biofertilizers for sustainable crop production. The integration of biological processes in nutrient management practices should be devised to prevent/unavoidable losses of chemical fertilizers. This will not only safeguard the ecological environment due to the impact of excessive fertilizers but also affect the cost–benefit ratio and population, which may ensure food security and sustainability of the environment.

The application of microbial consortia can reduce the negative effects that arise due to abiotic stress conditions on crops. But for their effective application in the crops, novel approaches are required to explore bacteria–bacteria and plant–bacteria interactions or bacteria–fungi interactions. Isolating and identifying the stress-tolerant or resistant microbes to recalcitrant agrochemicals and heavy metals is important, as per the advocacies of recent publications (Xia et al. 2020; Katiyar et al. 2021).

14.2.1 Microbial Consortia in Lowering of Chemical Fertilizers

A microbial consortium interacts with different abiotic and biotic factors that apply under field conditions. Apart from these stresses, its success also influenced the different crop varieties due to the involvement of plant microbes' interaction. The consortium efficiently colonized the rhizosphere, improved plant tolerance to abiotic stresses such as water deficit or drought (Ehteshami et al. 2007; Shahzad et al. 2013), tolerance to salinity (Ahmad et al. 2013a, b), heavy metal tolerance accumulated in soil (Sheng et al. 2012), temperature, and other stresses. Various workers (Idris 2003; Ayala and Rao 2002; Kumar et al. 2006) observed from different cropping systems that exhibited the positive interaction of the integrated use of mineral fertilizers, organic manures, biofertilizers, etc., for maintaining the growth throughout the crop duration.

On the other hand, bacterial consortium acts as a substitute to chemical fertilizers such as urea, DAP, etc., because decreased application of chemical fertilization along with bacterial consortium exhibited a similar effect on plant growth and yield as revealed while applying the recommended doses of chemical fertilizers (Kumar et al. 2010; Da Costa et al. 2013).

In addition, the bacterial consortium offered an alternative allowing efficient use of half of the recommended dose of nitrogen fertilizer. The use of the microbial consortium allowed the lowering of a 50% mineral N application and generated beneficial agronomic practices along with the lower cost to the cultivars (Molina-Romero et al. 2021). Recently, a new approach has been devised wherein the effect of microbial consortia is applied as fertilizer coating. For this, the use of next-generation sequencing (NGS) via Illumina high-throughput sequencing (IHS) technology has been understood that fertilizers influence the bulk soil and rhizosphere microbial community applied to potato fields (Overbeek et al. 2021). This concept may serve as a tool for the development of new microbial inoculants for the benefit and improvement of crop plants for their sustainability by using agro-practices.

14.2.2 Microbial Consortia in Salinity Stress Conditions

Strategy to evaluate the desirable plant growth promoting traits of microbial consortium is to assess their compatibility with plants under stress. In a healthy plant, all the physio-biochemical pathways are in a state of equilibrium, but if any deviation occurs in any of the pathways, it leads to plants becoming sick resulting in plant-growth inhibition and productivity loss. Since the soil is a habitat of plants and microbes, it plays a leading role from seed germination to crop maturity and harvesting. Salt stress is one of the major abiotic stresses that result in a decline in plant productivity worldwide, especially in arid and semi-arid regions (El-Katony et al. 2019).

According to Flowers et al. (1977), plants can be divided into glycophytes and halophytes based on their abilities to grow in different salt concentrations and complete their life cycle on a high concentration of salt, e.g., *Atriplex*, *Vesicaria*. Most terrestrial plants, including crops, are glycophytic and cannot tolerate high concentrations of salt. Plant growth and development are hampered due to salinity stress through (i) low osmotic potential of the soil solution (water stress), (ii) nutritional imbalance, (iii) specification effect (salt stress), or (iv) a combination of these factors (Ashraf 1994) (Fig. 14.1a). The following are the factors responsible for soil salinity: for primary salinity: (i) weathering of rocks, (ii) capillary rise from shallow brackish groundwater, (iii) the intrusion of seawater along the coast, and (iv) salt-laden sand blown by sea winds (Fig.14.1b). Impeded drainage secondary salinization is due to human activities like (i) introduction of irrigation without proper drainage system, (ii) industrial effluents, (iii) overuse of fertilizers, (iv) removal of natural plant cover, (v) flooding with salt-rich waters, and (vi) high water table and the use of poor-quality groundwater for irrigation (Ahmad et al. 2013a, b) (Fig. 14.1c).

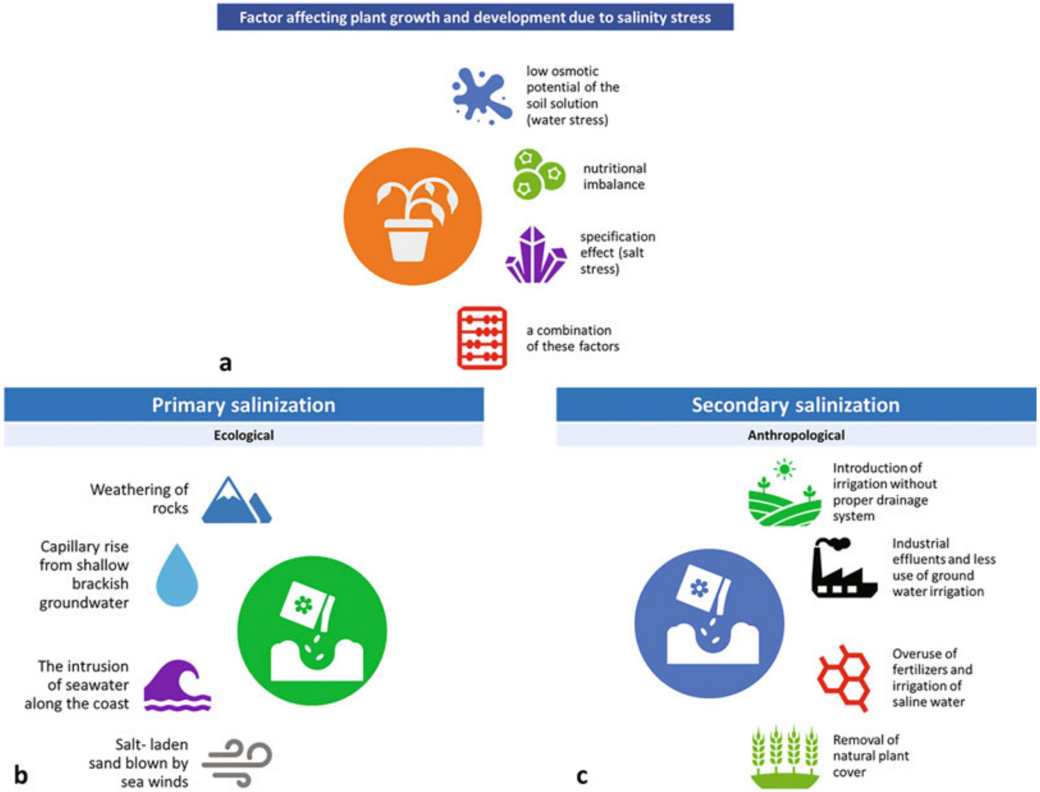


Fig. 14.1 Effect of salinity on plants and soils: (a) measures of salinity impacting on the plant health; (b) Ecological measures of primary salinity; (c) Anthropological measures of secondry salinity

During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolisms are affected (Ahmad et al. 2013a, b). It is, therefore, essential that stress in soil, mainly salt stress or sodic soil, has a direct impact on plants. Strogonov (1962) and Waisel (1972) described salinity as the induction of numerous structural changes: increase of succulence, changes in the number and size of stomata, thickening of the cuticle, extensive development of tyloses, earlier occurrence of lignification, inhibition of differentiation, and changes in the diameter and number of xylem vessels. Poor yield is due to photosynthesis efficiency, chlorophyll, total protein, biomass, stomata closure, and increased oxidative stress (Gupta and Huang 2014). Salinity also affects the germination rate of seeds, due to which germination rate declines (Soltabayeva et al. 2021). The earliest response is a reduction in the rate of leaf surface expansion. These effects often manifest as plant death or deterioration of plant growth, increased respiration rate, and decreased photosynthesis efficiency (Sudhir and Murthy 2004). A decrease in photosynthesis rates results in reduced plant growth, including root growth, which leads to a reduction in yield (Umego et al. 2020).

Various stresses impair plant morphology, embryogenesis, physiology, biochemistry, and gene regulation besides altering soil–microbial community, edaphic factors, etc., resulting in crop yield loss significantly. In general, abiotic stresses occur due to extremes of temperature, drought, environmental contaminants, and soil salinity. Most of the cultivated plants are sensitive to salt stress, in which NaCl-salinity causes a reduction in carbohydrates that are needed for cell growth. Carbohydrates are supplied mainly through the process of photosynthesis and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Ashraf and Harris 2004; Parida and Das 2005), and this would furthermore lead to restriction in water availability and imbalance in nutrient uptake by plants (Pessarakli and Tucker 1988; Katerji et al. 2004; Arzani 2008) with inhibition in seed germination due to ionic disturbance, osmotic, and toxic effects. Leaf area in bean plants decreased by approximately 20–40% in saline soil and reduced the total amount of vascular tissue. For example, stomatal resistance and proline and total chlorophyll (chlorophyll a + b) concentrations of maize plants were significantly influenced by salinity. In contrast, NaCl treatment decreased total chlorophyll concentration and increased stomatal resistance and proline concentration of maize plants. On the other hand, while stomatal resistance and proline concentration of plants was increased by salinity, total chlorophyll concentration decreased in salinity stress (Turan et al. 2009). Microbial consortium–plant interactions can overcome the losses that occur due to both biotic and abiotic factors.

14.2.3 *Effect of Microbial Cocktail*

Nowadays, plant growth and health-promoting beneficial bacterial genera are used for inoculation to promote plant growth directly and indirectly via the production of phytohormones enhancement of nutrient uptake and involvement of their beneficial traits conferred by the bacterial genera (Maheshwari 2010). To overcome the adverse impact of soil salinity, the use of PGPR-mediated salinity tolerance in plants has recently been reviewed by Mishra et al. (2021). Microbial consortia are bestowed through multifaceted features to counteract salinity stress in plants. Both similar and dissimilar rhizobacterial genera in consortia show their compatibility to boost the growth in sodic soil. Goswami et al. (2019) developed a non-rhizobacterial consortium for salt stress management in solanaceous crops. Earlier, Gupta and Pandey (2020) described the enhanced salinity tolerance in *Phaseolus vulgaris* due to inoculation of twin ACC deaminase-producing rhizobacteria. Arora et al. (2020) reported an interaction between *Piriformos poraindica* co-inoculated with *A. chroococcum* in diminishing the effect of salt stress in *Artemisia annua* L. This is mainly due to indirect enzymatic and nonenzymatic antioxidants. Kapadia et al. (2021) observed diverse genera of microbes (*Bacillus* spp. + *Delftia* spp. + *Enterobacter* spp.) to overcome the salinity stress in tomatoes. Another important feature of using microbial consortia is in improving soil salinization and climate change condition. In this scenario, recently, Gomez et al. (2021) noticed the importance of consortia of PGPR isolated from halophytes, which exhibited plant improvement due to soil salinity stress that adversely affects plant growth and crop productivity.

Scientific evidence revealed that abiotic stresses inhibit plant growth and development due to oxidative damage attacking DNA and cellular membranes. The antioxidant enzymes neutralize the reactive molecules; thus, cells are protected. PGPB cock-tailed with catalase and peroxidase properties are proven protective in nature. The beneficial bacteria also produce trehalose, which also benefits the plants to abiotic stress (Glick 2015; Kumar and Verma 2018). Microbial production of phytohormones also protects plants by the involvement of various physiological actions. PGPB induces the level of proline in plants. Proline scavenges reactive oxygen molecules and acts to stabilize proteins through molecular chaperons under stress conditions (Hossain et al. 2016).

Climate change is created due to unfavorable stress conditions in agroecosystems (Vimal et al. 2017). There is a growing challenge in plant–microbe interaction in stressed agriculture management. The characteristics such as strain evaluation to salinity stress and water drought resistance are also essential features to stimulate crop growth and improve tolerance to abiotic stresses and to prove more effective in extreme climate change conditions. Microbial inoculants may improve salt tolerance by altering hormonal root–shoot signaling that manages IAA production in plants by bacterial action, thus having the potential to enhance salt tolerance (Etesami and Maheshwari 2018). Such an approach is beneficial for a realistic assessment of the potential of microbial consortia in a climate-changed world.

The effect of consortium of *Bacillus cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21 was reported to develop healthy cucumber plants, with much darker green leaves containing increased proline and chlorophyll contents and inducing superoxide dismutase activity (Wang et al. 2012). An increase in ethylene levels is injurious to plants causing senescence and another deleterious effect that occur due to the accumulation of a consortium of ACC-deaminase-producing bacteria (*Ochrobactrum pseudogrigninense*, *Pseudomonas* sp., and *B. subtilis*) significantly increased early vegetative growth plant parameters in *Vigna mungo* and *P. sativum*.

14.3 Microbial Consortia in Soil Management

Microbial inoculation directly impacts the soil microbial community to increase the relative abundance of inoculated microbial genera. The rhizospheric microbial community composition differed substantially from the bulk soil microbial community composition (Overbeek et al. 2021). For example, in the case of potato roots, enrichment of the rhizosphere community over bulk soil was observed for *Proteobacteria*, and in *Eurotiomyces*, such difference in the microbial community was observed by several workers (Berendsen et al. 2012; Xue et al. 2018). The external input of microorganisms closely associated with the rhizosphere contributes as core microorganisms, and the alteration in the rhizospheric microbiome helps in designing microbial inoculants beneficial to the plants growing under a variety of soil conditions (Sathya et al. 2017).

14.4 Consortia Constructions and Applications

The construction of simplified microbial consortia to degrade recalcitrant materials based on enrichment and dilution to extinction culture was carried out by Kang et al. (2020). Microbial consortia are also used to control and optimize various industrial processes. Puentes-Tellez and Falaco Salles (2018) described the construction of effective minimal active microbial consortia for lignocellulose degradation. The simplification of the microbial community makes it easier to help and understand the individual roles of the strains in the consortia. Skariyachan et al. (2018) worked on polymer degradation by novel thermophilic consortia of *Brevibacillus* spp. and *Aneurinibacillus* sp. associated with waste management landfills and sewage treatment plants.

Earlier, the authors formulated bacterial consortia from plastic-contaminated cow dung. It is interesting to note that Subhashchandrabose et al. (2011) studied the biotechnology potential of consortia of cyanobacteria/microalgae and bacteria. A proper description of the consortium, the taxonomic affiliation of the strains, and identification protocols, the process of formulation, the effect of edaphic and other related parameters, and the population of most desirable species in the consortium that impact the consortium's success are various factors involved to work under nonstress conditions sustainably.

Brassica campestris (Indian mustard) healthy seeds of similar shape and size were bacterized with different rhizobia isolates and by different consortia, as given in

Table 14.1 In vitro interaction among selected PGPR for the preparation of microbial consortium

Strain	KRP1	KRP7	GRC1	KRB1	MTCC 429	RMP1	MTCC 97
KRP1	+	−	−	+	−	+	−
KRP7	−	+	+	−	+	−	+
GRC1	−	+	+	+	+	−	+
KRB1	+	−	+	+	−	+	−
MTCC 429	−	+	+	−	+	−	−
RMP1	+	−	−	+	−	+	+
MTCC 97	−	+	+	−	−	+	+

Abbreviation: KRP1, KRP7 and GRC1: *P. areuginosa*; MTCC-429: *B. licheniformis*; KRB1: *B. licheniformis*; RMP1: *Sinorhizobium meliloti*; MTCC-97: *Serratia marcescens*; (+): growth present; (−): growth was inhibited

Table 14.2 In vitro interaction studies of *P. aeruginosa* KRP1, *B. licheniformis* KRB1, and *Sinorhizobium meliloti* RMP1 for the biocontrol of *Sclerotina sclerotiorum* and *Fusarium oxysporum*

	Inhibition (%)	
Bacterial isolates and consortium	*F. oxysporum*	*S. sclerotiorum*
KRP1	79	81
KRB1	70	76
KRP1 + KRB1	82	83
KRP1 + RMP1	80	82
KRB1 + RMP1	74	78
Consortium (KRP1 + KRB1 + RMP1)	85	89

Table 14.1. The maximum enhancement of vegetative growth parameters was observed in the consortium in comparison to those that emerged due to individual treatment with KRP1, KRB1, and RMP1. Thus, the application of bacterial consortium proved to be most desirable for plant growth and development of *B. campestris*.

The PGP strains are to be evaluated for their native to inhibit the growth of each other by the “cross streaking” method of Pierson and Weller (1994). This was further confirmed by the filter paper disc method as given by Sindhu et al. (1999). The strains are further listed for their consortium-forming abilities following the spectrophotometric method of Shanmugam et al. (2002). The combined effect of different bacterial strains showed decreased suppression in comparison to that of individual application. Consortium declined disease incidence of skin blight caused by *Sclerotinia sclerotiorum* and *Fusarium oxysporum* more effectively in comparison to their isolates. The combined effect (KRP1 + KRB1 + RMP1) showed a more pronounced effect and proved much more effective in checking both the deleterious phytopathogens causing disease incidence in *B. campestris* (Table 14.2).

Thus, to sustain the overall benefits, biofertilizers in the form of use of beneficial microorganisms (single or in combined form) play an important role in INM because they not only (i) contain a wide range of natural, chelated plant nutrients and trace elements, carbohydrates, and amino acid and (ii) produce growth promoting substances, but also act as the soil conditioner by stimulating microbial activity resulting in an improved air–water relationship in soil and improved fertility and makes soil less prone to compaction and erosion.

For sustainable soil fertility, blending chemical fertilizers with chemical adaptive beneficial plant growth-promoting bacterial strains is one of the approaches that may derive synergistic benefits (Vargas et al. 2000; Joshi et al. 2006). For sustainability in agriculture, India does not need more fertilizer or total organic approaches, but a blend of both is required (Ayala and Rao 2002). Long back, Krishnamurthi and Kumar (1987) highlighted the establishment of a judicious combination of organic and inorganic approaches for an increase in the improvement of crops and restoration of physical, chemical, and microbiological properties beneficial to the plant and soil. It takes systemic and simultaneous account of the environmental aspects, the quality of the product, and the profitability of agriculture. Molina-Romero et al. (2021) observed the potential of a second-generation consortium formulated with *Azospirillum brasilense* SP7, *Pseudomonas putida* KT2440, *Acinetobacter* sp. EMM02, and *phingomonas* sp. OF 178A. The bacterial strains of the consortium proved compatible, resistant to desiccation, and efficient for field applications.

Certain fertilizer-adapted variants of *P. aeruginosa* GRC2 and *Azotobacter chroococcum* AC1 exhibited inhibition of *M. phaseolina* causing charcoal rot of *Brassica juncea.* Similar reports were obtained by Kumar et al. (2009) while working on rhizospheric competent *P. aeruginosa* LES4 wherein the reduction of chemical fertilizer occurred due to the application of LES4, and the yield obtained was almost like that of the recommended dose of chemical fertilizer in *Sesamum indicum* L. The enhancement of sesame growth was recorded under the influence of indigenous and nonindigenous rhizospheric competent fluorescent pseudomonads (Aeron et al. 2010). The co-inoculation of urea and DAP-tolerant *S. meliltoti* and *P. aeruginosa* acted due to an integrated approach to the growth enhancement of *B. juncea* (Maheshwari 2010). Such a concept proved eco-friendly for disease management as well as economically viable INM on oil seed crops (Dubey and Maheshwari 2011; Kumar et al. 2011; Maheshwari et al. 2012). In another study, a consortium of *P. aeruginosa* BUFF 12, *Proteus mirabilis* BUFF 14, and *Enterobacter xiangfangensis* BUFF 38 strains were used for bioformulation preparation based on the increase in PGP activity. The consortium of strain increases in PGP attributes (in vitro) and P-solubilization, K-solubilization, and increase in S oxidation were also noticed. The most potential dung slurry proved to be an effective supportive material with a mixture of strains exhibited incredible *Foeniculum vulgare* Mill. growth. This research disseminates a successful technology to develop an eco-friendly bio-formulation augmenting the growth leading to sustainable agriculture (Dhiman et al. 2022). On the other hand, bacterial consortium acts as a substitute to chemical fertilizers such as urea, DAP, etc., because decreased application of chemical fertilization along with bacterial consortium exhibited a similar effect on plant growth and yield as revealed while applying the recommended doses of chemical fertilizers (Kumar et al. 2010; Da Costa et al. 2013).

The application of biofertilizers and reduced doses of chemical fertilizers increased wheat yield, obtained from the single application of recommended doses of fertilizers (Stephens and Rask 2000). Saraf and Sood (2002) raised the pesticide-adoptive mutants of rhizobia strains and observed PGP activities to use them as bioinoculants. Reasonable results were achieved from the fertilized field using fertilizers/adaptive indigenous strains (Vargas et al. 2000). Bakhshandeh et al. (2020) described the early vegetative growth enhancement of soybean due to PGPB in salt stress conditions. LiH, La, et al. (2021) studied salt-induced recruitment of specific root-associated bacterial consortia able to enhance adaptation to salt stress. Furthermore, the authors demonstrated from a series of experiments that the consortium, but not individual isolate of the salt-induced root-derived bacteria, provided enduring resistance to that salt stress condition in soil, thus evidencing the critical role of salt-induced root desired bacteria in enhancing adaptation to plant against salt stress (Table 14.3).

Table 14.3 Examples of PGPR consortia and crop tolerant to salinity-stressed soil

PGPR	Crop	Reference
Pseudomonas sp. B14, *Microbacterium* sp. B19	*Brassica rapus* L.	Swiontek Brzezinska et al. (2022)
Bacillus sp., *Acinetobacter* sp.	*Zea mays* L.	Shabaan et al. (2022)
Bacillus sp., *Delftia* sp., *Enterobacter* sp., *Achromobacter* sp.	*Solanum lycopersicum* L.	Kapadia et al. (2021)
Pseudomonas sp., *Serratia proteamaculans*, *Alcaligenes* sp., *Bacillus* sp.	*Zea mays* L.	Xia et al. (2020)
Pseudomonas frederiksbergensis OB139, *Pseudomonas vancouverensis* OB155	*Capsicum annuum*	Samaddar et al. (2019)
Bacillus cereus strain Y5, *Bacillus* sp. Y14, *Bacillus subtilis* strain Y16	*Triticium aestivium* L.	Shahzad et al. (2017)
Bacillus safensis, *Ochrobactrum pseudogregnonense*	*Triticum aestium*	Chakraborty et al. (2013)
P. pseudoalcaligenes, *B. pumilus*	*O. sativa*	Jha et al. (2013)
B. subtilis, *Arthrobacter* sp.	*T. aestivum*	Upadhyay et al. (2012)
P. pseudoalcaligenes, *P. putida*	*Cicer arietinum*	Patel et al. (2012)
Brachybacterium saurashtrense, *Brevibacterium casei*, *Haererohalobacter* sp.	*Arachis hypogaea*	Shukla et al. (2012)
Bacillus, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Paenibacillus*	*T. aestivum*	Upadhyay et al. (2011)
P. fluorescens, *P. aeruginosa*, *P. stutzeri*	*Lycopersicon esculentum*	Tank and Saraf (2010)
Agrobacterium rubi, *Burkholderia gladii*, *P. putida*, *B. subtilis*, *B. megaterium*	*Raphanus sativus* L.	Kaymak et al. (2009)
Bacillus sp., *Ochrobactrum* sp.	*Z. mays*	Principe et al. (2007)
P. syringae, *P. fluorescens*, *E. aerogenes*	*Z. mays*	Nadeem et al. (2007)
Aeromonas hydrophila, *B. insolitus*, *Bacillus* sp.	*T. aestivum*	Ashraf and Harris (2004)
A. lipoferum, *A. brasilense*, *Azoarcus*, *Pseudomonas* sp.	*Leptochloafusca*	Malik et al. (1997)

14.5 Conclusions

For increasing crop productivity and for the maintenance and improvement of soil fertility for sustainable crop production, the multifunctional formulation will involve microbial consortium utilizing the PGPR and prove better and eco-friendly in comparison to that formulation alone. It is interesting to note the intricate network of natural interaction to engineer artificial microbial consortia that substantially and consistently benefit plant growth and health, increase crop production, and decrease the use of chemical fertilizers. The recommended use of microbial consortia will facilitate the production of plants in a more sustainable manner that eventually will not depend on agrochemicals. The biological management for the growth and development of plants is still at an early stage of development; while the approach appears to have tremendous potential and many of the basic concepts necessary for the implementation are in place, apparent obstacles such as information on biomass, formation of a product, and site of application and registration difficulties exist. They enhance the availability of mineral nutrients to plant application to seed or soil and offer an eco-friendly, economically viable, and socially acceptable means of reducing external input of chemical fertilizers.

References

Aeron A, Pandey P, Maheshwari DK (2010) Differential response of sesame under influence of indigenous and non-indigenous rhizosphere competent fluorescent pseudomonads. Curr Sci 99(2):166–168

Ahmad M, Zahir ZA, Khalid M, Nazli F, Arshad M (2013b) Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiol Biochem 63:170–176

Ahmad P, Azooz MM, Prasad MNV (2013a) Ecophysiology and responses of plants under salt stress. Springer, New York

Ankineedu G, Rao JV, Reddy BN (1983) Advances in fertiliser management for rainfed oilseeds. Fertiliser News, India

Arora NK, Fatima T, Mishra J, Mishra I, Verma S, Verma R, Bharti C (2020) Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils. J Adv Res 26:69–82

Arzani A (2008) Improving salinity tolerance in crop plants: a biotechnological view. In Vitro Cell Dev Biol Plant 44(5):373–383

Ashraf M (1994) Organic substances responsible for salt tolerance in *Eruca sativa*. Biol Plant 36: 255–259

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16

Ayala S, Rao EVSP (2002) Perspective of soil fertility management with a focus on fertilizer use of crop productivity. Curr Sci 82:797–807

Babich H, Stotzky G (1983) Further studies on environmental factors that modify the toxicity of nickel to microbes. Regul Toxicol Pharmacol 3(1):82–99

Bakhshandeh E, Gholamhosseini M, Yaghoubian Y, Pirdashti H (2020) Plant growth promoting microorganisms can improve germination, seedling growth and potassium uptake of soybean under drought and salt stress. Plant Growth Regul 90(1):123–136

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Bishnoi RK, Das A, Gaware UP, Baviskar PP, Bhupender (2020) A bird eye view of fertilizer industry of India. Curr Appl Sci Technol 39(14):9–18

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838

Chakraborty U, Chakraborty BN, Chakraborty AP, Dey PL (2013) Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World J Microbiol Biotechnol 29(5):789–803

Da Costa PB, Beneduzi A, De Souza R, Schoenfeld R, Vargas LK, Passaglia LMP (2013) The effects of different fertilization conditions on bacterial plant growth promoting traits: guidelines for directed bacterial prospection and testing. Plant Soil 368:267–280

Deshwal VK, Dubey RC, Maheshwari DK (2003) Isolation of plant growth-promoting strains of *Bradyrhizobium* (Arachis) sp. with biocontrol potential against *Macrophomona phaseolina* causing charcoal rot of peanut. Curr Sci 83(3):443–448

Dhiman S, Baliyan N, Maheshwari DK (2022) Appraisal of biofilm forming bacteria in developing buffalo dung-based bioformulation coupled to promote yield of *Foeniculum vulgare* Mill. 3Biotech 12(9):1–13

Dubey RC, Maheshwari DK (2011) Role of PGPR in integrated nutrient management of oil seed crops. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Heidelberg, pp 1–15

Duhoon SS, Jain HC, Deshmukh MR, Goswami U (2001) Integrated nutrient management in kharif sesame (*Sesamum indicum* L.). J Oilseed Res 18(1):88–99

Ehteshami SM, Aghaalikhani M, Khavazi K, Chaichi MR (2007) Effect of phosphate solubilizing microorganisms on quantitative and qualitative characteristics of maize (*Zea mays* L.) under water deficit stress. Pak J Biol Sci 10:3585–3591

El-Katony TM, El-Bastawisy ZM, El-Ghareeb SS (2019) Timing of salicylic acid application affects the response of maize (*Zea mays* L.) hybrids to salinity stress. Heliyon 5:e01547

Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Safe 156:225–246

Flowers TJ, Troke PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. Annu Rev Plant Biol 28(1):89–121

Glick BR (2015) Stress control and ACC deaminase. In: Principles of plant-microbe interactions. Springer, Cham, pp 257–264

Gomez JA, Höffner K, Barton PI (2021) Production of biofuels from sunlight and lignocellulosic sugars using microbial consortia. Chem Eng Sci 239:116615

Goswami SK, Kashyap PL, Awasthi S (2019) Deciphering rhizosphere microbiome for the development of novel bacterial consortium and its evaluation for salt stress management in solanaceous crops in India. Indian Phytopathol 72(3):479–488

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genom 2014:1–18

Gupta S, Pandey S (2020) Enhanced salinity tolerance in the common bean (*Phaseolus vulgaris*) plants using twin ACC deaminase producing rhizobacterial inoculation. Rhizosphere 16:100241

Hossain MA, Burritt DJ, Fujita M (2016) Abiotic stress response in plants. In: Tuteja N, Gill SS (eds) Cross-stress tolerance in plants: molecular mechanisms and possible involvement of reactive oxygen species and methylglyoxal detoxification systems. Wiley-VCH, Germany, pp 327–380

Hossain MS (2019) Present scenario of global salt affected soil, its management and importance of salinity research. Int Res J Biol Sci 1(1):1–3

Idris M (2003) Effect of integrated use of mineral, organic N and *Azotobacter* on yield, yield components and nutrition of wheat (*Triticum aestivum.* L.). Pak J Biol Sci 6(6):539–546

Jha SK, Damodaran T, Verma CL, Mishra VK, Sharma DK, Sah V, Rai RB, Dhama K (2013) Fluoride partitioning in rice (*Oryza sativa*) and wheat (*Triticum aestivum*) upon irrigation with fluoride-contaminated water and its risk assessment. South Asian J Exp Biol 3(3):137–144

Joshi KK, Kumar V, Dubey RC, Maheshwari DK (2006) Effect of chemical fertilizer adaptive variants, *Pseudomonas aeruginosa* GRC2 and *Azotobacter chroococcum* AC1 on *Macrophominaphaseolina* causing charcoal rot of *Brassica juncea*. Kor J Environ Agric 25(3):228–235

Kang D, Jacquiod S, Herschend J, Wei S, Nesme J, Sørensen SJ (2020) Construction of simplified microbial consortia to degrade recalcitrant materials based on enrichment and dilution-to-extinction cultures. Front Microbiol 10(3010):1–10

Kapadia C, Sayyed RZ, ElEnshashy HA, Vadhy H, Sharma D (2021) Halotolerant microbial consortia for sustainable mitigation of salinity stress, growth promotion, and mineral uptake in tomato plants and soil nutrients enhancement. Sustainability 13(15):8369

Katerji N, Van HJW, Hamdy A, Mastrorilli M (2004) Comparison of corn yield response to plant water stress caused by salinity and by drought. Agric Water Manag 65(2):95–101

Katiyar P, Dubey RC, Maheshwari DK (2021) ACC deaminase-producing *Ensifer adhaerens* KS23 enhances proximate nutrient of *Pisum sativum* L. cultivated in high altitude. Arch Microbiol 203(5):2689–2698

Kaymak HÇ, Güvenç İ, Yarali F, Dönmez MF (2009) The effects of bio-priming with PGPR on germination of radish (Raphanus sativus L.) seeds under saline conditions. Turk J Agri For 33 (2):173–179

Kesavan PC, Swaminathan MS (2006) Managing extreme natural disasters in coastal areas. Philos Trans R Soc A Math Phys Eng Sci 364(1845):2191–2216

Kesavan PC, Swaminathan MS (2020) Bio-village as a model for bioeconomy. In: Kataki R, Pandey A, Khanal SK, Pant D (eds) Current developments in biotechnology and bioengineering. Elsevier, Amsterdam, pp 453–474

Krishnamurthi KK, Kumar R (1987) Bull permanent manurial experiments. Tamil Nadu Agriculture University, Coimbatore

Kumar A, Verma JP (2018) Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52

Kumar B, Senthil Kumar M, Annapurna K, Maheshwari DK (2006) Genetic diversity of plant growth-promoting rhizobia isolated from a medicinal legume, *Mucuna pruriens* Linn. Curr Sci 91(11):1524–1529

Kumar H, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L) var. Manak by bacterial combinations amended with chemical fertilizer. Crop Protec 29(6):591–598

Kumar S, Kumar P, Dubey RC, Maheshwari DK (2011) Bioremediation of pollutants. In: Maheshwari DK, Dubey RC (eds) Ecofriendly economically viable integrated nutrient management for sustainable agriculture, I.K. International House, New Delhi, pp 279–298

Kumar T, Wahla V, Pandey P, Dubey RC, Maheshwari DK (2009) Rhizosphere competent *Pseudomonas aeruginosa* in the management of *Heteroderacajani* on Sesame. World J Microbiol Biotechnol 25:277–285

LiH, La S, Zhang X, Gao L, Tian Y (2021) Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress. ISME J 15(10): 2865–2882

Maheshwari DK (2010) Plant growth and health promoting bacteria. Springer Science and Business Media, Berlin/Heidelberg

Maheshwari DK (2011) Bacteria in agrobiology: crop ecosystems. Springer Science and Business Media, Berlin/Heidelberg

Maheshwari DK, Dheeman S (2019) Field crops: sustainable management by PGPR. Springer Nature Switzerland AG, p 458

Maheshwari DK, Dheeman S, Agarwal M (2015) Bacterial metabolites in sustainable agroecosystem. In: Maheshwari DK (ed) Phytohormone-producing PGPR for sustainable agriculture. Springer, Cham, pp 159–182

Maheshwari DK, Dubey RC, Aeron A, Kumar B, Kumar S, Tewari S, Arora NK (2012) Integrated approach for disease management and growth enhancement of *Sesamum indicum* L. utilizing *Azotobacter chroococcum* TRA2 and chemical fertilizer. World J Microbiol Biotechnol 28 (10):3015–3024

Malik KA, Bilal R, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. In: Ladha JK et al (eds) Opportunities for biological nitrogen fixation in rice and other non-legumes. Springer, Dordrecht, pp 37–44

Mishra P, Mishra J, Arora NK (2021) Plant growth promoting bacteria for combating salinity stress in plants. Recent developments and prospects: a review. Microbiol Res 252(126861):1–13

Molina-Romero D, Juárez-Sánchez S, Venegas B, Ortíz-González CS, Baez A, Morales-García YE, Muñoz-Rojas J (2021) A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. Front Sustain Food Syst 4:616757

Mukhopadhyay AN, Shrestha SM, Mukherjee PK (1992) Biological seed treatment for control of soil-borne plant pathogens. FAO Plant Protec Bull 40(110):21–30

Muntasir IM, Tauhiduzzaman S, Ishaque M (2001) Study on the effect of N fertilizer and green manure on the growth and yield of BR 22 rice. Indian J Agric Chem 34(1–2):15–21

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53(10):1141–1149

Overbeek W, Jeanne T, Hogue R, Smith DL (2021) Effects of microbial consortia, applied as fertilizer coating, on soil and rhizosphere microbial communities and potato yield. Front Agron 3(714700):1–13

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60(3):324–349

Patel D, Jha CK, Tank N, Saraf M (2012) Growth enhancement of chickpea in saline soils using plant growth-promoting rhizobacteria. J Plant Growth Regul 31(1):53–62

Paul IK, Savitri KE (2003) Effect of biofertilizer vs. perfected chemical fertilization for sesame grown in summer rice fallow. J Trop Agric 41:47–49

Pessarakli M, Tucker TC (1988) Dry matter yield and nitrogen 15 uptake by tomatoes under sodium and chloride stress. Soil Sci Soc Am J 52:698–700

Pierson EA, Weller DM (1994) To suppress take-all and improve the growth of wheat. Phytopathology 84(1):940–947

Principe A, Alvarez F, Castro MG, Zachi L, Fischer SE, Mori GB, Jofré E (2007) Biocontrol and PGPR features in native strains isolated from saline soils of Argentina. Curr Microbiol 55 (4):314–322

Puentes-Tellez PE, Falaco Salles J (2018) Construction of effective minimal active microbial consortia for lignocellulose degradation. Microb Ecol 76(2):419–429

Rana SS, Rana MC (2011) Cropping system. Department of Agronomy, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur

Roy RN, Ange AL (1991) Integrated plant nutrition system (IPNS) and sustainable agriculture. Annual seminar 1991. FAI, New Delhi, pp SV/1-1–SV/1-12

Samaddar S, Chatterjee P, Choudhury AR, Ahmed S, Sa T (2019) Interactions between Pseudomonas spp. and their role in improving the red pepper plant growth under salinity stress. Microbiol Res 219:66–73

Saraf M, Sood N (2002) Influence of monocrotophos on growth, oxygen uptake and exopolysaccharide production of *Rhizobium* NCIM 2271 on chickpea. J Indian Bot Soc 82: 157–164

Sathya A, Vijayabharathi R, Gopalakrishnan S (2017) Plant growthpromoting actinobacteria: A new strategy for enhancing sustainable production and protection of grain legumes. 3Biotech 7: 102–102

Shahzad SM, Arif MS, Riaz M, Iqbal Z, Ashraf M (2013) PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (*Zea mays* L.) under fertilized conditions. Eur J Soil Biol 57:27–34

Shahzad S, Khan MY, Zahir ZA, Asghar HN, Chaudhry UK (2017) Comparative effectiveness of different carriers to improve the efficacy of bacterial consortium for enhancing wheat production under salt affected field conditions. Pak J Bot 49(4):1523–1530

Shanmugam V, Senthil N, Raguchander T, Ramanathan A, Samiyappan R (2002) Interaction of *Pseudomonas fluorescens* with *Rhizobium* for their effect on the management of peanut root rot. Phytoparasitica 30(2):169–176

Shabaan M, Asghar HN, Zahir ZA, Sardar MF, Parveen R, Ali Q (2022) Halotolerant rhizobacterial consortium confers salt tolerance to maize under naturally salt-affected soil. Soil Sci Soc Am J 86(5):1264–1279

Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012) Promotion of growth and Cu accumulation of bio-energy crop (*Zea mays*) by bacteria: implications for energy plant biomass production and phytoremediation. J Environ Manag 103:58–64

Shukla PS, Agarwal PK, Jha B (2012) Improved salinity tolerance of *Arachis hypogaea* (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. J Plant Growth Regul 31 (2):195–206

Sindhu SS, Gupta SK, Dadarwal KR (1999) Antagonistic effect of *pseudomonas* spp. on pathogenic fungi and enhancement of growth of green gram (*Vigna radiata*). Biol Fertil Soil 29(1):62–68

Singh VR (2020) Critical analysis of new urea policy 2015-Way forward. Doctoral dissertation, Full Text-IIPA, New Delhi. http://14.139.53.35/jspui/bitstream/1/3688/1/4539.pdf

Skariyachan S, Patil AA, Shankar A, Manjunath M, Bachappanavar N, Kiran S (2018) Enhanced polymer degradation of polyethylene and polypropylene byb novel thermophilic consortia of *Brevibacillus* sps. And *Aneurinibacillus* sp. screened from waste management landfills and sewage treatment plants. Poly Degrad Stab 149:52–68

Soltabayeva A, Ongaltay A, Omondi JO, Srivastava S (2021) Morphological, physiological and molecular markers for salt-stressed plants. Plan Theory 10(2):243

Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crop Res 65:249–258

Strogonov BP (1962) Fisiologithcheskie Osnovy Soleustoitchivosti Rastenii (physiological bases of salt tolerance in plants). Akademia Nauk SSSR, Moskva

Subhashchandrabose SR, Ramakrishan B, Megharaj M, Venkatesawrlu K, Naidu R (2011) Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. Biotechnol Adv 29(6):896–907

Sudhir P, Murthy SDS (2004) Effects of salt stress on basic processes of photosynthesis: a review. Photosynthetica 42:481–486

Swiontek Brzezinska M, Świątczak J, Wojciechowska A, Burkowska-But A, Kalwasińska A (2022) Consortium of plant growth-promoting rhizobacteria enhances oilseed rape (*Brassica napus* L.) growth under normal and saline conditions. Arch Microbiol 204(7):393

Tank N, Saraf M (2010) Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J Plant Interact 5(1):51–58

Truyens S, Weyens N, Cuypers A, Vangronsveld J (2015) Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ Microbiol 7:40–50

Turan MA, El Karim AHA, Taban N, Taban S (2009) Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. Afr J Agric Res 4:893–897

Umego C, Ntui VO, Ita EE, Opara C, Uyoh EA (2020) Screening of rice accessions for tolerance to drought and salt stress using morphological and physiological parameters. Am J Plant Sci 11: 2080–2102

Upadhyay SK, Singh JS, Singh DP (2011) Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. Pedosphere 21(2):214–222

Upadhyay SK, Singh JS, Saxena AK, Singh DP (2012) Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. Plant Biol 14(4):605–611

Vargas MA, Mendes IC, Hungria M (2000) Response of field-grown bean (*Phaseolus vulgaris* L.) to Rhizobium inoculation and nitrogen fertilization in two Cerrados soils. Biol Fertil Soils 32(3): 228–233

Vimal SR, Singh JS, Arora NK, Singh S (2017) Soil-plant-microbe interactions in stressed agriculture management: a review. Pedosphere 27(2):177–192

Waisel Y (1972) Biology of halophytes. Academic Press, New York

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7(12):1–10

Xia M, Chakraborty R, Terry N, Singh RP, Fu D (2020) Promotion of saltgrass growth in a saline petroleum hydrocarbons contaminated soil using a plant growth promoting bacterial consortium. Int Biodeter Biodegrad 146(104808):1–8

Xue D, Christenson R, Genger R, Gevens A, Lankau RA (2018) Soil microbial communities reflect both inherent soil properties and management practices in Wisconsin potato fields. Am J Potato Res 95:696–708

Zorb C, Geifue CM, Dietz KJ (2019) Salinity and crop yield. Plant Biol 21(S1):31–38

Chapter 15
Bioencapsulation of Biocontrol Agents as a Management Strategy for Plant Pathogens

Clement Kiing Fook Wong

Abstract The biological control of plant pathogens is an environmentally friendly and low-cost method of managing plant pathogens. To extend the survivability of the biocontrol agents (BCAs) in the environment, they can be immobilized into protective polymeric carriers using the encapsulation method. Additives such as chitosan, clay materials, osmoprotectants, proteins, or carbon sources can be added to the polymer matrix to improve the viability of BCAs and impart slow-releasing properties of the formulation. Compared to the conventional dry and liquid formulation, encapsulated microbial cells are better protected from abiotic and biotic stresses. This chapter sought to provide an overview of the technologies available and current progress in the encapsulation of BCAs in plant disease management. Potential challenges and future research directions in encapsulation technology will also be discussed in detail.

Keywords Additives · Biocontrol · Encapsulation · Plant pathogen · Polymer

15.1 Introduction

The biological control of plant diseases using living organisms is a well-known technique that is environmentally and farmer-friendly compared to the use of synthetic pesticides. These organisms or otherwise known as biocontrol agents (BCAs) exhibited antagonistic properties against plant pathogens while at the same time they enhanced crop growth via the production of plant-growth-promoting hormones. Application of these BCAs has resulted in disease suppression, but inconsistent control has been reported (Bardin et al. 2015; Schulz et al. 2019). Unpredictable weather conditions, soil fertility, agricultural practices, and competition with native microbial communities are some of the major factors contributing to

---

C. K. F. Wong (✉)
Department of Agriculture and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Kampar, Perak, Malaysia
e-mail: kfwong@utar.edu.my

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_15

Table 15.1 A comparison between microbial formulations

Factor	Solid formulation	Liquid formulation	Encapsulation
Shelf-life/ microbial viability	Poor to average due to desiccation	Poor to average due to insufficient oxygen, accumulation of metabolic waste, and osmotic imbalance	Average to good since cells are protected. Nutrients and oxygen are accessible through diffusion
Production cost	Raw materials are easily available and low cost	Culture media and fermentation costs are higher	The cost for polymeric compounds is higher, but low-cost polymers are available
Microbial biomass	High biomass is needed to compensate for the washing-off and high mortality rate during field application	High biomass is needed as cell mortality is high in liquid formulations	Enables the slow release of cells which reduces the initial microbial biomass. Cells are protected from environmental stress
Contamination	Contamination is high when exposed	Contamination is common in liquid formulation	Encapsulated cells are protected from contaminants
Transportation	High cost due to bulkiness	Size of storage vessels reduced in concentrated formulations. Reduces transportation costs	Encapsulated beads are often small, applied in small volumes, and require minimum storage space. Reduces transportation costs
Seed application	Coating efficiency is low for seeds	The coating is possible with the addition of stickers	The coating is possible with microbeads of sizes 10–100 μM
Application method	Soil application	Soil, foliar application, or through fertigation	Soil application
Facilities	No or less sophisticated equipment needed	No or less sophisticated equipment is needed	Requires equipment for spraying and solidification for microcapsule production

the reduced viability of the BCAs to exert their full potential in crop protection (Bardin et al. 2015; Besset-Maszoni et al. 2019; Pirttilä et al. 2021). In other words, sustaining or improving microbial viability after the introduction of BCAs into the field is crucial to ensure their bioactivity against plant pathogens is not hampered. To protect the BCAs, the incorporation of these microbes into suitable carriers supplemented with additives remains a topic of interest among researchers to develop a stable, effective, and reliable formulation that could maintain crop health and yield (Seema et al. 2018).

Conventional biofertilizers are developed and commercialized as powder, granular, and liquid formulations (Table 15.1). Typical carriers used for solid formulations include clay or peat-based materials, polysaccharides, proteins, plant by-products, synthetic silicates, and polymers (John et al. 2011; Lobo et al. 2020).

The incorporation of microbes into dry carriers is often characterized by poor BCA viability due to exposure to desiccation stress during the drying process (Berninger et al. 2018). The survival of bacterial BCA is also inferior in liquid formulations such as suspension, concentrates, oil-based, and emulsion since microbial cells are left exposed to harsh environmental conditions without protectants (Alori and Babalola 2018). Hence, improving the shelf life of biofertilizers is an important factor that determines the bioactivity of the microbes. As the global population is increasing, intensified agriculture activities have led to skyrocketing production costs, especially in fertilizer application. To date, controlled-released fertilizers are favored by most growers since less cost and input are required compared to conventional fertilizers (Majeed et al. 2015; Raimondi et al. 2021).

The encapsulation technology is a method that immobilizes microbial cells or active ingredients within a protective shell or capsules, which results in better viability during storage and improved survivability in soil, rhizosphere, or phyllosphere after application (Chen et al. 2021). In addition, microbial cells are released gradually from the encapsulation material, which makes this method a promising alternative in the production of slow-releasing biofertilizers (Vassilev et al. 2020; Mitter et al. 2021). In this chapter, emphasis will be directed on the current progress, challenges, and future directions of applying encapsulation technology to immobilize BCAs for plant disease management.

15.2 Progress in Encapsulation Technology to Sustain BCA Viability

Various encapsulation technologies (Fig. 15.1) were critically reviewed for their practicality in immobilizing BCAs (Winder et al. 2003; John et al. 2011; Schoebitz et al. 2013; Vemmer and Patel 2013; Vassilev et al. 2020; Saberi-Riseh et al. 2021). In general, most BCAs are loaded onto polymeric substances through ionic gelation in which an alginate–microbial mixture is dropped into a calcium chloride solution to form beads. Other methods could immobilize BCAs onto microparticles through spray drying or fluidized bed spraying. Each method has its advantages and

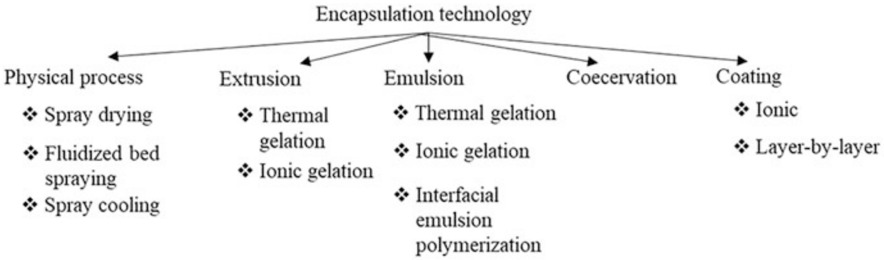


Fig. 15.1 Recent available technologies for encapsulation

Table 15.2 Advantages and limitations of available technologies in the encapsulation of BCAs

Current technologies	General mechanism	Advantages	Limitations
Extrusion	Microbial–polymer mixture is passed through a nozzle into oil suspension and/or calcium chloride solution forming beads Bead size depends on the size of the nozzle, distance from the nozzle to the polymerization solution, viscosity, and concentration of the polymer	A simple and cheap method with minimal sophisticated machines and procedures	Poor mechanical strength due to chelation to cations present in the soil causing cells to be released Large pore size Slow for large-scale production as bead formation is slow Additives such as chitosan are added to provide better integrity and strength
Emulsion	Microbial–polymer mixture added to oil suspension followed by solidification in calcium chloride solution Size of beads controlled by agitation speed	Improves microbial shelf-life compared to the extrusion method Rapid bead formation suitable for scaling up	Higher cost of production due to the use of vegetable oil in the emulsification process
Spray drying	Atomization of the microbial–polymer mixture into a high-temperature chamber leading to drying and the formation of microcapsules	Rapid formation of dried capsules	High temperature can potentially reduce the viability of non-spore-forming microbes Requires costly equipment Variation in particle shape and size
Fluidized spray drying	Coating materials (inoculants) are sprayed onto particles fluidized with hot air followed by a series of wetting and drying	Less stressful for cells than the spray-drying method Particle size is uniform	Requires costly equipment

limitations in sustaining the viability of BCAs and their bioactivity against plant pathogens (Table 15.2).

In general, most BCAs are encapsulated in microbeads within the size of 1 to 1000 μM (Lengyel et al. 2019). With the help of nanotechnology, the bead size can be further reduced to the size of 1–1000 nm (Mittal et al. 2020). Nanoencapsulation of microbes brings several advantages over conventional microencapsulation. The nano-sized beads have a larger surface area, higher solubility, and bioavailability, which could improve the bioactivity of the microbes toward the target host. Such technology has been applied in probiotic bacteria using various nano-carriers, which demonstrated better protection of the encapsulated cells and microbial viability than microencapsulation (Misto et al. 2018; Pateiro et al. 2021; Razavi et al. 2021). Nevertheless, the nanoencapsulation of agriculturally important microbes is still in its infancy. Pour et al. (2019) indicated that the nano-encapsulated plant growth-promoting rhizobacteria (PGPR) and its microbial metabolite were able to improve the vegetative growth of in-vitro-grown pistachio plants. Metabolites derived from different *Bacillus* strains were also encapsulated into chitosan nanoparticles to control *Radophilus similis*, a plant parasitic nematode of bananas (Ureña-Saborío et al. 2017). In other words, the nanoencapsulation technology should be explored further to evaluate its microbial encapsulation and biocontrol efficacy against plant pathogens.

15.3 Encapsulation of BCAs in Plant Disease Management

Encapsulation of BCAs remained a topic of interest for most researchers as cells are protected within a polymer matrix, which results in improved microbial viability and production of antimicrobial metabolites or enzymes. Most of the studies preferred the use of the ionic gelation or extrusion method of encapsulation simply because of its cost-effectiveness and low production cost (Table 15.3). Additives can also be added to modify the capsule features such as additional nutrients for microbial growth, cell protectants for improving survivability, and clay materials for producing a slow-releasing formulation. The following section describes the current progress of the application of encapsulation technology in prolonging the viability and bioactivity of BCAs against plant pathogens.

One of the earliest alginates encapsulations of BCAs was performed by Fravel et al. (1985). The study reported the dried alginate formulation sustained the viability of fungal BCAs compared to *Pseudomonas cepacia*, a bacterial BCA, possibly due to the ability of fungal spores to tolerate desiccation stress during the drying process. To improve the viability of non-spore-forming bacteria in alginate beads, Russo et al. (1996) stored the fresh beads of *P. fluorescens* without drying in distilled water or sealed containers under dry conditions and successfully sustained the microbial viability after 8 weeks of storage at 4, 12, and 28 °C. The production of antifungal metabolite phloroglucinol was also greatly enhanced in encapsulated cells resulting in the growth inhibitory effect against the in-vitro-grown *Pythium ultimum*. El-Katatny et al. (2003) also indicated alginate encapsulated *Trichoderma harzianum* supplemented with 0.5% chitin or dried mycelium of the pathogenic *Fusarium oxysporum* increased the production of hydrolytic enzymes such as chitinase and beta 1,3 glucanase by 5- and 3-folds, respectively. These enzymes are commonly produced by BCAs to lyse the cell wall component of fungal pathogens. The production of the enzymes was affected by the concentration of alginate, incubation temperature, and bead size. A 2% alginate was suitable for chitinase production, whereas glucanase production was optimal at 4% alginate. This study also indicated that optimal temperatures for chitinase and -1,3-glucanase production were 40 and 35 °C, respectively, and larger bead size (8 mm) enhanced the production of the enzyme as well (El-Katatny et al. 2003). Kumaravel and Gopal (2010) also explained that bead size and alginate-to-cell ratio play an important role in antimicrobial metabolite production. Large bead size (2.7-3.0 mm) showed better production of these metabolites, whereas the increased ratio of cells will cause cell

Table 15.3 Previous encapsulation formulation of BCAs in the management of plant diseases

Carriers	Additives	Method	BCAs	Pathogen	Application method	Shelf-life study	Remarks	Reference
Sodium alginate	Pyrophyllite	Ionic gelation	*Talaromyces flavus* *Gliocadium virens* *Penicillium oxalicum* *Trichoderma viride* *Pseudomonas cepacia*	n.d.	n.d.	Viability sustained for all fungi for up to 12 weeks at room temperature except for non-spore-forming bacteria *P. ceapacia*	Use of calcium gluconate as a crosslinker improved microbial viability after drying	Fravel et al. (1985)
Sodium alginate	Not used	Ionic gelation	*Pseudomonas fluorecens* F113	*Pythium ultimum*	n.d.	Sustained viability when stored in distilled water or sealed container in a dry condition for up to 8 weeks at 4, 12, and 28 °C	Encapsulated cells showed enhanced production of antimicrobial metabolite—phloroglucinol • Inhibited the in-vitro growth of *P. ultimum*	Russo et al. (1996)
Sodium alginate	Chitin or dried mycelium of *Fusarium oxysporum*	Ionic gelation	*Trichoderma harzianum*	n.d.	n.d.	n.d.	Enhanced production of chitinase and glucanase of *T. harzianum* in alginate beads added with chitin or dried mycelium Immobilized cultures incubated at 40 and 35 °C are optimal for enzyme production	El-Katatny et al. (2003)

Sodium alginate	LB broth	Ionic gelation	*Pantoea agglomerans E325*	*Erwinia amylovora*	Floral dip	n.d.	Core–shell beads indicated cell release within 6 h at 4, 15, and 25 °C Alginate concentration ratio on the core and shell and bead size influence the release efficiency and cell viability No antagonistic assay was conducted	Kim et al. (2012)
Sodim alginate	Not used	Ionic gelation	*Pseudomonas fluorecens 134*	*Rhizoctonia solani*	Soil drenching and soil application	100% microbial viability in larger beads (2 mm) when stored as dried beads for 31 days at 4 °C compared to 0.5 mm and 1 mm beads	Biocontrol activity against *R. solani* is greater when alginate beads are dissolved in sodium citrate buffer and applied as soil drenching Slow release of encapsulated BCAs reduces root colonization efficiency leading to increased disease severity	Russo et al. (2008)
Maltodextrin	Not used	Spray drying	*Bacillus subtilis B99-2*	*Rhizoctonia solani*	Soil drenching	Stable up until 18 months at room temperature	Maltodextrin protects microbial cells during spray	Ma et al. (2015)

(continued)

Table 15.3 (continued)

Carriers	Additives	Method	BCAs	Pathogen	Application method	Shelf-life study	Remarks	Reference
					and foliar spray		drying with a survival rate of more than 90% Achieved 79.91% disease suppression compared to fungicide thiram	
Sodium alginate	Starch	Ionic gelation	*Metarhizium brunneum* and *Saccharomyces cerevisiae*	n.d.	n.d.	After 12 weeks at 35 °C: *M. brunneum*—9.06% and *S. cerevisiae*—30.7%	Calcium gluconate in place of calcium chloride as a crosslinker improved viability after drying and reduced hygroscopic property and shelf life Gluconate is metabolized by microbes to improve survivability after drying	Humbert et al. (2017)
Sodium alginate	Chitosan, peat powder, or skim milk	Ionic gelation	*Burkholderia cepacia*, *Bacillus* spp., *Trichoderma virens*	*Fusarium oxysporum* f. sp. *lycopersici*	Mix with infected soil at 0.5 g beads/kg soil	Wet microbeads have higher microbial viability compared to lyophilized beads for up to 6 months	Additives did not affect the encapsulation efficiency of microbes Peat sustained microbial viability during storage of wet microbeads	Szczech and Maciorowski (2016)

							and reduced contamination Control of tomato Fusarium wilt using beads loaded with *Bacillus* spp. is comparable to the fungicide thiram	
Chitosan-alginate	Copper cations	Ionic gelation	*Trichoderma viride*	n.d.	n.d.	n.d.	Copper ions and *T. viride* incorporated within chitosan/alginate matrix without growth inhibition of spores	Vinceković et al. (2016)
Sodium alginate	ChitosanStarch	Ionic gelation	*Bacillus velezensis* NH-1	*Fusarium oxysporum*	20 g of beads buried in soil	n.d.	Microbial viability in alginate beads alone is higher after storage at room temperature for 65 days Alginate beads without additives showed 100% control efficiency on Fusarium wilt in the field	Luo et al. (2019)
Starch, alginate, and poly(*N*-	Kaolin	Ionic gelation	Nontoxigenic *Aspergillus flavus*	Toxigenic *Aspergillus flavus*	n.d.	n.d.	Kaolin allows the slow release of spores Spore release from	Feng et al. (2020)

(continued)

Table 15.3 (continued)

Carriers	Additives	Method	BCAs	Pathogen	Application method	Shelf-life study	Remarks	Reference
isopropyl acrylamide)							the alginate matrix is temperature-responsive (30 °C)	
Sodium alginate	Soy protein isolate	Spray drying	*Meyerozyma guilliermondii*	*Colletotrichum gloeosporioides*	Liquid spray application	n.d.	Reduce severity of mango anthracnose by up to 65% without compromising postharvest quality	López-Cruz et al. (2020)
Sodium alginate	Not used	Ionic gelation	*Streptomyces palmae CMU-AB204*	*Ganoderma boninense*	Soil application	Maximum shelf life of 103 days when stored at 4 ° C as hydrogels	Reduced severity of basal stem rot of oil palm from 75.8% to 81.6%	Sujarit et al. (2020)
Sodium alginate	Not used	Ionic gelation	*Trichoderma harzianum*	*Sclerotiana sclerotiorum*	n.d.	Maintained viability stored as wet particles at 5 or 30 °C up to 120 days	Encapsulation improves the chitinolytic activityMicrobial viability sustained under UV light exposure Showed better in-vitro growth inhibition of *S. sclerotium* when encapsulated *T. harzianum* used	Maruyama et al. (2020)

Starch-alginate	Montmorillonite	Ionic gelation	*Trichoderma harzianum*	n.d.	n.d.	Maintained viability stored as dried particles for up to 7 months at 5 °C	Starch and montmorillonite protect fungal spores from desiccation stress and increase swelling for the efficient release of microbes	Adzmi et al. (2021)

n.d. not determined

leakage from the alginate matrix leading to reduced metabolite production. Until now, the exact mechanism of polymers in the production of antimicrobial metabolites was only briefly studied (Clermont et al. 2010).

To extend the shelf-life and viability of encapsulated BCAs, various modifications were introduced. Alginate beads loaded with *T. harzianum* were able to inhibit the in-vitro growth of *Sclerotiana sclerotium*, and the spore viability was retained after 7 days of UV radiation exposure (Maruyama et al. 2020). Additives such as montmorillonite clay fillers and carbon source, respectively, were added into the *T. harzianum*-loaded alginate capsules to improve the swelling degree of the dried beads so that the spore release efficiency can be improved. Starch was also added as filler to reduce the shrinkage of beads and, thus, improve the spore viability for up to 7 months when stored at 5 °C (Adzmi et al. 2021). Developing a slow-releasing formulation is desirable as labor and application costs can be greatly reduced. The release of *Bacillus velezensis* NH-1 in alginate capsules coated with chitosan was increased gradually as compared to the direct application of the microbe into the soil. Chitosan was found to bind with the alginate matrix forming a dense structure, which, in turn, reduced the permeability of BCAs while at the same time increasing the microbial encapsulation efficiency (Luo et al. 2019). In the same study, the double coating of chitosan and cassava-modified starch on the alginate capsules could further increase the encapsulation efficiency of *B. velezensis*, but the viability and permeability of the microbes into the environment were greatly affected. Slow-releasing encapsulated cells, in some cases, might not be that beneficial to crops when plant pathogens are established in the field. Russo et al. (2008) pointed out that the high population of pathogens might take the opportunity to outcompete the slow-releasing BCA from the capsules. In fact, the dried beads containing *P. fluorescens* 134 did not result in the suppression of *Rhizoctonia solani* compared to the use of a liquid formulation where microbial-loaded beads were dissolved in sodium citrate solution. Nonetheless, the application of slow-releasing encapsulated cells can still be beneficial in crop protection, especially when plant pathogen severity is low, or they can be applied as preventive means to keep crops healthy.

Kim et al. (2012) have also developed slow-releasing alginate capsules at the size of 100 μM that were made up of a shell and a core that was loaded with *Pantoea agglomerans* E325 suspended in nutrient-rich LB broth. The shell protects the cells from adverse environmental conditions while the softcore allows the cell to multiply and permeate through the core to control the fire blight of apple. Smaller capsule size was also found to improve the viability of *P. agglomerans* than larger capsules, perhaps because oxygen can better penetrate and distribute within the capsule. The release efficiency of the BCA was the greatest within 24 to 72 h, which allows efficient colonization of the apple blooms (i.e., they only last for 3 to 10 days), leading to better control of the fire blight disease (Kim et al. 2012). In contrast, Russo et al. (2008) discovered that larger dried beads (2 mm) were able to retain 100% viability of *P. fluorescens* 134 after 31 days of storage at 4 °C compared to 0.5–1.0 mm beads. Since the bead size affects the viability of different microbial genera, optimizing the bead size can be an important parameter to be included in future encapsulation studies. In another study, a temperature-responsive polymer—

poly(*N*-isopropyl acrylamide) (PNIPAAm)—was added to the starch–alginate matrix loaded with nontoxigenic *Aspergillus flavus* to control the aflatoxin contamination in peanut plants. The polymer will only disintegrate at 30 °C during the onset of flowering to better control the fungal pathogen from infecting and colonizing the blooms (Feng et al. 2020). Both studies demonstrated that understanding the ecology of plant pathogens is essential to designing an encapsulation technology that could protect crops at the disease-susceptible stage.

The addition of peat into alginate capsules loaded with *Bacillus* sp. and *T. virens* resulted in better microbial viability for up to 6 months as wet capsules (Szczech and Maciorowski 2016). Peat was found to reduce contamination with other microbes, although the reason behind this was not clearly mentioned. Cations such as copper ions were also added to chitosan–alginate beads containing *T. viride* to improve micronutrient deficiency in soils (Vinceković et al. 2016). The spore viability was not affected by the presence of copper ions. Such a study could possibly open new research revenues in the encapsulation of both BCAs and other essential plant nutrients to manage plant disease as well as to reduce the cost of purchasing chemical fertilizers. Humbert et al. (2017) replaced $CaCl_2$ with calcium gluconate (CG) for the polymerization of alginate. CG was discovered to be a potential substitute as a crosslinker, and it has improved microbial viability after drying and rehydration, which corroborated the findings of Fravel et al. (1985). It was proposed that microbes can metabolize gluconic acid as a nutrient source. CG was also proposed to have osmoprotectant properties that stabilize the cell membrane during the drying process. However, gentle rehydration must be conducted at high humidity to ensure maximum viability of the cells before the capsules are immersed for complete rehydration (Humbert et al. 2017).

Microencapsulation of BCAs through spray drying is a method that produces dried powder formulation, reduces contamination from other microbial species, and prolongs the shelf-life of microbes. As compared to using alginate as the sole polymer in the ionic gelation method, various low-cost carriers can be used as substitutes in the spray drying method. The use of maltodextrin as a carrier in spray drying of *B. subtilis* B99-2 indicated that a more than 90% survival rate in these microcapsules was reported. Maltodextrin served as a protectant against desiccation stress and high temperature during spray drying without compromising the biocontrol efficacy of the BCA in the suppression of *R. solani* in the field (Ma et al. 2015). Similarly, Ishak et al. (2020) used the same carrier maltodextrin on *T. asperellum* spores and found that they can be stored at 4 °C for up to 40 weeks. Jin and Curtis (2011) also achieved the highest survival percentage (about 80%) when a 2% sucrose solution was used in the spray drying process to produce microcapsules containing *T. harzianum* conidia. Sucrose microencapsulation formed a coating, which served as a plasma membrane stabilizer on the hydrophilic conidia during the rapid dehydration process of spray drying and during rehydration when it is applied to the field. The use of alginate and soy protein during spray drying also improved the swelling of beads during rehydration which caused the relaxation of polymer chains to increase the diffusion of *Meyerozyma guilliermondii*. Soy protein has also improved the microbial viability by up to 97.8%, which eventually led to the reduction of disease severity of mango anthracnose (López-Cruz et al. 2020).

15.4 Potential Challenges and Future Research Directions

One of the few crucial reasons to encapsulate BCAs is to protect the cells from external environmental factors, which could potentially reduce viability and bioactivity. When developing an encapsulation study, there are several factors (Fig. 15.2) that should be included to ensure the consistency of the encapsulated BCAs against plant pathogens. Several articles have extensively reviewed the importance of these factors in microbial encapsulation (Berninger et al. 2018; Vassilev et al. 2020; Saberi-Riseh et al. 2021). Therefore, the following section includes other challenges or factors that researchers can consider in improving the encapsulation and biocontrol efficiency of the BCAs for future studies.

15.4.1 Choice of Microbes

Most studies were conducted on the encapsulation efficiency of a single BCA strain. In general, the outcomes of these studies are often positive, with extended microbial viability during storage and reduced disease severity in crops infected with plant pathogens. The use of microbial consortia has received attention lately due to better disease suppression as compared to the use of a single BCA strain (Czajkowski et al. 2020; Niu et al. 2020). The exact mechanism is not known, but some studies have proposed that the suppression is likely because of multiple modes of antagonism expressed by the respective strains present in the microbial consortia (Thakkar and Saraf 2015; Mukherjee et al. 2021). In fact, the successful co-encapsulation of plant growth-promoting microbes such as *Rhizobium* and mycorrhizal fungi has been widely reported (John et al. 2011). So far, there are limited studies available on the encapsulation of multiple BCA strains. Wong et al. (2019) reported the co-encapsulation of *P. aeruginosa* and *T. harzianum* in alginate beads, but the microbial viability of both compatible BCA strains can be sustained up to 150 days of storage at 4 °C, but the viability dropped drastically after 30 days of

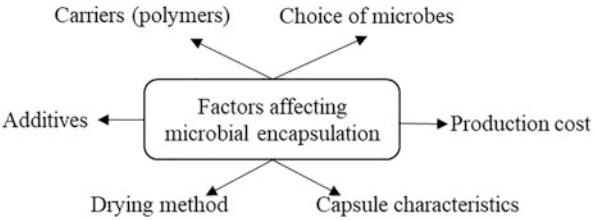


Fig. 15.2 Factors affecting encapsulation of BCAs

storage at room temperature. Further research is needed to investigate the encapsulation efficiency of a consortium of BCAs to achieve stable microbial viability and biocontrol efficacy against plant pathogens.

15.4.2 Alternative Low-Cost Carrier Materials

Although the immobilization of BCAs seemed promising as controlled-release biopesticides, the higher cost of polymer-based carriers than solid and liquid formulations is the major setback for large-scale production and farmers' application in the field (Bashan et al. 2016; Vassilev et al. 2020). For instance, low-cost carriers such as chitosan-starch-based polymeric carriers were used to produce macro-beads containing two genera of plant growth-promoting bacteria with the controlled-release property. Microbial viability in these beads was also sustained for up to 12 months at 25 °C with 70–80% relative humidity (Perez et al. 2018). Nevertheless, the preparation of the beads was relatively time-consuming and required different chemicals as compared to the preparation of alginate beads. Moreover, most of the low-cost carriers such as gum arabic, gellan gum, cornflour, malt dextrin, skim milk, and whey protein require a high initial cost of procuring spray dryer equipment for microencapsulation (Saberi-Riseh et al. 2021). Perhaps, a cost–benefit ratio analysis of using different encapsulation carrier materials should be conducted to provide a holistic overview of which carrier materials are cost-effective for the agriculture industry in the long run.

15.4.3 Features of Capsules

Very often, microbial-loaded capsules are characterized by their size, swelling characteristics, and encapsulation efficiency. For instance, microbial viability depends on the capsule size. Chandramouli et al. (2004) found that a larger capsule size (500–1000 μM) was found to improve the viability of microbes, but Sønderholm et al. (2017) reported that oxygen diffusion reduces greatly after reaching a 100 μM depth of 1000 μM alginate beads. The anoxia condition was relieved by providing nitrate anions, which allowed the bacteria to grow deeper into the beads. Understanding and improving the oxygen diffusion rates and concentration in the capsules could provide some useful insights for researchers to improve the encapsulation efficiency as well as to prolong microbial viability.

15.4.4 Scaling Up of Encapsulated Microbes

Scaling up immobilized microbial cells is still a topic of interest among researchers searching for low-cost and optimal methods for use in the biofertilizer industry. Developing an optimal microbial biomass production requires careful planning to ensure cost-effectiveness in the use of culture medium and culturing conditions so that the production of biomass can meet the supply and demand (Crater and Lievense 2018; Lobo et al. 2020). To produce microbial-loaded encapsulated beads, liquid-state fermentation is preferred over solid-state fermentation due to the issues that cause contamination, and the culture conditions can be better controlled when microbes are cultured in the submerged state (Cumagun 2014; Mascarin et al. 2019). For instance, the conidia and microsclerotia of *T. asperellum* were mass-produced in a liquid fermentation and were encapsulated in alginate beads with microbial viability recorded up to 120 days when stored at 8 °C (de Oliveira Lopes et al. 2020). The only limitation of using such a fermentation method is the use of costly chemicals used in the nutrient medium, and the search for affordable alternatives is, therefore, necessary (Lobo et al. 2020).

15.5 Conclusions

Attempts to improve the encapsulation technology of BCAs are ongoing with the aim of producing a formulation that has a longer shelf-life and bioactivity against plant pathogens. Hence, the design of the BCA-loaded capsules should be able to address most of the critical factors that could affect microbial viability, if not all. The understanding of the crop physiology and ecology of plant pathogens should also be incorporated into microbial encapsulation studies so that disease severity can be greatly reduced during a crop's most susceptible stage. Finally, nanoencapsulation technology presents exciting potential for researchers to evaluate its control-release properties, encapsulation, and biocontrol efficacy against plant pathogens compared to microencapsulation. The impacts of such nanoparticles on the environment and biodiversity should be elucidated as well to ensure such technology can be widely adopted in the agriculture sector. As technology advances, microbial encapsulation is likely to remain a technique that presents unlimited potential for improvements and commercialization in the foreseeable future.

References

Adzmi F, Musa MH, Siddiqui Y, Yun WM, Hamid HA, Abdu A, Abiri R (2021) Development of alginate-montmorillonite-starch with encapsulated *Trichoderma harzianum* and evaluation of conidia shelf life. Int J Agric Biol 26:87–96. https://doi.org/10.17957/IJAB/15.1812

Alori ET, Babalola OO (2018) Microbial inoculants for improving crop quality and human health in Africa. Front Microbiol 9:2213. https://doi.org/10.3389/fmicb.2018.02213

Bardin M, Ajouz S, Comby M, Lopez-Ferber M, Graillot B, Siegwart M, Nicot PC (2015) Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Front Plant Sci 6:566. https://doi.org/10.3389/fpls.2015.00566

Bashan Y, de-Bashan LE, Prabhu SR (2016) Superior polymeric formulations and emerging innovative products of bacterial inoculants for sustainable agriculture and the environment. In: Singh HB, Sarma BK, Keswani C (eds) Agriculturally important microorganisms—commercialization and regulatory requirements in Asia. Springer, Berlin, pp 15–46

Berninger T, López OG, Bejarano A, Preininger C, Sessitsch A (2018) Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. Microb Biotechnol 11: 277–301. https://doi.org/10.1111/1751-7915.12880

Besset-Maszoni Y, Joly P, Brutel A, Gerin F, Soudière O, Langin T, Prigent-Combaret C (2019) Does in vitro selection of biocontrol agents guarantee success in planta? A study case of wheat protection against Fusarium seedling blight by soil bacteria. PLoS One 14(12):e0225655. https://doi.org/10.1371/journal.pone.0225655

Chandramouli V, Kailasapathy K, Peiris P, Jones M (2004) An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. J Microbiol Methods 56:27–35. https://doi.org/10.1016/j.mimet.2003.09.002

Chen S, Arnold WA, Novak PJ (2021) Encapsulation technology to improve biological resource recovery: recent advancements and research opportunities. Environ Sci Water Res Technol 7: 16–23. https://doi.org/10.1039/D0EW00750A

Clermont N, Legault G, Lerat S, Beaulieu C (2010) Effect of biopolymers on geldanamycin production and biocontrol ability of *Streptomyces melanosporofaciens* strain EF-76. Canadian J Plant Pathol 32:481–489. https://doi.org/10.1080/07060661.2010.512121

Crater JS, Lievense JC (2018) Scale-up of industrial microbial processes. FEMS Microbiol Lett 365:fny138. https://doi.org/10.1093/femsle/fny138

Cumagun CJR (2014) Advances in formulation of Trichoderma for biocontrol. In: Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG (eds) Biotechnology and biology of Trichoderma. Elsevier, Amsterdam, pp 527–531

Czajkowski R, Maciag T, Krzyzanowska DM, Jafra S (2020) Biological control based on microbial consortia – from theory to commercial products. In: De Cal A, Melgarejo P, Magan N (eds) How research can stimulate the development of commercial biological control against plant diseases. Progress in biological control, vol 21. Springer, Cham, pp 183–202

de Oliveira Lopes AR, Locatelli GO, de Melo BR, Junior LM, Mascarin GM, Finkler CLL (2020) Preparation, characterisation and cell viability of encapsulated *Trichoderma asperellum* in alginate beads. J Microencapsul 37:270–282. https://doi.org/10.1080/02652048.2020.1729884

El-Katatny MH, Hetta AM, Shaban GM, El-Komy HM (2003) Improvement of cell wall degrading enzymes production by alginate encapsulated *Trichoderma* spp. Food Technol Biotechnol 41: 219–225. https://hrcak.srce.hr/118617

Feng J, Dou J, Zhang Y, Wu Z, Yin D, Wu W (2020) Thermosensitive hydrogel for encapsulation and controlled release of biocontrol agents to prevent peanut aflatoxin contamination. Polymers 12:547. https://doi.org/10.3390/polym12030547

Fravel DR, Marois JJ, Lumsden RD, Connick WJ Jr (1985) Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathology 75:774–777. https://doi.org/10.1094/Phyto-75-774

Humbert P, Przyklenk M, Vemmer M, Patel AV (2017) Calcium gluconate as cross-linker improves survival and shelf life of encapsulated and dried *Metarhizium brunneum* and *Saccharomyces cerevisiae* for the application as biological control agents. J Microencapsul 34:47–56. https://doi.org/10.1080/02652048.2017.1282550

Ishak AA, Zulkepli FRA, Hayin NFM, Md Zain NH, Sapak Z (2020) Effect of high inlet temperature of spray dryer on viability of microencapsulated *Trichoderma asperellum* conidia. IOP Conf Ser Earth Environ Sci 757:012023. https://doi.org/10.1007/s42770-022-00832-z

Jin X, Curtis D (2011) Microencapsulating aerial conidia of *Trichoderma harzianum* through spray drying at elevated temperatures. Biol Control 56:202–208. https://doi.org/10.1016/j.biocontrol.2010.11.008

John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D (2011) Bio-encapsulation of microbial cells for targeted agricultural delivery. Crit Rev Biotechnol 31:211–226. https://doi.org/10.3109/07388551.2010.513327

Kim I-Y, Pusey PL, Zhao Y, Korban SS, Choi H, Kim KK (2012) Controlled release of *Pantoea agglomerans* E325 for biocontrol of fire blight disease of apple. J Control Release 161:109–115. https://doi.org/10.1016/j.conrel.2012.03.028

Kumaravel V, Gopal SR (2010) Immobilization of *Bacillus amyloliquefaciens* MBL27 cells for enhanced antimicrobial protein production using calcium alginate beads. Biotechnol Appl Biochem 67:97–103. https://doi.org/10.1042/BA20100252

Lengyel M, Kállai-Szabó N, Antal V, Laki AJ (2019) Microparticles, microspheres, and microcapsules for advanced drug delivery. Sci Pharm 87:20. https://doi.org/10.3390/scipharm87030020

Lobo CB, Tomás MSJ, Viruel E, Ferrero MA, Lucca ME (2020) Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. Microbiol Res 219:12–25. https://doi.org/10.1016/j.micres.2018.10.012

López-Cruz R, Ragazzo-Sánchez JA, Calderón-Santoyo M (2020) Microencapsulation of *Meyerozyma guilliermondii* by spray drying using sodium alginate and soy protein isolate as wall materials: a biocontrol formulation for anthracnose disease of mango. Biocontrol Sci Tech 30:1116–1132. https://doi.org/10.1080/09583157.2020.1793910

Luo W, Liu L, Qi G, Yang F, Shi X, Zhao X (2019) Embedding *Bacillus velezensis* NH-1 in microcapsules for biocontrol of cucumber Fusarium wilt. Appl Environ Microbiol 85:e03128–e03118. https://doi.org/10.1128/AEM.03128-18

Ma X, Wang X, Cheng J, Nie X, Yu X, Zhao Y, Wang W (2015) Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficacy against *Rhizoctonia solani* in tomato. Biol Con 90:34–41. https://doi.org/10.1016/j.biocontrol.2015.05.013

Majeed Z, Ramli NK, Mansor N, Man Z (2015) A comprehensive review on biodegradable polymers and their blends used in controlled-release fertilizer processes. Rev Chem Eng 31:69–95. https://doi.org/10.1515/revce-2014-0021

Maruyama CR, Bilesky-José N, de Lima R, Fraceto LF (2020) Encapsulation of *Trichoderma harzianum* preserves enzymatic activity and enhances the potential for biological control. Front Bioeng Biotechnol 8:225. https://doi.org/10.3389/fbioe.2020.00225

Mascarin GM, Lopes RB, Delalibera I Jr, Fernandes EKK, Luz C, Faria M (2019) Current status and perspectives of fungal entomopathogens used for microbial control of arthropod pests in Brazil. J Invertebr Pathol 165:46–53. https://doi.org/10.1016/j.jip.2018.01.001

Misto YIP, Sitorus RDM, Permatasari ID, Noor E, Sunarti TC (2018) Formulation and storage stability of nanoencapsulated *Proteus penneri* and *Bacillus aerophilus*. IOP Conf Ser Earth Environ Sci 209:012040. https://doi.org/10.1088/1755-1315/209/1/012040

Mittal D, Kaur G, Singh P, Yadav K, Ali SA (2020) Nanoparticle-based sustainable agriculture and food science: recent advances and future outlook. Front Nanotechnol 2:579954. https://doi.org/10.3389/fnano.2020.579954

Mitter EK, Tosi M, Obregón D, Dunfield KE, Germida JJ (2021) Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies. Front Sustain Food Syst 5:606815. https://doi.org/10.3389/fsufs.2021.606815

Mukherjee A, Chouhan GK, Gaurav AK, Jaiswal DK, Verma JP (2021) Development of indigenous microbial consortium for biocontrol management. In: Verma JP, Macdonald CA, Gupta VK, Podile AR (eds) New and future developments in microbial biotechnology and bioengineering: phytomicrobiome for sustainable agriculture. Elsevier, India, pp 91–104

Niu B, Wang W, Yuan Z, Sederoff RR, Sederoff H, Chiang VL, Borriss R (2020) Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. Front Microbiol 11:585404. https://doi.org/10.3389/fmicb.2020.585404

Pateiro M, Gómez B, Munekata PES, Barba FJ, Putnik P, Kovačević DB, Lorenzo JM (2021) Nanoencapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products. Molecules 26:1547. https://doi.org/10.3390/molecules26061547

Perez JJ, Francois NJ, Maroniche GA, Borrajo MP, Pereyra MA, Creus CM (2018) A novel, green, low-cost chitosan-starch hydrogel as potential delivery system for plant growth-promoting bacteria. Carbohydr Polym 202:409–417. https://doi.org/10.1016/j.carbpol.2018.07.084

Pirttilä AM, Tabas HMP, Baruah N, Koskimäki JJ (2021) Biofertilizers and biocontrol agents for agriculture: how to identify and develop new potent microbial strains and traits. Microorganisms 9:817. https://doi.org/10.3390/microorganisms9040817

Pour MM, Saberi-Riseh R, Mohammadinejad R, Hosseini A (2019) Nano-encapsulation of plant growth-promoting rhizobacteria and their metabolites using alginate-silica nanoparticles and carbon nanotube improves UCB1 Pistachio micropropagation. J Microbiol Biotechnol 29: 1096–1103. https://doi.org/10.4014/jmb.1903.03022

Raimondi G, Maucieri C, Toffanin A, Renella G, Borin M (2021) Smart fertilizers: what should we mean and where should we go? Italian J Agron 16:1794. https://doi.org/10.4081/ija.2021.1794

Razavi S, Janfaza S, Tasnim N, Gibson DL, Hoorfar M (2021) Nanomaterial-based encapsulation for controlled gastrointestinal delivery of viable probiotic bacteria. Nanoscale Adv 3:2699–2709. https://doi.org/10.1039/D0NA00952K

Russo A, Moënne-Loccoz Y, Fedi S, Higgins P, Fenton A, Dowling DN, O'Regan M, O'Gara F (1996) Improved delivery of biocontrol *Pseudomonas* and their antifungal metabolites using alginate polymers. Appl Microbiol Biotechnol 44:740–745

Russo A, Basaglia M, Casella S, Nuti MP (2008) *Pseudomonas fluorescens* 134 as a biological control agent (BCA) model in cell immobilization technology. Biotechnol Prog 21:309–314. https://doi.org/10.1021/bp040030w

Saberi-Riseh R, Moradi-Pour M, Mohammadinejad R, Thakur VK (2021) Biopolymers for biological control of plant pathogens: advances in microencapsulation of beneficial microorganisms. Polymers 13:1938. https://doi.org/10.3390/polym13121938

Schoebitz M, López MD, Roldán A (2013) Bioencapsulation of microbial inoculants for better soil-plant fertilization. A review. Agron Sustain Dev 33:751–765. https://doi.org/10.1007/s13593-013-0142-0

Schulz AN, Lucardi RD, Marsico TD (2019) Successful invasions and failed biocontrol: the role of antagonistic species interactions. BioSci 69:711–724. https://doi.org/10.1093/biosci/biz075

Seema K, Mehta K, Singh N (2018) Studies on the effect of plant growth promoting rhizobacteria (PGPR) on growth, physiological parameters, yield and fruit quality of strawberry cv. Chandler J Pharmacogn Pytochem 7:383–387

Sønderholm M, Kragh KN, Koren K, Jakobsen TH, Darch SE, Alhede M, Jensen PØ, Whiteley M, Kühl M, Bjarnsholt T (2017) *Pseudomonas aeruginosa* aggregate formation in an alginate bead model system exhibits *in vivo*-like characteristics. Appl Environ Microbiol 83:e00113–e00117. https://doi.org/10.1128/AEM.00113-17

Sujarit K, Pathom-aree W, Mori M, Dobashi K, Shiomi K, Lumyong S (2020) *Streptomyces palmae* CMU-AB204^T, an antifungal producing-actinomycete, as a potential biocontrol agent to protect palm oil producing trees from basal stem rot disease fungus, *Ganoderma boninense*. Biol Control 148:104307. https://doi.org/10.1016/j.biocontrol.2020.104307

Szczech M, Maciorowski R (2016) Microencapsulation technique with organic additives for biocontrol agents. J Hort Res 24:111–122. https://doi.org/10.1515/johr-2016-0013

Thakkar A, Saraf M (2015) Development of microbial consortia as a biocontrol agent for effective management of fungal diseases in Glycine max L. Arch Phytopathol Plant Protect 48:457–474. https://doi.org/10.1080/03235408.2014.893638

Ureña-Saborío H, Madrigal-Carballo S, Sandoval J, Vega-Baudrit JR, Rodríguez-Morales A (2017) Encapsulation of bacterial metabolic infiltrates isolated from different Bacillus strains in chitosan nanoparticles as potential green chemistry-based biocontrol agents against *Radophilus similis*. J Renew Mater 5:290–299. https://doi.org/10.7569/JRM.2017.634119

Vassilev N, Vassileva M, Martos V, Garcia del Moral LF, Kowalska J, Tylkowski B, Malusá E (2020) Formulation of microbial inoculants by encapsulation in natural polysaccharides: focus on beneficial properties of carrier additives and derivatives. Front Plant Sci 11:270

Vemmer M, Patel AV (2013) Review of encapsulation method suitable for microbial biological control agents. Biol Con 67:380–389. https://doi.org/10.1016/j.biocontrol.2013.09.003

Vinceković M, Jalšenjak N, Topolovec-Pintarić S, Đermić E, Bujan M, Jurić S (2016) Encapsulation of biological and chemical agents for plant nutrition and protection: chitosan/alginate microcapsules loaded with copper cations and *Trichoderma viride*. J Agric Food Chem 64: 8073–8083. https://doi.org/10.1021/acs.jafc.6b02879

Winder RS, Wheeler JJ, Conder N, Otvos IS, Nevill R, Duan L (2003) Microencapsulation: a strategy for formulation of inoculum. Biocontrol Sci Tech 13:155–169. https://doi.org/10.1080/0958315021000073439

Wong CKF, Saidi NB, Vadamalai G, Teh CY, Zulperi D (2019) Effect of bioformulations on the biocontrol efficacy, microbial viability and storage stability of a consortium of biocontrol agents against fusarium wilt of banana. J Appl Microbiol 127:544–555. https://doi.org/10.1111/jam.14310

Chapter 16
Designing Tailored Bioinoculants for Sustainable Agrobiology in Multi-stressed Environments

Eloísa Pajuelo, José A. Carrasco, Noris J. Flores-Duarte,
Ignacio D. Rodríguez-Llorente, Jennifer Mesa-Marín,
Enrique Mateos-Naranjo, Susana Redondo-Gómez,
and Salvadora Navarro-Torre

Abstract The increasing demand of plant-origin products (food, animal feed, wood, paper, biofuels, etc.), combined with soil degradation and climate change, depict a scenario of low plant productivity unable to satisfy such demands. Raising use of agrochemicals compromises the environment through pollution and eutrophication. At regulatory level, new agro-environmental regulations recognize the use of biofertilizers, and companies show increasing interest in plant growth-promoting and biocontrol microorganisms (PGPR, endophytes, mycorrhizal fungi). In this review, the steps for the development of autochthonous biofertilizers are revised. After the isolation of cultivable strains, a core consortium of microorganisms with plant growth-promoting activities is selected focusing in the following characteristics: (a) high tolerance toward biotic stresses (salt, high temperatures, drought, metals, xenobiotics, cold, etc.); (b) presence of multifarious plant growth-promoting traits (nitrogen fixation, phosphate and potassium solubilization, production of IAA and other phytohormones, HCN, aminocyclopropane carboxylic acid ACC deaminase activity, degrading activities such as cellulases, pectinases, proteases, etc.); (c) high competitiveness in the rhizosphere (motility, formation of biofilms, quorum

---

E. Pajuelo (✉) · N. J. Flores-Duarte · I. D. Rodríguez-Llorente · S. Navarro-Torre
Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain
e-mail: epajuelo@us.es; nflores@us.es; irodri@us.es; snavarro1@us.es

J. A. Carrasco (✉)
Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, Seville, Spain

Earlham Institute, Norwich, Norfolk, UK
e-mail: jcarrascol@us.es

J. Mesa-Marín · E. Mateos-Naranjo · S. Redondo-Gómez
Department of Plant Biology and Ecology, Faculty of Biology, University of Seville, Seville, Spain
e-mail: jmesam@us.es; emana@us.es; susana@us.es

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_16

sensing and quenching, secretion of siderophores and extracellular enzymes, cellulase, etc.); and (d) safety to humans, animals, and the environment. Omics offer tremendous possibilities at the time of selecting the best candidates for the consortium, and culturomics can increase the number of cultivable strains based on specific plant-based culture media. Scaling up experiments from greenhouse to pilot scale and then to field must be accompanied of stabilization and long-life of the inoculant, which must be optimized in each case. In this particular, the development of local, tailored biofertilizers can be envisioned as an opportunity for local biotechnology companies and social development.

Keywords Climate change · Soil degradation · Plant productivity · Biofertilizers · PGPR · Endophytes · Omics

16.1 Introduction

In the film "The Martian" directed by Ridley Scott in 2015, astronaut Mark Watney—played by Matt Damon—is trapped alone on Mars watching his last potato stocks run out. He decides growing the last remaining potatoes rather than eating them but, to ensure successful cultivation, he uses the frozen and vacuum-packed feces of himself and his deceased companions. Bacteria present in them as well as nutrients such as organic matter, P, N, K, or S improved plant grow and yield allowing his survival. Despite being a futuristic science fiction film, this is not a novel approach since animal manure has been used as fertilizer since time immemorial (Sheldrick et al. 2003). Human excreta have been also used, with previous composting treatment in order to eliminate pathogenic microorganisms (Heinonen-Tanski and van Wijk-Sijbesma 2005; Phuc et al. 2006).

However, the use of excrement without prior treatment and control may pose a risk to food safety due to the possibility of transmission of pathogens (Gwara et al. 2021). In contrast, biofertilizers based on the cultivation and large-scale production of particular and well-known microorganisms with plant growth-promoting properties (PGPR) and safe (Generally Recognized As Safe: GRAS) constitute an alternative to the indiscriminate and generally oversized use of chemical fertilizers, which end up contaminating aquifers (Menéndez and García-Fraile 2017; Srivastav 2020). In this sense, eutrophication has become one of the main water quality problems for most of the world's freshwater and coastal marine ecosystems (Griffith and Gobler 2020). A recent example of this is frequent eutrophication of Mar Menor in Murcia (Spain) with the death of almost 5 tons of fish in summer 2021 (https://elpais.com/clima-y-medio-ambiente/2021-08-22/removed-45-tons-of-dead-fish-en-el-mar-minor.html).

In this sense, the use of biofertilizers is being promoted through the adoption of agro-environmental policies such as the Regulation (EU) 2019/1009, laying down rules on the market of EU fertilizing products (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1009 & from=EN). Moreover, the availability of agricultural soil is limited due to the demand of food and feed for

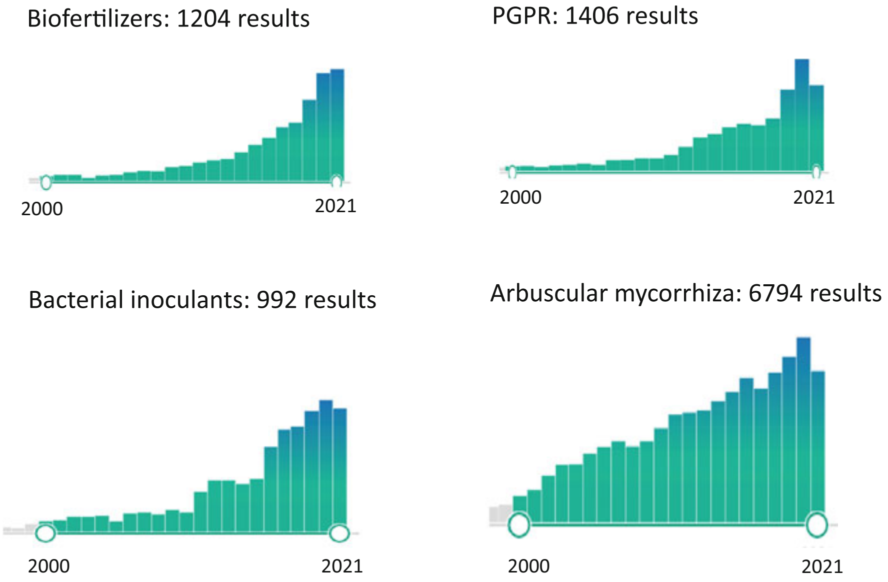


Fig. 16.1 Publications found in Pubmed in the last 20 years after using as keywords: biofertilizers, bacterial inoculants, arbuscular mycorrhiza, and PGPR

increasing human population and animals, together with the competing use of agricultural land for other uses such as wood, fibers, paper, biofuels, etc. (FAO 2015; Borrelli et al. 2020). This will finally lead to the utilization of marginal soils for plant cultivation. In fact, future scenarios depict climate change, with severe drought periods, high temperatures, desertification, increased salinity or contamination of soils, floods, etc. These stress conditions have a wide range of effects on the morphology, physiology, and biochemistry of plants (Sammauria et al. 2020). Biofertilizers will surely constitute an alternative to chemical fertilizers and help to counteract their impact by reducing the amount necessary to maintain the productivity of crops under predictable stress situations (García-Fraile et al. 2015; Chakraborty and Akhtar 2021; Mahanty et al. 2017). For these reasons, it is a very active focus of research and scientific production, as reflects in Fig. 16.1, which shows the increases in the total number of publications found in Pubmed in the last 20 years after using as keywords: biofertilizers, bacterial inoculants, arbuscular mycorrhiza, and PGPR.

16.2 Plant Allies: How Do They Work?

The plant microbiome is composed by microorganisms that colonize internal plant tissues (endophytes) and external surfaces and organize themselves into microbial communities (Trivedi et al. 2020). Most of these microorganisms obtain their carbon sources from the host plant and supply the host with essential nutrients such as nitrogen, phosphorus, potassium, and sulfur, besides other benefits, such as water retention. Such host–microbiome interactions are crucial for plant health, growth and development, and plant defense against biotic and abiotic stresses (Qiu et al. 2019; Nosheen et al. 2021). Based on their interaction with plants, microorganisms associated with them can be classified into three groups: beneficial, harmful, and neutral (Kumar and Verma 2018). Beneficial microorganisms, termed PGPM (plant growth-promoting microorganisms) include rhizobacteria, endophytes, and mycorrhizal fungi and are known to exert a stimulant effect on plant growth, yield, or resilience toward an array of stress conditions (Nadeem et al. 2014; Du Jardin 2015; García-Fraile et al. 2015). According to the last author, "a plant biostimulant is any substance or microorganism applied to plants with the aim of improving nutritional efficiency, tolerance to abiotic stress and/or quality traits of the crop, regardless of its nutrient content" (Du Jardin 2015).

Currently, abundant information is being produced on the mechanisms by which beneficial microorganisms exert their stimulant action on plants. These mechanisms of action can be classified into two types: (a) direct mechanisms, including nutrient uptake, degradation of organic matter in the rhizosphere and nutrient recycling, and regulation of plant growth and development via production of phytohormones, and (b) indirect mechanisms, such as biocontrol, secretion of siderophores or antibiotics, production of ACC deaminase for alleviating plant stress, induction of the systemic resistance, and the systemic acquired resistance or bio/phytoremediation (Etesami and Maheshwari 2018; Martínez-Hidalgo et al. 2019).

16.2.1 Improving Nutrient Acquisition

Typically, chemical fertilizers include three elements crucial for plant development and growth, i.e., NPK (Roba 2018), so it is not surprising that nitrogen fixation as well as potassium and phosphorous solubilization are some of the properties required for PGPM. Besides, mobilization of micronutrients such as Cu or Zn is also desired (Tariq et al. 2007; Teotia et al. 2017).

16.2.1.1 Dinitrogen Fixation

Regarding N acquisition, the utilization of rhizobial inoculants for legumes crops is one of the oldest practices (Arora et al. 2017). The first patent was granted in the US in 1896 to inoculate legume field crops with nitrogen-fixing species of *Rhizobium* (Nobbe and Hiltner 1896). On its hand, the first non-symbiotic microorganism (*Azospirillum*) to promote plant growth was launched commercially in 1982 (Alexandre 2017). Diazotrophs are microorganisms that can fix nitrogen in free-living conditions, in the rhizosphere, as endophyte microorganisms, or in symbiosis (collectively known as rhizobia) (Kaschuk and Hungria 2017). Nitrogen fixation, i.e., the reduction of atmospheric N_2 to ammonia is an energy-consuming reaction carried out by the nitrogenase enzymatic complex and requires 16 magnesium ATP (MgATP), eight protons, and eight electrons (Seefeldt et al. 2009). This enzymatic complex is composed of two component proteins called the Fe protein and the MoFe protein. The structural genes of the nitrogenase complex (*nif*) are highly conserved among all nitrogen-fixing bacteria (Steenhoudt and Vanderleyden 2000). However, after comparing symbiotic and free-living strains of *Bradyrhizobium*, authors have concluded that the free-living *nif* cluster represents a more ancestral version compared to that in symbiotic lineages and horizontal gene transfer is the driving mechanism for evolution of free-living nitrogen fixation (Tao et al. 2021). On their side, rhizobia, in specific association with legumes, represent the most effective group in supplying N to agricultural systems (Kaschuk and Hungria 2017). The effect of diazotrophs as PGPM goes beyond atmospheric N_2 fixation and many of them have other PGP properties such as IAA (indoleacetic acid) production, induction of the tolerance of abiotic stresses, and promotion of defense strategies such as the expression of pathogenesis-related genes (Fukami et al. 2018). Table 16.1 shows examples of diazotrophs for growth promotion in several plant species. Besides, there is increasing evidence that bacteria other than rhizobia nodulate legume in stress conditions such as arid or semiarid environments, heavy metal pollution, etc., including *Ochrobactrum*, *Paenibacillus*, *Devosia*, *Cupriavidus*, etc. (Balachandar et al. 2007, Table 16.1). In these stress situations, it is also frequent the presence of nodulating helper bacteria (NHP) that enter the plant at the same time as rhizobia and occupy the nodules (Etesami and Adl 2020; Flores-Duarte et al. 2022a). Another mechanism for action of NHB is the diminution of ethylene levels by means of the ACC deaminase activity, which finally leads to better nodulation and nitrogen fixation in stressed plants (Nascimento et al. 2019; Flores-Duarte et al. 2022b).

16.2.1.2 Phosphate Solubilization

Phosphate is one of the most limiting elements in soils, so P fertilization is compulsory. The exhaustion of mines of phosphate used for chemical fertilization is aggravating the problem of low levels of P in soils (Alewell et al. 2020). Even in soils where the total P concentration could be sufficient, usually the amount of available P is low due to precipitation of phosphate salts, binding to organic fraction of soils, etc. (Ara et al. 2018). Microorganisms can mobilize phosphorous from soils in different ways. Inorganic P can be mobilized by releasing organic acids such as lactic, maleic, malic, oxalic, ketobutyric, acetic, etc., releasing protons (acidolysis) or CO_2 (Alori et al. 2017). Then, phosphate can be transported into the bacterial cell (or the plant root) by high and low affinity phosphate transporters (Wang et al. 2017). Besides, P can form part of organic compounds in C–P bonds, which can be hydrolyzed by extracellular phosphatases (Alori et al. 2017). Special mention deserve compounds containing inositol–P bonds (phytates) are not readily available to plants but can be hydrolyzed by microbial phytases (Singh and Satyanarayana 2011). The huge battery of P hydrolyzing enzymes and transporters reveals the

Table 16.1 Examples of diazotrophs for growth promotion in several plant species

N_2-fixing microorganisms	Compartment	Bacteria	Host plants	References
Associated with plant roots	Rhizosphere	*Bacillus* *Pseudomonas* *Enterobacter* *Sphingomonas* *Paenibacillus*	Sugarcane Sugarcane Wheat *Dendrobium officinale* Different plants	Singh et al. (2020) Li et al. (2017) Ji et al. (2020) Yang et al. (2014) Liu et al. (2019a)
Endophytes	Endosphere, Phyllosphere (Endophytes)	*Azotobacter* *Azospirillum* *Herbaspirillum* *Gluconacetobacter*	Rice Rice Tropical grasses Sugarcane	Banik et al. (2019) Kaneko et al. (2010) Pedrosa et al. (2011) Bertalan et al. (2009)
Symbiotic (rhizobia)	Nodules of legumes	*Bradyrhizobium* *Ensifer* (*Sinorhizobium*) *Rhizobium* *Mesorhizobium* *Azorhizobium*	Soybean, *Cytisus*, *Lupinus* Alfalfa Pea, *Trifolium*, *Phaseolus* *Lotus*, chickpea *Sesbania rostrata*	Andrews and Andrews (2017) Rivas et al. (2009)
Symbiotic (non-rhizobia)	Nodules of legumes	*Methylobacterium* *Devosia* *Ochrobactrum* *Cupriavidus* *Paraburkholderia* *Microvirga* *Variovorax* (commensal)	*Lupinus* *Medicago* *Cytisus* *Calicotome villosa*	Andrews and Andrews (2017) Rivas et al. (2009) Bessadok et al. (2020)
Cyanobacteria Free-living	(Rhizosphere)	*Calothrix*, *Westiellopsis*, *Hapalosiphon* and *Nostoc*	Wheat	Karthikeyan et al. (2009)
Cyanobacteria (symbiotic)	Endophyte	*Anabaena*	Azolla (fern)	Pereira (2017)
		Nostoc	Rice	Álvarez et al. (2020)

scarcity and importance of this nutrient, for which bacteria have multiple ways of acquisition (Pajuelo et al. 2021).

Table 16.2 shows several examples of phosphate solubilizing microorganisms. In this case, both rhizosphere and endophytic bacteria display this PGP activity. Besides, it is important to highlight the relevance of mycorrhizal associations for the biogeochemical cycle of this element. Mycorrhiza are able to establish non-specific symbiotic interactions with around 90% of terrestrial plants (Bonfante

Table 16.2 Phosphate solubilizing microorganisms from the rhizosphere and endophytes of plants

P solubilizing microorganisms	Compartment	Examples of PGPM	Host plants	References
Associated with plant roots	Rhizosphere	*Bacillus* *Pseudomonas* *Enterobacter* *Proteus*, *Pseudomonas*, *Rhizobium*	*Mentha arvensis* Wheat Rice Alfalfa	Prakash and Arora (2019) Liu et al. (2019b) Singh (2018) Raklami et al. (2019)
	Endosphere, Phyllosphere (Endophytes)	*Pantoea ananatis* *Bacillus*, *Lysinibacillus* *Pseudomonas* *Burkholderia* *Rhizobium* (nodules)	Rice Banana trees *Pisum sativum* *Populus* *Cassia absus*, *Sesbania rostrata*	Lu et al. (2021) Matos et al. (2017) Otieno et al. (2015) Varga et al. (2020) Sridevi and Mallaiah (2009)
Mycorrhizal fungi	Endophytes (arbuscular mycorrhiza)	*Rhizophagus irregularis* *Glomus fasciculate* *Glomus intraradices*	*Cajanus cajan* *Acacia nilotica* *Hordeum vulgare*	Garg and Singh (2018) Giri et al. (2007) Bayani et al. (2015)

and Genre 2010) and are able to mobilize P for plants. The interaction leads to the formation of internal hyphae which develop specific structures known as arbuscules (arbuscular mycorrhiza) where the delivery of P into the plant cell is performed by specific transporters in exchange for carbon sources such as sugars (Ferrol et al. 2019). Besides the macronutrients N and P, mycorrhizal fungi have been reported to increase the uptake of micronutrients such as Zn and Cu by plants (Liu et al. 2000; Goicoechea and Antol 2017) and to contribute to abiotic stress management in plants, such as salinity, drought, etc. (Igiehon and Babalola 2017; Begum et al. 2019), as well as to soil stabilization and maintenance of humidity through the secretion of aggregating substances such as glomalin and other soil proteins (Rillig 2004).

16.2.1.3 Potassium Solubilization

Soils contain large amounts of K but most of it is also unavailable for plant uptake. In this regard, potassium solubilizing bacteria (KSB) can solubilize K-bearing minerals such as biotite, feldspar, illite, muscovite, orthoclase, and mica, for plant uptake (Etesami et al. 2017). K mobilization can be achieved through the production of organic and inorganic acids, acidolysis, complexolysis, chelation of other elements

Table 16.3 Potassium solubilizing microorganisms isolated from the rhizosphere of different plants or as endophytes. Many of these microorganisms display additional plant growth-promoting activities

K solubilizing microorganisms	Compartment		Host plants	References
Bacteria	Rhizosphere	*Acidithiobacillus ferrooxidans* *Paenibacillus* *Bacillus mucilaginosus* *Burkholderia cepacia* *Pseudomonas* *Enterobacter*	Tobacco Wheat Black pepper Tobacco Sorghum, Maize Sunflower	Zhang and Kong (2014) Parmar and Sindhu (2013) Sangeeth et al. (2012) Zhang and Kong (2014) Archana et al. (2013) Shahid et al. (2012)
	Endosphere, Phyllosphere, Nodules (Endophytes)	*Alcaligenes* *Streptomyces* *Rhizobium* *Burkholderia*	Moso Bamboo Corn Chick pea Corn	Yuan et al. (2015) Aallam et al. (2021) Sindhu et al. (2019) Baghel et al. (2020)
Fungi	Endophytes (arbuscular mycorrhiza)	*Cladosporium* *Glomus intraradices*, and *G. mosseae*	Tobacco Maize	Zhang and Kong (2014) Wu et al. (2005)

present in K-bearing minerals, secretion of polysaccharides, and exchange reactions (Meena et al. 2014; Etesami et al. 2017).

Many bacteria from different phyla (α-, β- and γ-Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes) have capacity to solubilize K minerals (Verma et al. 2017; Bashir et al. 2019). Regarding their habitat, these include acidophiles, alkaliphiles, mesophiles, thermophiles, and psychrophiles. Some examples are given in Table 16.3. Besides, many fungi are able to mobilize K (Verma et al. 2017); of particular interest are arbuscular mycorrhiza such as *Glomus intraradices*, *G. mosseae*, and *Cladosporium* improving K nutrition in several plants (Verma et al. 2017).

16.2.1.4 Promotion of Plant Growth and Development Via Phytohormones

Another mechanism for plant growth promotion is direct secretion of growth stimulating hormones. The most important are auxins, in particular, indole acetic acid (IAA). IAA regulates various aspects of plant growth and development, in particular in roots, it promotes the elongation of the main root and also the appearance of lateral roots and increases the number of root hairs ameliorating absorption of nutrients by roots (Overvoorde et al. 2010; Tian et al. 2014). Besides, it has also effects on shoot development (Ferguson and Beveridge 2009). Up to five different IAA synthesis pathways have been described in bacteria, most of them from tryptophan: indol-3-acetamide pathway, indole-3-pyruvate pathway, tryptamine pathway, tryptophan side-chain oxidase pathway, and indole-3-acetonitrile pathway (Li et al. 2018; Duca and Glick 2020). Both rhizosphere and endophytic microorganisms are able to produce IAA and have been probed to increase plant growth and yield in a battery of plants (Grover et al. 2021; Table 16.4). The importance of fungi as IAA producers is being recognized and exploited for root growth and development (Fu et al. 2015).

Another important aspect is the fact that many of IAA producing microorganisms display additional plant growth-promoting activities, such as phosphate and/or potassium solubilization, siderophores production and biocontrol properties, ACC deaminase activity, etc. (Wagi and Ahmed 2019). Moreover, in many cases the occurrence of stress conditions such as salinity or metals not only does not affect negatively the production of IAA, but also increase it (Myo et al. 2019; Paredes-Páliz et al. 2016a; Flores-Duarte et al. 2022b).

Besides auxins, some PGPB are able to produce other phytohormones such as gibberellic acid, cytokinins, abscisic acid, jasmonic acid, and salicylic acid (Tsukanova et al. 2017). For instance, cytokinin-producing strains alter plant cytokinin homeostasis in plants, increasing plant growth and elongation of the main root, whereas it inhibits lateral roots formation in *Brassica napus* (Pallai et al. 2012). Analogously, gibberellins-producing PGPR alter the levels of this hormone and improve shoot and root growth of rice (Kang et al. 2014; Shahzad et al. 2016). On their side, ABA-producing PGPR also improve plant growth and regulate stomatal conductance for adaptation to drought (Jiang et al. 2012; Salomon et al. 2014). The production of jasmonic acid and salicylic acid by endophytes (PGPE) ameliorates the plant defense against pathogens by mechanisms known as the induced systemic resistance (ISR) and the systemic acquired resistance (SAR). The first one is dependent on the levels of salicylic acid and ethylene, whereas the second one depends on jasmonate and ethylene (Choudhary et al. 2007; Gao et al. 2015). Many endophytes produce jasmonate and salicylic acid (Forchetti et al. 2007; Bordiec et al. 2011) increasing the levels of these phytohormones and helping plants in situations of biotic stress (Tsukanova et al. 2017).

16.2.2 Indirect Mechanisms

16.2.2.1 ACC Deaminase Activity

This PGP property is particularly desirable in bacteria when they are to be used in stressful situations. Ethylene is the main stress-related hormone in plants (Pattyn

Table 16.4 Phytohormones producing microorganisms from the rhizosphere of plants or endophytes

Phytohormones producing microorganisms	Compartment		Host plants	References
IAA				
Bacteria	Rhizosphere	*Bacillus cereus* and *B. subtilis*	*Solanum nigrum* and *Malvastrum tricuspidatum*	Wagi and Ahmed (2019)
		Pseudomonas stutzeri, *Bacillus subtilis*, *Stenotrophomonas maltophilia*, and *Bacillus amyloliquefaciens*	Cucumber	Islam et al. (2016)
		Phyllobacterium, *Bacillus*, *Agrobacterium* and *Rhizobium*	*Acacia cyanophylla*	Lebrazi et al. (2020)
	Endophytes	*Azospirillum*, *Rhizobium*, *Variovorax*, *Microbacterium*	Duckweed	Gilbert et al. (2018)
		Sphingomonas, *Bacillus*, *Methylobacterium*	*Solanum lycopersicum*	Khan et al. (2016)
Fungi	Endophytes	*Trichoderma virens*	*Arabidopsis*	Contreras-Cornejo et al. (2009)
		Tricholoma vaccinum	Spruce	Krause et al. (2015)
		Candida tropicalis	*Zea maize*	Mukherjee and Sen (2015)
Gibberellic acid		*Leifsonia soli*	*Solanum lycopersicum*	Kang et al. (2014)
		Bacillus amyloliquefaciens	*Oryza sativa*	Shahzad et al. (2016)
Cytokinins		*Pseudomonas fluorescens*	*Brassica napus*	Pallai et al. (2012)
ABA		*Bacillus licheniformis* and *Pseudomonas fluorescens*	*Vitis vinifera*	Salomon et al. (2014)
ABA		*Variovorax paradoxus*	*Pisum sativum*	Jiang et al. (2012)

et al. 2021), and its levels increase enormously under these conditions. It is also involved in processes such as senescence and fruit ripening (Noushina et al. 2017). The synthesis of this gaseous plant hormone is carried out from

S-adenosylmethionine (SAM) in two steps: in the first one, SAM is transformed into aminocyclopropane carboxylic acid (ACC) by the action of the enzyme ACC synthase, and in the second one, ACC, the direct precursor of ethylene, is transformed into the hormone by the reaction catalyzed by the enzyme ACC oxidase (Pattyn et al. 2021).

The enzyme ACC deaminase interrupts the synthesis of ethylene by hydrolyzing the amino group of the direct precursor ACC (Nascimento et al. 2014). This activity has been shown to be essential in stressful situations (salinity, drought, presence of heavy metals, hypoxia and anoxia, etc.) since plants regulate ethylene levels and prevent the appearance of stress symptoms such as growth inhibition, premature senescence, etc. (Glick 2014; Yang et al. 2009). In this regard, the rhizosphere or the tissues of plants grown under multiple stress conditions could be a source of novel and biotechnologically important PGPB (Timmusk et al. 2011; Misra et al. 2017; Garcia-Teijeiro et al. 2020). Not only within rhizosphere bacteria, but among the endophytes, ACC deaminase-producing bacteria have been found (Orozco-Mosqueda et al. 2020). Studies of overexpression of the *acdS* gene encoding this enzyme (Liu et al. 2017; Nascimento et al. 2018; Subramanian et al. 2015) and knock-out of the same gene (Liu et al. 2021) confirm the fundamental role of this mechanism in plant protection against multiple stresses. Table 16.5 shows PGPR bacteria and endophytes with ACC deaminase activity and their beneficial effects on various plants.

16.2.2.2 Production of Siderophores

Fe is essential for all living beings and is involved in diverse functions including the transport of electrons in the respiratory chain, cofactor of a plethora of enzymes, nitrogen fixation, etc. (Cornelis and Andrews 2010). In addition to specific transporters of high and low affinity for Fe^{2+} and Fe^{3+} (Wyckoff et al. 2006; Lau et al. 2016), microorganisms ensure the uptake of this element through the secretion of siderophores, complexing molecules that show a high affinity for this element and are transported into the microorganisms by specific transporters (Ahmed and Holmström 2014). Among the great variety of these molecules, heme, hemin, ferritin, bacterioferritin, enterobactin, achromobactin, ferrioxamine, bacillibactin, rhizobactin, pioverdin, etc. are included (Ahmed and Holmström 2014; Pajuelo et al. 2021). Competition for Fe is also an advantageous factor in the rhizosphere, as well as a biocontrol mechanism by sequestering this element, making it not available for other competitors (Sayyed et al. 2013; Kramer et al. 2020). Table 16.6 shows siderophores-producing PGPR and endophytes and their beneficial effects on plant growth and as biocontrol agents (biotic stress). The production of siderophores is, not only is a basic mechanism for competition and biocontrol, but also favor plant growth by providing Fe or other metals than can be mobilized, particularly under stress situations (Ferreira et al. 2019).

Table 16.5 PGPR bacteria and endophytes with ACC deaminase activity and their beneficial effects on various plants

ACC-producing microorganisms	Bacteria	Host plant	Effect	Reference
Rhizosphere	*Aneurinibacillus*, *aneurinilyticus*, and *Paenibacillus* sp.	*Phaseolus vulgaris*	Ameliorates salt stress, better growth, induction ISR	Gupta and Pandey (2019)
	P. fluorescens	*Zea mays*	Decreases stress by drought	Zarei et al. (2020)
	Leclercia adecarboxylata	*Solanum lycopersicum*	Improve resilience toward salt stress	Kang et al. (2019)
	Alcaligenes sp., *Bacillus* sp. and *Ochrobactrum* sp	*Oryza sativa*	Improve resilience toward salt stress	Bal et al. (2013a)
	Bacillus, *Microbacterium*, *Methylophaga*, *Agromyces*, and *Paenibacillus*	*Oryza sativa*	Improve resilience toward salt stress	Bal et al. (2013b)
Endophytes	*Pseudomonas* spp.	*Solanum lycopersicum*	Improved growth, photosynthetic performance, and ionic balance	Win et al. (2018)
	Pseudomonas azotoformans	*Solanum lycopersicum*	Amelioration of salinity stress	Liu et al. (2021)
	Enterobacter sp. and *Kosakonia* sp.	*Brassica oleracea*	Alleviation of salt stress	Liu et al. (2017)

16.2.2.3 Other PGP Properties

Besides the production of siderophores, a plethora of mechanisms are involved in biocontrol, which can be classified in different categories according to the mode of action (Table 16.7): (a) competition for Fe and other nutrients; (b) secretion of substances with bactericidal and antifungal properties; (c) secretion of extracellular hydrolytic enzymes able to break down glycosidic linkages present in the cell wall of fungal pathogens causing cell death, such as chitinase, glucanase, protease (Bhadrecha et al. 2020); (d) parasitism; (e) production of volatile compounds such as HCN; and (f) induction of the induced systemic resistance (ISR) and the systemic acquired resistance (SAR). Other extracellular enzymes such as cellulases, pectinases, xylanases, etc. are key to facilitate the entrance and progression of endophytes inside the phyllosphere (Dogan and Taskin 2021; Bhadrecha et al. 2020). Besides, inhibition of quorum sensing signals of other microorganimsms is proposed as a interesting trait when searching for bacteria with phytotechnological applications (Rodríguez et al. 2020).

Table 16.6 Siderophores-producing microorganisms isolated form the rhizosphere of different plants or as endophytes of diverse plant hosts and their effect on plant growth or as biocontrol agents. Many of these microorganisms display addition PGP properties

Producers of siderophores	Compartment		Host plants	References
Bacteria	Rhizosphere	*Pseudomonas fluorescens*	*Arachis hypogea*	Subramanium and Sundaram (2020)
		Bacillus subtilis	*Coriandrum sativum*	Kumari et al. (2021)
		Pantoea agglomerans and *Bacillus aryabhattai*	*Spinacia oleracea*, *Lactuca sativa*, *Brassica napus*	Pajuelo et al. (2021)
		Pseudomonas spp., *Enterobacter* spp., and *Bacillus sporothermodurans*	*Helianthus annus*	Pourbabaee et al. (2018)
	Endophytes	*Pantoea ananatis*	*Oryza sativa*,	Loaces et al. (2011)
		Jeotgalicoccus huakuii, and *Bacillus amyloliquefaciens*	*Cynodon dactylon*, and *Eleusine indica*	Ustiatik et al. (2021)
		Pseudomonas	*Oryza sativa*	Walitang et al. (2017)
Fungi	Endophytes	*Penicillium chrysogenum*, *Aspergillus sydowii*, *Aspergillus terreus*,	*Cymbidium aloifolium*	Chowdappa et al. (2020)
		Epichloe festucae	*Lolium perenne*	Koulman et al. (2012)

16.2.3 Tolerance of Bacterial Inoculants Toward Abiotic Stresses

The increase in human population together with expected abiotic multi-stress scenarios in near future will compromise food quality and security (Molotoks et al. 2021). In this regard, the development of multi-stress tolerant bioinoculants for sustainable and environmentally responsible food production is of the outmost importance (Mitter et al. 2021). From 1896 to 2020, the use of PGPR has grown in parallel with the creation of new inoculant industries and concern for the ecosystem fosters good practices in agriculture (Soumare et al. 2020). As previously discussed, PRPM activities maintain the soil rich in micro- and macronutrients through nitrogen fixation, solubilization, or mineralization of phosphate and potassium, the release of plant growth regulating substances, the production of antibiotics, and the biodegradation and recycling of organic matter in the soil (Bhardwaj et al. 2014). These beneficial effects are much obvious under multiple stress conditions. In fact, comparative meta-analyses of a large number of studies with biofertilizers have

Table 16.7 Biocontrol mechanisms exerted by microorganisms of the rhizosphere

Mechanisms	Mode of action	Examples	Reference
Production of siderophores	Competition for Fe	Heme, hemin, ferritin, bacterioferritin, enterobactin, achromobactin, ferrioxamine, bacillibactin, rhizobactin, pioverdin, etc.	Kramer et al. (2020)
Competition	Competition for nutrients and niche	Competition for N, C, P, and micronutrients	Kuzyakov and Xu (2013)
Parasitism	Parasitism	*Trichoderma* has been widely used as biocontrol, it parasitizes fungal pathogens	Contreras-Cornejo et al. (2016)
Antibiosis	Secretion of bactericidal and antifungal compounds	2,4-Diacetyl phloroglucinol (DAPG), amphisin, oomycin A, phenazine, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, oligomycin A, zwittermicin A, kanosamine, xanthobaccin, toxins	Balthazar et al. (2021)
Extracellular enzymes	Degradation of cell walls of phytopathogens	Chitinase Glucanase Proteases	Jadhav et al. (2017)
Production of volatile substances	Synthesis of HCN	The direct effect on phytopathogens is being questioned; rather acts as complexing of metals or regulator of the availability of phosphorous	Rijavec and Lapanje (2016)
Induced systemic resistance (ISR)	Dependent on jasmonate and ethylene	Elicited by non-pathogenic bacteria such as PGPR	Kamle et al. (2020)
Systemic acquired resistance (SAR)	Dependent on salicylic acid and Pathogen Related (PR) proteins	Elicited by salicylic acid and pathogens	Kamle et al. (2020)

shown their greatest usefulness in conditions of dry climates and soils with low organic matter (Schütz et al. 2018; Flores-Duarte et al. 2022c). However, most PGPR products are marketed as biocontrol (70%) and biofertilizers (25%), and only 5% are formulated to improve the stress tolerance of plants (Tabassum et al. 2017). In this context, the selection of PGPM with high tolerance toward several stresses is needed. Bacteria with elevated resistance toward salt, drought, high or low temperature, metals, xenobiotics, etc., are prospected and used as biofertilizers with remarkable beneficial effects on plants growing under these constraints (Yang et al. 2009; Enebe and Babalola 2019; Abbas et al. 2019).

Some of the most sensitive steps in plant growth under abiotic stress are seed germination and initial plant establishment. Inoculation with salt and metal tolerant rhizobacteria improved seed germination of *Spartina maritima* (Paredes-Páliz et al. 2016b). In the same way, inoculation with halotolerant PGPR improved seed germination in wheat (Albdaiwi et al. 2019) and canola (Siddikee et al. 2015).

Numerous examples are reported on the use of salt-tolerant beneficial microorganisms, which promote plant growth on saline soils (Abbas et al. 2019; Quamruzzaman et al. 2021). In the same way, thermotolerant and psychrophilic PGPB ameliorate plant growth at high or low temperatures (Sharma et al. 2020; Yarzábal 2020). On its side, multi-stress resistant PGPR and rhizobia (salt, high temperature, drought, and metals) ameliorated alfalfa growth in the presence of metals and, at the same time, blocked the entrance of metals in plant tissues for safe cultivation in polluted soils (Raklami et al. 2019). Some examples of multi-stress tolerant PGPM and their effect on plants are summarized in Table 16.8. The mechanisms are being deciphered and include the production of auxins, HCN, phytohormones, and the induction of the Induced Systemic Response (ISR) and the Systemic Acquired Response (SAR). In general, inoculation correlates with the synthesis of phytohormones and by eliciting the expression of genes related to the antioxidant system (enzymes such as catalase, peroxidases, superoxide dismutase, glutathione transferase, etc.) and pathogenesis-related genes (Fukami et al. 2017; Lafuente et al. 2015; Paredes-Páliz et al. 2018; Kang et al. 2019, 2021; Zarei et al. 2020). However, the complex interplay between edaphic characteristics, plant genotype, and inoculum (composition, stability, timing of application, etc.) sometimes prevent good results in field experiments in spite of previous data at the greenhouse (Quamruzzaman et al. 2021). In this context, the presence of ACC-deaminase in selected PGPM is highly desired, in order to diminish plant stress and improve plant resilience to harsh environments as discussed in previous section (Glick 2014; Siddikee et al. 2015; Kang et al. 2019, 2021; Zarei et al. 2020; Bessadok et al. 2020).

16.2.4 Competition in the Rhizosphere and Root Colonization

In addition to good PGP properties and resistance to a variety of stresses, the microorganisms to be selected for an inoculant must be competitive in the rhizosphere and have the ability to colonize plant roots (Santoyo et al. 2021). The presence of flagella that allows motility and positive taxis toward plant roots is a factor that helps colonization (Böhm et al. 2007; Fernández-Llamosas et al. 2021), although not-motile bacteria have been used successfully as inoculants at least in greenhouse experiments. Adhesion to the root is facilitated by structures such as fimbriae, pili, flagella, exopolysaccharides, presence of capsule, etc. (Wheatley and Poole 2018; Santoyo et al. 2021). Attachment occurs in two phases: a first in which the union is weak but in which PGP effects can already be exerted in the plant rhizosphere, and another phase in which binding is strong, necessary for the penetration of endophytes and pathogenic microorganisms (Wheatley and Poole 2018). Once attached, the microorganisms must compete in the rhizosphere and colonize the root. Biofilm formation appears to be a determining factor for strong and effective attachment and colonization (Pandit et al. 2020). Other mechanisms include the production of siderophores already discussed and the synthesis of bacteriocins, antibiotics, or toxins (Beneduzi et al. 2012; Subramanian and Smith

Table 16.8 Alleviation of multifarious stresses in several plants by PGPR, endophytes, and mycorrhizal fungi

Compartment	Bacteria	Host plant	Type of stress	Mechanisms	Reference
Rhizosphere	*Aneurinibacillus aneurinilyticus*, and *Paenibacillus* sp.	*Phaseolus vulgaris*	Ameliorates salt stress	Production of ACC, IAA, siderophore, ammonia, HCN and P and Zn solubilization.	Gupta and Pandey (2019)
	P. fluorescens	*Zea mays*	Decreases stress by drought	Production of ACC and	Zarei et al. (2020)
	Leclercia adecarboxylata	*Cucurbita peppo*	Improve resilience toward Zn stress	Production of ACC and siderophores. Better plant growth, induction of ISR, decreased Zn uptake in polluted soil	Kang et al. (2021)
	Kosakonia sacchari	*Vigna radiata*	Zn stress	Halotolerant bacterium. Improves the ionic balance, stressor metabolites, antioxidant status, and yield	Shahid et al. (2021)
	Ensifer medicae, *Pseudomonas fluorescens*, and *Ochrobactrum cytisi*	*Lupinus luteus*	Improve resilience toward metal stress, field experiments	Metal resistant rhizobacteria. Decreased metal uptake and improved growth.	Dary et al. (2010)
Endophytes	*Cronobacter sakazakii* and *Kocuria rhizophila*	*Triticum aestivum*	Alleviation of salt stress	Better growth, induction of antioxidant system	Afridi et al. (2019)
	Lysinibacillus fusiformis Cr33	*Solanum lycopersicum*	Diminution of cadmium stress	Increase of jasmonic acid, decrease of NO levels, and downregulation of Fe uptake	Zhu et al. (2021)
Mycorrhizal fungi	*Funneliformis mosseae*	*Zea mays*	Water-deficit stress	Positive effect on plant growth, chlorophyll index, and nutrients uptake. Induction of ISR (antioxidant enzymes and total phenolics content)	Bahraminia et al. (2020)

2015; Pajuelo et al. 2021) as well as the resistance toward antibiotics produced by competing strains (Pajuelo et al. 2021). The "biofilm way of life" provides higher resistance toward antibiotics, bacteriocins or toxins secreted by competitors, as compared to the "planktonic life" (Lerch et al. 2017). On the other hand, since many rhizosphere processes including biofilm formation, pathogenesis, etc. depend on *quorum sensing*, many microorganisms are capable of degrading the communication molecules (AHLs: acyl-homoserine lactones) of other species, known as quorum quenching, thus preventing colonization by these other microorganism (pathogens or simply competitors) and ensuring a better competence in the rhizosphere (Podile et al. 2014; Rodríguez et al. 2020). Moreover, *Serratia liquefaciens* and *Pseudomonas putida* strains producing AHL elicited induced systemic resistance (ISR) in tomato against *Alternaria alternata*, whereas AHL-null mutant strains resulted in reduced ISR (Schuhegger et al. 2006). In turn, some microorganisms can prevent and/or prejudice colonization by other PGPR. Such is the case of *Bacillus subtilis* UD1022 which alters nodulation efficiency of *Sinorhizobium meliloti* on *Medicago truncatula* (Rosier et al. 2021). In this regard, careful examination of the interaction between partners of bacterial consortia must be addressed in order not to have antagonistic effects.

16.3 How Can Omics Help Designing Inoculants?

The use of omics technologies has been a boost to biofertilizers' engineering (Martínez-Hidalgo et al. 2019). Metagenomics studies of the rhizosphere allow us to know microbial populations and their dynamics in respect to environmental changes and especially in response to root exudates secreted by the plants (Hayat et al. 2017; Zhang et al. 2015), which are in turn related to environmental changes. In this way, not only the most abundant species in the rhizosphere can be identified, but all of them, since sometimes minority species can exert a greater beneficial effect than more represented ones (Dawson et al. 2017). These comparisons are very interesting from an ecological perspective, since the same plant species can select certain microorganisms to better adapt to certain edaphic or climatic conditions of an area (Na et al. 2018).

When selecting the bacteria that will form part of the inoculant, it must be taken into account that they must be robust microorganisms, with good capacity for competition in the rhizosphere. They must be versatile bacteria able to use a large number of C, N, P, and S sources (Luziatelli et al. 2020; Shariati et al. 2017; Pajuelo et al. 2021). In addition, these microorganisms must have tolerance to multiple stress situations including salinity, heavy metals, heat or cold, UV radiation, wide pH range, etc. (Weilharter et al. 2011; Shariati et al. 2017; Pajuelo et al. 2021). At the same time, some of these bacteria possess the ability to degrade diverse xenobiotics (Azaizeh et al. 2011; Pajuelo et al. 2021). It is also important looking for traits for antioxidant enzymes such as catalase, peroxidases, superoxide dismutase, glyoxylase, glutathione reductase, glutathione transferase, etc. (Kusale et al. 2021).

All these traits are indicative of high resilience and competitiveness in the rhizosphere. Searching for a core-genome including these characteristics may better guarantee the effectiveness of the inoculant (Belbahri et al. 2017; Zboralski and Filion 2020). In particular, three key aspects for application as a bioinoculant will be focused: (a) traits related to resistance to heavy metals and salt (high osmolarity), high temperatures, cold, etc.; (b) traits for PGP properties; and (c) traits related to competition in the rhizosphere and root colonization.

16.3.1 Traits for Resistance Toward Abiotic Stresses

In polluted soils, it could be interesting to look for operons that encode resistance toward metals and metalloids (Nies 2003). In particular, metal efflux pumps such as *copA* for Cu, *zntB* and *zitB* for Zn, *rcnA* for Co and Ni and the arsenic resistance operon *ars* (Grass and Rensing 2001; Rodrigue et al. 2005; Fekih et al. 2018) could be prospected depending on the specific contamination in the polluted area. Concerning soils affected by salinity, a battery of genes regulate the resistance to salt (osmotic stress) (Leontidou et al. 2020). Genes involved in the synthesis and degradation of osmoprotectants, such as *doex* for ectoine (Schwibbert et al. 2011), *betIABT* for betaine (Cánovas et al. 2000), trehalose (*otsAB*) (Kaasen et al. 1994) could be sought. Moreover, these microorganisms have permeases such as *proPVWXY*, *osmVWXY*, *yehXYZ*, and *ousA* for the uptake of osmoregulatory substances such as proline, glycine choline betaine and proline betaine, ectoine, and pipecolic acid (Checroun and Gutierrez 2004; Frossard et al. 2012). Recycling of osmoregulatory metabolites as carbon source once the osmotic stress conditions disappear can be achieved by the activity of trehalases codified by *treAF*, which could be also prospected (Carroll et al. 2007). The selection of drought resistant strains can be done on media containing polyethylene glycol (Niu et al. 2018) and usually present the gene *acdS* for AAC deaminase, as well a production of exopolysaccharides. Other genes related to drought are aquaporin Z (*aqpZ*) and glycerol uptake (*glpF*) (Nordstedt and Jones 2021). Besides, genes for osmoprotectants previously described are involved in drought tolerance. Analogously, strains tolerant to high and low temperatures can be prospected in the adequate conditions. Different mechanisms have been associated with protection toward heat stress, including synthesis of auxins, organic acids, and gibberellins (Khan et al. 2020).

16.3.2 Traits for PGP Properties

Regarding synthesis of siderophores, genes for the production and/or transport of a great diversity of siderophores can be prospected, including heme (Otto et al. 1992), hemin (Hornung et al. 1996), several bacterial ferritins (Yao et al. 2011), enterobactin (Reitz et al. 2017), achromobactin (Berti and Thomas 2009), ferrioxamine (Sauer et al. 1987), and ferri-bacillibactin (Miethke et al. 2006). Besides, PGPB have low and high affinity transporters for free ion, both in the form of ferrous (EfeO, FeoAB, EfeU) (Lau et al. 2016) and ferric ions (FbpBC) (Wyckoff et al. 2006).

With regard to phosphorous solubilization, genes for extracellular phosphatases that hydrolyze C–P bonds can be searched, particularly *phoD* encoding alkaline phosphatase (Hegyi et al. 2021). Besides, the operon for phosphonase *phn* can be investigated (Stasi et al. 2019). This operon is involved in the degradation/assimilation of P form phosphonates, which are organophosphorous compounds derived from phosphonic acid. Besides, the gene *phtA* encoding phytase (Jorquera et al. 2013) can be prospected. Phytates are other of the organic forms of P present in soils derived from rest of vegetal origin. However, it is a form of P with low bioavailability since not many microorganisms have the enzyme phytase, able to degrade the bond C(myoinositol)-P (Lei et al. 2013). The captured phosphorus can accumulate in the form of polyphosphate granules in the cytoplasm thanks to the Ppk polyphosphate kinase (Shiba et al. 2000) and be mobilized when necessary by means of the inorganic pyrophosphatase Ppa (Kajander et al. 2013). Besides, PGPB can transport inorganic forms of P by transporters whose encoding genes *pst* and *pit* can be prospected (Zheng et al. 2016).

With respect to the production of auxins, genes involved in the tryptophan biosynthesis and indole-3-acetic acid (IAA) biosynthesis can be sought. The main precursor in the IAA synthesis is tryptophan, and five different pathways to synthetize IAA have been studied: indol-3-acetamide pathway, indole-3-pyruvate pathway, tryptamine pathway, tryptophan side-chain oxidase pathway, and indole-3-acetonitrile pathway (Li et al. 2018; Duca and Glick 2020). In this regard, the screening of all the pathways may be complex since a huge amount of genes are involved. As the most representative candidates are genes *iah* encoding the indole-acetamide hydrolase and *iad* codifying the indole-acetaldehyde dehydrogenase (Li et al. 2018).

16.3.3 Traits Related to Competition in the Rhizosphere and Root Colonization

Genes involved in rhizosphere processes important for plant–bacterium interaction can be analyzed, including genes for synthesis, rotation, and regulation of the flagella *fli* and *flh* (Nakamura and Minamino 2019), genes involved in chemotaxis such as *che* and *Tsr/Tar* (Feng et al. 2018). Genes involved in biofilm formation such as *ariR* and *BssS* seem to be conserved among PGPB (Anupama et al. 2018). For all these rhizosphere processes to occur, there must be a minimum cell density, detected by *quorum sensing* systems such as Qse, Lux, and Rhi (Altaf et al. 2017). Finally, gene *aiiA* for lactonase (Zhang et al. 2007; Mat-Amin et al. 2016) able to degrade acyl-homoserine lactones (AHLs) and inhibit *quorum sensing* signals of other bacteria ensures the competition of the bacterium in the rhizosphere, together with siderophores as previously discussed.

16.4 Designing Inoculants Adapted to Poly-Stress Situations: The Core-Microbiome Approach

For the design of bioinoculants with desired functionality, it could be useful to perform a previous metagenomics analysis (Marco and Abram 2019). This would allow knowing the composition and dynamics of the microbial community associated with a particular plant species (or to multiple plant species) in a particular environment subjected to one or several stress constraints (Taghavi et al. 2009; Medina-Córdoba et al. 2021).

There are two strategies for designing phytomicrobiomes: “top-down,” which modifies an existing microbiome by modifying variables such as pH, temperature, redox potential, nutrients, and “bottom-up,” which build artificial or engineered microbiomes (e.g., synthetic communities or SynComs) from individual isolated microorganisms (Mitter et al. 2021). For the design of biofertilizers, the “bottom-up” method is the most frequently used (Mitter et al. 2021). It is based on producing an inoculant constituted by an affordable number of strains with the best performance, typically 2–4 selected strains. So in spite that a great diversity of bacteria can be identified in the rhizosphere or as endophytes of plants, the production of the inoculant is finally dependent on cultivation.

The isolation of bacteria from the rhizosphere and endophytes is then the next step in the design of biofertilizers. Culturomics, i.e., the fine adaptation of media and culture conditions based on previous genomic information has allowed increasing significantly the number of species isolated from gut microbiome, as well as the identification of new species (Lagier et al. 2017). In an analogous manner, besides using general media such as nutrient growth, TSA or YMA for rhizobia, information provided by metagenomics can help selecting the appropriate culture media for the subsequent isolation of particular candidates. For instance, if halophilic bacteria are found in metagenomics, specific salt-containing media can be included in the initial screening. Analogously, thermophilic, psychrophilic, acidophilic, or alkaliphilic bacteria can be more specific isolated in the appropriate incubation conditions (Torbaghan et al. 2017; Yadav et al. 2018). The Culturomics approach is gaining significance in many recent studies for the isolation of specific plant interacting microbes (Sarhan et al. 2019).

In our hands, typically between 30 and 80 morphologically different isolates can be obtained from the rhizosphere of plants (Andrades-Moreno et al. 2014; Paredes-Páliz et al. 2016a; Navarro-Torre et al. 2016a) whereas the number of endophytes is much lower (Mesa et al. 2015; Navarro-Torre et al. 2016b). Figure 16.2 shows a scheme of selection of the bacteria that would constitute the final inoculum. All

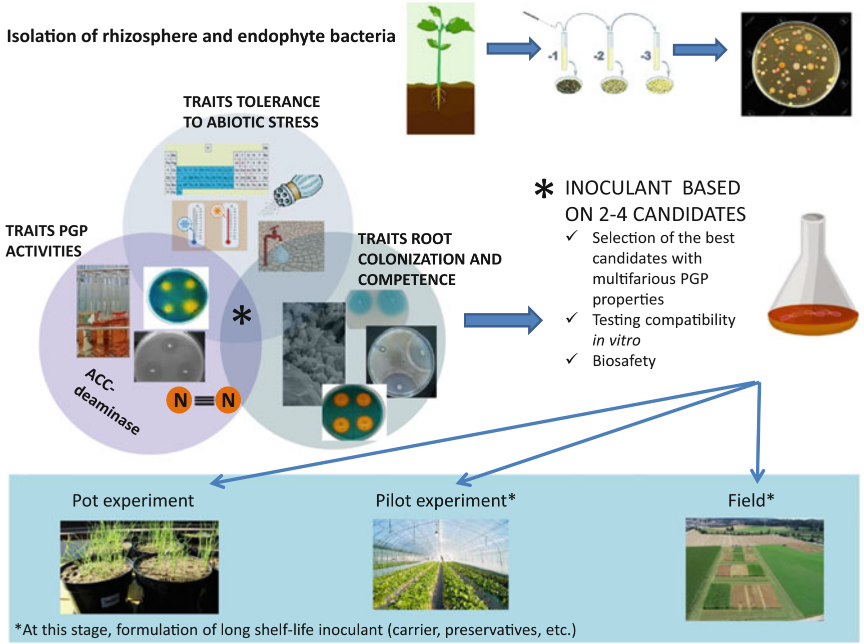


Fig. 16.2 Main steps in the process of designing an inoculant useful for stress situations steps. After isolation on the appropriate media, all strains are tested for: (**a**) resistance to abiotic stress (metals, salt, high or low temperatures, drought); (**b**) PGP properties (P and K solubilization, siderophores, auxins, N_2 fixation, extracellular enzymes, etc.); and (**c**) competition in the rhizosphere and root colonization. Two-four strains with the best performance are selected for the inoculum, after testing compatibility and biosecurity. Tests are performed in pots, green houses, and field pilot experiments. At this stage, formulations ensuring longer shelf-life must be developed

strains would be analyzed for PGP activities (phosphate and potassium solubilization, nitrogen fixation, siderophores production, ACC deaminase activity, extracellular enzymes like cellulases and chitinases, etc.), tolerance toward multiple stresses (salt, drought, heavy metals, high or low temperature) and competence in the rhizosphere (formation of biofilms, secretion of antibiotics, tolerance against antibiotics, exopolysaccharides). The different activities will be plotted and the best candidates with multiple properties selected (Medina-Córdoba et al. 2021). Finally, after testing compatibility in vitro, the final inoculum will be produced based on the selection of 2–4 of the best candidates with multifarious PGP properties. These microorganisms together constitute a core-microbiome that represent all beneficial traits investigated, which guarantees positive effects on plants (Mitter et al. 2021). It is possible to develop a preliminary test for plant growth promotion on agar plates before testing the inoculum in pots in greenhouse plants, pilot experiments in small parcels or orchards, and finally field experiments (Pajuelo et al. 2021). Before this stage, formulations for stabilization and prolonged shelf-life of the inoculum (carriers, preservatives, etc.) must be assessed, as discussed below. In this way, customization of inoculants adapted to particular plants/environments combinations can be achieved (Kumar et al. 2019; Du et al. 2020). Besides, this activity implies social, economic, and ecologic aspects, such as the development of local biotechnological industries for the development of tailored inoculants, the protection of the intellectual property, and the diminution of chemical fertilizers for the protection of the environment (Pajuelo et al. 2014).

16.5 Bottlenecks to Commercialization: Stability, Competitiveness, Regulatory Issues

In Fig. 16.1, we have seen the large number of publications produced in the last 20 years related to PGPM. Comparing these results with licensed patents (Singh et al. 2019), it can be seen that the research is still far from having a real scope. Considering also that many patents are not commercialized, it can finally be concluded that there really is a bottleneck for a large-scale application of biofertilizers. The constraints that hold back the application are technical, economic, of infrastructure and training of specialized biotechnologists, as well as regulatory issues, since the legislation is different according to the countries. This, together with production prices, creates mistrust among farmers (Lobo et al. 2019; Basu et al. 2021). The use of non-polluting residues together with inoculants may offer new possibilities for the use of wastes and partially compensate the price of production (Ghosh and Singh 2005; Raklami et al. 2021).

Main problems associated with extended use of biofertilizers and increased field application are stability, durability, competitiveness, price, etc. (Backer et al. 2018; Basu et al. 2021). Inoculants can be applied as liquid, slurry, or solid formulations. Liquid formulations may be suitable on a small scale, but they have the problems of stability since the shelf-life of microorganisms in liquid medium is short (Bashan et al. 2014). Additives such as alginate, carrageenan (a sulphated polysaccharide extracted from red seaweed), or molasses can be used for further stabilization in liquid formulations (Cortés-Patiño and Bonilla 2015; Berninger et al. 2018). Encapsulation in alginate or polyacrylamide beads increases longevity and allows slow releasing of the inoculant (Santos et al. 2019). In the case of spore-forming bacteria such as *Bacillus* species, dried spores can be directly used as biocontrol agents (Wu et al. 2015). However, gram negative microorganisms with good PGP properties are extremely sensitive to desiccation (Berninger et al. 2018). For this reason, inoculants have been developed in the form of slurry or immobilized solids on a carrier. In the case of rhizobia, it is common to use peat and improve the adherence of bacteria to the carrier by adding sticking substances such as sucrose (Casteriano et al. 2013). Other carriers are charcoal, lignite, and biochar (Egamberdieva et al. 2018; Basu et al. 2021). For acid soils, it is recommended to mix the inoculant with lime or rock phosphate (Nabahungu et al. 2007; Bakari et al. 2020). PGPM have recently been immobilized in talc or kaolin, increasing the shelf-life of the inoculant up to 4 months

Fig. 16.3 SWOT analysis of the current situation of biofertilizers as real alternative to agrochemicals

(Ei et al. 2018; Myo et al. 2019). Other problems refer to the competitiveness of the microorganisms in real conditions of application (soils, climates, plant genetics, etc.). To increase the efficacy of *Azospirillum*, culture media enriched in exopolysaccharides (EPS) and polyhydroxybutyrate (PHB) have been used (Oliveira et al. 2017).

Additional concerns come from the point of view of biosecurity. In this regard, only group 1 microorganisms are authorized by European legislation to be used as inoculants (GRAS microorganisms, which stand for Generally Recognized as Safe). Many species with good PGP properties belong to biosecurity group 2 and cause opportunistic infections, particularly in nosocomial or immunocompromised patients (Cruz et al. 2007). In this sense, a search for genes related to pathogenicity must be performed, including determinants of resistance to multiple antibiotics, synthesis of antibiotics, beta-lactamases, toxins (hemolysins, RNAase), together with siderophores and biofilm formation traits, which may play important roles as virulence factors (Pajuelo et al. 2021). In this regard, the comparison of genomics analysis of strains isolated from plants and from patients can establish the presence of disease determinants in the former strains (Keswani et al. 2019). In some cases, regulatory issues prevent the use of particular strains as inoculant. Strategies have been designed to take advantage of the PGP characteristics in cell-free extracts. In this way, the bacteria are grown and only the culture supernatant is used to inoculate the plants although effectiveness is lower as compared to the bacteria-containing formulation (Luziatelli et al. 2020).

In conclusion, many issues must be still addressed before full implementation of PGPMs as alternative to chemical fertilizers. Research must fulfill the gaps of knowledge and public-private inversions are needed. But the opportunities are immense from ecological, economic, social and biotechnological points of view. Strengths, weaknesses, threads, and opportunities (Fig. 16.3) must be considered and optimized.

16.6 Concluding Remarks

The increasing demand on plant products together with degradation of soils and climate change will limit global agricultural production. It is clear that both economic and ecologic reasons advise on the utilization of biofertilizers as alternative to agrochemicals. Further development and commercialization of biofertilizers are hindered by both theoretical and practical aspects. Considering the former ones, in spite of our growing knowledge on plant–PGPM interactions, further progress depends on filling gaps in several aspects that need to be investigated in depth, such as (a) the study of the signals involved in the plant-microorganisms dialogue; (b) the regulation of gene expression in plants due to the effect of inoculation; (c) changes in plant metabolome due to inoculation with beneficial microorganisms, which can modulate, not only growth but metabolites affecting the final quality and/or applications of plants. On its side, practical application is limited by the optimization of better formulations in order to increase shelf-life of inoculants and decrease production prices, the way that this technology could be attractive to farmers.

References

Aallam Y, Maliki BE, Dhiba D et al (2021) Multiple potential plant growth promotion activities of endemic *Streptomyces* spp. from Moroccan sugar beet fields with their inhibitory activities against *Fusarium* spp. Microorganisms 9:1429. https://doi.org/10.3390/microorganisms9071429

Abbas R, Rasul S, Aslam K et al (2019) Halotolerant PGPR: a hope for cultivation of saline soils. J King Saud Univ Sci 31(4):1195–1201. https://doi.org/10.1016/j.jksus.2019.02.019

Afridi MS, Amna S et al (2019) Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: involvement of ACC deaminase and antioxidant enzymes. Plant Physiol Biochem 139:569–577. https://doi.org/10.1016/j.plaphy.2019.03.041

Ahmed E, Holmström SJ (2014) Siderophores in environmental research: roles and applications. Microb Biotechnol 7(3):196–208. https://doi.org/10.1111/1751-7915.12117

Albdaiwi RN, Khyami-Horani H, Ayad JY et al (2019) Isolation and characterization of halotolerant plant growth promoting rhizobacteria from durum wheat (*Triticum turgidum* subsp. durum) cultivated in saline areas of the dead sea region. Front Microbiol 10:1639. https://doi.org/10.3389/fmicb.2019.01639

Alewell C, Ringeval B, Ballabio C et al (2020) Global phosphorus shortage will be aggravated by soil erosion. Nat Commun 11:4546. https://doi.org/10.1038/s41467-020-18326-7

Alexandre G (2017) *Azospirillum brasilense*, a beneficial soil bacterium: Isolation and cultivation. Curr Protoc Microbiol 47:3E.1.1–3E.1.10. https://doi.org/10.1002/cpmc.40

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971. https://doi.org/10.3389/fmicb.2017.00971

Altaf MM, Khan MSA, Abulresh HH et al (2017) Quorum sensing in plant growth-promoting rhizobacteria and its impact on plant-microbe interaction. In: Singh D, Singh H, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 311–331. https://doi.org/10.1007/978-981-10-5813-4_16

Álvarez C, Navarro JA, Molina-Heredia FP et al (2020) Endophytic colonization of rice (*Oryza sativa* L.) by the symbiotic strain *Nostoc punctiforme* PCC 73102. Mol Plant-Microbe Interact 33(8):1040–1045. https://doi.org/10.1094/MPMI-01-20-0015-SC

Andrades-Moreno L, Del Castillo I, Parra R et al (2014) Prospecting metal-resistant plant-growth promoting rhizobacteria for rhizoremediation of metal contaminated estuaries using *Spartina densiflora*. Environ Sci Pollut Res Int 21(5):3713–3371. https://doi.org/10.1007/s11356-013-2364-8

Andrews M, Andrews ME (2017) Specificity in legume-rhizobia symbioses. Int J Mol Sci 18(4): 705. https://doi.org/10.3390/ijms18040705

Anupama R, Mukherjee A, Babu S (2018) Gene-centric metagenome analysis reveals diversity of *Pseudomonas aeruginosa* biofilm gene orthologs in fresh water ecosystem. Genomics 110(2): 89–97. https://doi.org/10.1016/j.ygeno.2017.08.010

Ara I, Islam S, Kashem A et al (2018) A comparative study of phosphorus availability in an acidic soil and an alkaline soil amended with organic and inorganic phosphorus sources. J Soil Sci Plant Nutr 18:2. https://doi.org/10.4067/S0718-95162018005001402

Archana DS, Nandish MS, Savalagi VP et al (2013) Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil. BIOINFOLET Q J Life Sci 10(1b):248–257

Arora N, Verma M, Mishra J (2017) Rhizobial bioformulations: past, present and future. In: Mehnaz S (ed) Rhizotrophs: plant growth promotion to bioremediation. Springer, Singapore, pp 69–99. https://doi.org/10.1007/978-981-10-4862-3_4

Azaizeh H, Castro PML, Kidd P (2011) Biodegradation of organic xenobiotic pollutants in the rhizosphere. In: Organic xenobiotics. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9852-8_9

Backer R, Rokem JS, Ilangumaran G et al (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473. https://doi.org/10.3389/fpls.2018.01473

Baghel V, Thakur Y, Yadav S et al (2020) Phosphorus and potassium solubilization from rock minerals by endophytic *Burkholderia* sp. strain FDN2-1 in soil and shift in diversity of bacterial endophytes of corn root tissue with crop growth stage. Geomicrobiol J 37:1–14. https://doi.org/10.1080/01490451.2020.1734691

Bahraminia M, Zarei M, Ronaghi A et al (2020) Ionomic and biochemical responses of maize plant (Zea mays L.) inoculated with Funneliformis mosseae to water-deficit stress. Rhizosphere 16: 100269. https://doi.org/10.1016/j.rhisph.2020.100269

Bakari R, Mungai N, Thuita M et al (2020) Impact of soil acidity and liming on soybean (*Glycine max*) nodulation and nitrogen fixation in Kenyan soils. Acta Agric Scand B Soil Plant Sci 70(8): 667–678. https://doi.org/10.1080/09064710.2020.1833976

Bal HB, Das S, Dangar TK et al (2013a) ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. J Basic Microbiol 53(12):972–984. https://doi.org/10.1002/jobm.201200445

Bal HB, Nayak L, Das S et al (2013b) Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 366(1/2):93–105. https://doi.org/10.1007/s11104-012-1402-5

Balachandar D, Raja P, Kumar K et al (2007) Non-rhizobial nodulation in legumes. Biotechnol Mol Biol Rev 2(2):49–57. https://doi.org/10.13140/2.1.1775.2960

Balthazar C, Novinscak A, Cantin G et al (2021) Biocontrol activity of *Bacillus* spp. and *Pseudomonas* spp. against *Botrytis cinerea* and other cannabis fungal pathogens. Sci Soc Phytopathol. https://doi.org/10.1094/PHYTO-03-21-0128-R

Banik A, Dash GK, Swain P et al (2019) Application of rice (*Oryza sativa* L.) root endophytic diazotrophic Azotobacter sp. strain Avi2 (MCC 3432) can increase rice yield under green house and field condition. Microbiol Res 219:56–65. https://doi.org/10.1016/j.micres.2018.11.004

Bashan Y, de-Bashan L, Prabhu SR et al (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). Plant Soil 378:1–33. https://doi.org/10.1007/s11104-013-1956-x

Bashir Z, Zargar M, Vishwakarma D (2019) Chapter 2: Potassium-solubilizing microorganisms for sustainable agriculture. In: Kumar R, Singh VP, Jhajharia D et al (eds) Applied agricultural practices for mitigating climate change. CRC Press, Boca Raton, FL, pp 17–28. https://doi.org/10.1201/9780429326400-2

Basu A, Prasad P, Das SN et al (2021) Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. Sustainability 13:1140. https://doi.org/10.3390/su13031140

Bayani R, Saateyi A, Faghani E (2015) Influence of arbuscular mycorrhiza in phosphorus acquisition efficiency and drought-tolerance mechanisms in barley (*Hordeum vulgare* L.). Int J Biosci 7(1):86–94. https://doi.org/10.12692/ijb/7.1.86-94

Begum N, Qin C, Ahanger MA et al (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 10:1068. https://doi.org/10.3389/fpls.2019.01068

Belbahri L, Chenari-Bouket A, Imen R et al (2017) Comparative genomics of *Bacillus amyloliquefaciens* Strains reveals a core genome with traits for habitat adaptation and a secondary metabolites rich accessory genome. Front Microbiol 8:1438. https://doi.org/10.3389/fmicb.2017.01438

Berninger T, González-López O, Bejarano A et al (2018) Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. Microb Biotechnol 11(2): 277–301. https://doi.org/10.1111/1751-7915.12880

Beneduzi A, Ambrosini A, Passaglia LM (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051. https://doi.org/10.1590/S1415-47572012000600020

Bertalan M, Albano R, de Pádua V et al (2009) Complete genome sequence of the sugarcane nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* Pal5. BMC Genomics 10:450. https://doi.org/10.1186/1471-2164-10-450

Berti AD, Thomas MG (2009) Analysis of achromobactin biosynthesis by *Pseudomonas syringae* pv. syringae B728a. J Bacteriol 191:4594–4604. https://doi.org/10.1128/JB.00457-09

Bessadok K, Navarro-Torre S, Pajuelo E et al (2020) The ACC-deaminase producing bacterium *Variovorax* sp. CT7.15 as a tool for improving *Calicotome villosa* nodulation and growth in arid regions of Tunisia. Microorganisms 8:E541. https://doi.org/10.3390/microorganisms8040541

Bhadrecha P, Bala M, Khasa YP et al (2020) *Hippophae rhamnoides* L. rhizobacteria exhibit diversified cellulase and pectinase activities. Physiol Mol Biol Plants 26:1075–1085. https://doi.org/10.1007/s12298-020-00778-2

Bhardwaj D, Ansari MW, Sahoo RK et al (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13:66. https://doi.org/10.1186/1475-2859-13-66

Böhm M, Hurek T, Reinhold-Hurek B (2007) Twitching motility is essential for endophytic rice colonization by the N_2-fixing endophyte *Azoarcus* sp. strain BH72. Mol Plant-Microbe Interact 20:526–533. https://doi.org/10.1094/MPMI-20-5-0526

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48. https://doi.org/10.1038/ncomms1046

Bordiec S, Paquis S, Lacroix H et al (2011) Comparative analysis of defense responses induced by the endophytic plant growth-promoting rhizobacterium *Burkholderia phytofirmans* strain PsJN and the non-host bacterium *Pseudomonas syringae* pv. *pisi* in grapevine cell suspensions. J Exp Bot 62:595–603. https://doi.org/10.1093/jxb/erq291

Borrelli P, Robinson DA, Panagos P et al (2020) Land use and climate change impacts on global soil erosion by water (2015-2070). Proc Natl Acad Sci 117(36):21994–22001. https://doi.org/10.1073/pnas.2001403117

Cánovas D, Vargas C, Kneip S et al (2000) Genes for the synthesis of the osmoprotectant glycine betaine from choline in the moderately halophilic bacterium Halomonas elongata DSM 3043, USA. Microbiology 146:455–463. https://doi.org/10.1099/00221287-146-2-455

Carroll JD, Pastuszak I, Edavana VK et al (2007) A novel trehalase from *Mycobacterium smegmatis*—purification, properties, requirements. FEBS J 274:1701–1714. https://doi.org/10.1111/j.1742-4658.2007.05715.x

Casteriano A, Wilkes MA, Deaker R (2013) Physiological changes in rhizobia after growth in peat extract may be related to improved desiccation tolerance. Appl Environ Microbiol 79(13):3998–4007. https://doi.org/10.1128/AEM.00082-13

Chakraborty T, Akhtar N (2021) Chapter 20: Biofertilizers: prospects and challenges for future. In: Inamuddin MIA, Rajender B, Mashallah R (eds) Biofertilizers: study and impact. Wiley, Hoboken, NJ. https://doi.org/10.1002/9781119724995.ch20

Checroun C, Gutierrez C (2004) Sigma(s)-dependent regulation of *yehZYXW*, which encodes a putative osmoprotectant ABC transporter of *Escherichia coli*. FEMS Microbiol Lett 236:221–226. https://doi.org/10.1016/j.femsle.2004.05.046

Choudhary DK, Prakash A, Johri BN (2007) Induced systemic resistance (ISR) in plants: mechanism of action. Indian J Microbiol 47(4):289–297. https://doi.org/10.1007/s12088-007-0054-2

Chowdappa S, Jagannath S, Konappa N et al (2020) Detection and characterization of antibacterial siderophores secreted by endophytic fungi from *Cymbidium aloifolium*. Biomol Ther 10:1412. https://doi.org/10.3390/biom10101412

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C et al (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol 149:1579–1592. https://doi.org/10.1104/pp.108.130369

Contreras-Cornejo HA, Macías-Rodríguez L, del-Val E et al (2016) Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiol Ecol 92(4):fiw036. https://doi.org/10.1093/femsec/fiw036

Cornelis P, Andrews SC (2010) Iron uptake and homeostasis in microorganisms. Caister Academic Press. https://doi.org/10.21775/9781910190791

Cortés-Patiño S, Bonilla RR (2015) Polymers selection for a liquid inoculant of *Azospirillum brasilense* based on the Arrhenius thermodynamic model. Afr J Biotechnol 14(33):2547–2553. https://doi.org/10.5897/AJB2015.14777

Cruz AT, Cazacu AC, Allen CH (2007) *Pantoea agglomerans*, a plant pathogen causing human disease. J Clin Microbiol 45:1989–1992. https://doi.org/10.1128/JCM.00632-07

Dary M, Chamber-Pérez MA, Palomares AJ et al (2010) "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177(1–3):323–330. https://doi.org/10.1016/j.jhazmat.2009.12.035

Dawson W, Hör J, Egert M et al (2017) A small number of low-abundance bacteria dominate plant species-specific responses during rhizosphere colonization. Front Microbiol 8:975. https://doi.org/10.3389/fmicb.2017.00975

Dogan G, Taskin B (2021) Hydrolytic enzymes producing bacterial endophytes of some poaceae plants. Pol J Microbiol 70(3):297–304. https://doi.org/10.33073/pjm-2021-026

Du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Sci Hortic 196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021

Du J, Li Y, Yin Z et al (2020) High-throughput customization of plant microbiomes for sustainable agriculture. Front Plant Sci 11:569742. https://doi.org/10.3389/fpls.2020.569742

Duca DR, Glick BR (2020) Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. Appl Microbiol Biotechnol 104:8607–8619. https://doi.org/10.1007/s00253-020-10869-5

Egamberdieva D, Ma H, Reckling M et al (2018) Potential effects of biochar-based microbial inoculants in agriculture. Environ Sustain 1. https://doi.org/10.1007/s42398-018-0010-6

Ei SL, Lwin KM, Padamyar KHO et al (2018) Study on IAA producing rhizobacterial isolates and their effect in talc-based carrier on some plants. J Soil Sci Plant Health 2:1

Enebe MC, Babalola OO (2019) The impact of microbes in the orchestration of plants' resistance to biotic stress: a disease management approach. Appl Microbiol Biotechnol 103:9–25. https://doi.org/10.1007/s00253-018-9433-3

Etesami H, Adl SM (2020) Can interaction between silicon and non-rhizobial bacteria help in improving nodulation and nitrogen fixation in salinity-stressed legumes? A review. Rhizosphere 15:100229. https://doi.org/10.1016/j.rhisph.2020.100229

Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Saf 156:225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013

Etesami H, Emami S, Alikhani HA (2017) Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects—a review. J Soil Sci Plant Nutr 17(4): 897–911. https://doi.org/10.4067/S0718-95162017000400005

FAO (Food and Agriculture Organization of the United Nations) (2015) Healthy soils are the basis for healthy food production. pp. 1–4. http://www.fao.org/documents/card/en/c/645883cd-ba28-4b16-a7b8-34babbb3c505

Fekih BI, Zhang C, Li YP et al (2018) Distribution of arsenic resistance genes in prokaryotes. Front Microbiol 9:2473. https://doi.org/10.3389/fmicb.2018.02473

Feng H, Zhang N, Du W et al (2018) Identification of chemotaxis compounds in root exudates and their sensing chemoreceptors in plant-growth-promoting rhizobacteria *Bacillus amyloliquefaciens* SQR9. Mol Plant-Microbe Interact 31:995–1005. https://doi.org/10.1094/MPMI-01-18-0003-R

Ferguson BJ, Beveridge CA (2009) Roles for auxin, cytokinin, and strigolactone in regulating shoot branching. Plant Physiol 149:1929–1944. https://doi.org/10.1104/pp.109.135475

Fernández-Llamosas H, Díaz E, Carmona M (2021) Motility, adhesion and c-di-GMP influence the endophytic colonization of rice by *Azoarcus* sp. cib. Microorganisms 9:554. https://doi.org/10.3390/microorganisms9030554

Ferreira MJ, Silva H, Cunha A (2019) Siderophore-producing rhizobacteria as a promising tool for empowering plants to cope with iron limitation in saline soils: a review. Pedosphere 29(4): 409–420. https://doi.org/10.1016/S1002-0160(19)60810-6

Ferrol N, Azcón-Aguilar C, Pérez-Tienda J (2019) Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: an overview on the mechanisms involved. Plant Sci 280:441–447. https://doi.org/10.1016/j.plantsci.2018.11.011

Flores-Duarte NJ, Caballero-Delgado S, Pajuelo E et al (2022a) Enhanced legume growth and adaptation to degraded estuarine soils using Pseudomonas sp. nodule endophytes. Front Microbiol 13:1005458. https://doi.org/10.3389/fmicb.2022.1005458

Flores-Duarte NJ, Pérez-Pérez J, Navarro-Torre S et al (2022b) Improved Medicago sativa nodulation under stress assisted by Variovorax sp. endophytes. Plan Theory 11(8):1091. https://doi.org/10.3390/plants11081091

Flores-Duarte NJ, Mateos-Naranjo E, Redondo-Gómez S et al (2022c) Role of nodulation-enhancing rhizobacteria in the promotion of Medicago sativa development in nutrient-poor soils. Plan Theory 11:1164. https://doi.org/10.3390/plants11091164

Forchetti G, Masciarelli O, Alemano S et al (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76:1145–1152. https://doi.org/10.1007/s00253-007-1077-7

Frossard SM, Khan AA, Warrick EC et al (2012) Identification of a third osmoprotectant transport system, the *osmU* system, in *Salmonella enterica*. J Bacteriol 194:3861–3871. https://doi.org/10.1128/JB.00495-12

Fu SF, Wei JY, Chen HW et al (2015) Indole-3-acetic acid: a widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav 10(8):e1048052. https://doi.org/10.1080/15592324.2015.1048052

Fukami J, Ollero FJ, Megías M et al (2017) Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. AMB Express 7:153. https://doi.org/10.1186/s13568-017-0453-7

Fukami J, Cerezini P, Hungria M (2018) *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Exp 8:73. https://doi.org/10.1186/s13568-018-0608-1

Gao Q-M, Zhu S, Kachroo P et al (2015) Signal regulators of systemic acquired resistance. Front Plant Sci 6:228. https://doi.org/10.3389/fpls.2015.00228

García-Fraile P, Menéndez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng 2(3):183–205. https://doi.org/10.3934/bioeng.2015.3.183

Garcia-Teijeiro R, Belimov AA, Dodd IC (2020) Microbial inoculum development for ameliorating crop drought stress: a case study of *Variovorax paradoxus* 5C-2. New Biotechnol 56:103–113. https://doi.org/10.1016/j.nbt.2019.12.006

Garg N, Singh S (2018) Arbuscular mycorrhiza Rhizophagus irregularis and silicon modulate growth, proline biosynthesis and yield in Cajanus cajan L. Millsp. (pigeonpea) genotypes under cadmium and zinc stress. J Plant Growth Regul 37:46–63. https://doi.org/10.1007/s00344-017-9708-4

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18. https://doi.org/10.15666/aeer/0301_001018

Gilbert S, Xu J, Acosta K et al (2018) Bacterial production of indole related compounds reveals their role in association between duckweeds and endophytes. Front Chem 6:265. https://doi.org/10.3389/fchem.2018.00265

Giri B, Kapoor R, Mukerji KG (2007) Improved tolerance of *Acacia nilotica* to salt stress by Arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. Microb Ecol 54(4):753–760. https://doi.org/10.1007/s00248-007-9239-9

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39. https://doi.org/10.1016/j.micres.2013.09.009

Goicoechea N, Antol MC (2017) Increased nutritional value in food crops. Microbiol Biotechnol 10:1004–1007. https://doi.org/10.1111/1751-7915.12764

Grass G, Rensing C (2001) CueO is a multi-copper oxidase that confers copper tolerance *in Escherichia coli*. Biochem Biophys Res Commun 286:902–908. https://doi.org/10.1006/bbrc.2001.5474

Griffith AW, Gobler CJ (2020) Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. Harmful Algae 91:101590. https://doi.org/10.1016/j.hal.2019.03.008

Grover M, Bodhankar S, Sharma A et al (2021) PGPR mediated alterations in root traits: way toward sustainable crop production. Front Sustain Food Syst 4:618230. https://doi.org/10.3389/fsufs.2020.618230

Gupta S, Pandey S (2019) ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. Front Microbiol 10:1506. https://doi.org/10.3389/fmicb.2019.01506

Gwara S, Wale E, Odindo A, Buckley C (2021) Attitudes and perceptions on the agricultural use of human excreta and human excreta derived materials: a scoping review. Agriculture 11:153. https://doi.org/10.3390/agriculture11020153

Hayat S, Faraz A, Faizan M (2017) Chapter 10: Root exudates: composition and impact on plant–microbe interaction. In: Ahmad I, Husain FM (eds) Biofilms in plant and soil health. Wiley, Hoboken, NJ, pp 179–193. https://doi.org/10.1002/9781119246329.ch10

Hegyi A, Nguyen TBK, Posta K (2021) Metagenomic analysis of bacterial communities in agricultural soils from Vietnam with special attention to phosphate solubilizing bacteria. Microorganisms 9:1796. https://doi.org/10.3390/microorganisms9091796

Heinonen-Tanski H, van Wijk-Sijbesma C (2005) Human excreta for plant production. Bioresour Technol 96(4):403–411. https://doi.org/10.1016/j.biortech.2003.10.036

Hornung JM, Jones HA, Perry RD (1996) The *hmu* locus of *Yersinia pestis* is essential for utilization of free haemin and haem–protein complexes as iron sources. Mol Microbiol 20: 725–739. https://doi.org/10.1111/j.1365-2958.1996.tb02512.x

Igiehon NO, Babalola OO (2017) Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. Appl Microbiol Biotechnol 101(12):4871–4881. https://doi.org/10.1007/s00253-017-8344-z

Islam S, Akanda AM, Prova A et al (2016) Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol 6:1360. https://doi.org/10.3389/fmicb.2015.01360

Jadhav HP, Shaikh SS, Sayyed RZ (2017) Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: an overview. In: Mehnaz S (ed) Rhizotrophs: plant growth promotion to bioremediation, Microorganisms for sustainability, vol 2. Springer, Singapore. https://doi.org/10.1007/978-981-10-4862-3_9

Ji C, Liu Z, Hao L et al (2020) Effects of *Enterobacter cloacae* HG-1 on the nitrogen-fixing community structure of wheat rhizosphere soil and on salt tolerance. Front Plant Sci 11:1094. https://doi.org/10.3389/fpls.2020.01094

Jiang F, Chen L, Belimov AA et al (2012) Multiple impacts of the plant growth-promoting rhizobacterium Variovorax paradoxus 5C-2 on nutrient and ABA relations of Pisum sativum. J Exp Bot 63:6421–6430. https://doi.org/10.1093/jxb/ers301

Jorquera MA, Saavedra N, Maruyama F et al (2013) Phytate addition to soil induces changes in the abundance and expression of *Bacillus* ß-propeller phytase genes in the rhizosphere. FEMS Microbiol Ecol 83(2):352–360. https://doi.org/10.1111/j.1574-6941.2012.01480.x

Kaasen I, McDougall J, Strøm AR (1994) Analysis of the *otsBA* operon for osmoregulatory trehalose synthesis in *Escherichia coli* and homology of the OtsA and OtsB proteins to the yeast trehalose-6-phosphate synthase/phosphatase complex. Gene 145:9–15. https://doi.org/10.1016/0378-1119(94)90316-6

Kajander T, Kellosalo J, Goldman A (2013) Inorganic pyrophosphatases: one substrate, three mechanisms. FEBS Lett 587:1863–1869. https://doi.org/10.1016/j.febslet.2013.05.003

Kamle M, Borah R, Bora H et al (2020) Systemic acquired resistance (SAR) and induced systemic resistance (ISR): role and mechanism of action against phytopathogens. In: Hesham AL, Upadhyay R, Sharma G et al (eds) Fungal biotechnology and bioengineering, Fungal biology. Springer, Cham. https://doi.org/10.1007/978-3-030-41870-0_20

Kaneko T, Minamisawa K, Isawa T et al (2010) Complete genomic structure of the cultivated rice endophyte *Azospirillum* sp. B510. DNA Res 17(1):37–50. https://doi.org/10.1093/dnares/dsp026

Kang SM, Khan AL, You YH et al (2014) Gibberellin production by newly isolated strain *Leifsonia soli* SE134 and its potential to promote plant growth. J Microbiol Biotechnol 24:106–112. https://doi.org/10.4014/jmb.1304.04015

Kang SM, Shahzad R, Bilal S et al (2019) Indole-3-acetic-acid and ACC deaminase producing *Leclercia adecarboxylata* MO1 improves *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. BMC Microbiol 19:80. https://doi.org/10.1186/s12866-019-1450-6

Kang SM, Shahzad R, Khan MA et al (2021) Ameliorative effect of indole-3-acetic acid- and siderophore-producing *Leclercia adecarboxylata* MO1 on cucumber plants under zinc stress. J Plant Interact 16(1):30–41. https://doi.org/10.1080/17429145.2020.1864039

Karthikeyan N, Prasanna R, Sood A et al (2009) Physiological characterization and electron microscopic investigation of cyanobacteria associated with wheat rhizosphere. Folia Microbiol (Praha) 54(1):43–51. https://doi.org/10.1007/s12223-009-0007-8

Kaschuk G, Hungria M (2017) Chapter 12: Diversity and importance of diazotrophic bacteria to agricultural sustainability in the tropics. In: de Azevedo J, Quecine M (eds) Diversity and benefits of microorganisms from the tropics. Springer, Cham, pp 269–292. https://doi.org/10.1007/978-3-319-55804-2_12

Keswani C, Prakash O, Bharti N et al (2019) Re-addressing the biosafety issues of plant growth promoting rhizobacteria. Sci Total Environ 690:841–852. https://doi.org/10.1016/j.scitotenv.2019.07.046

Khan A, Halo B, Elyassi A et al (2016) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64. https://doi.org/10.1016/j.ejbt.2016.02.001

Khan MA, Asaf S, Khan AL et al (2020) Thermotolerance effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. BMC Microbiol 20:175. https://doi.org/10.1186/s12866-020-01822-7

Koulman A, Lee TV, Fraser K et al (2012) Identification of extracellular siderophores and a related peptide from the endophytic fungus *Epichloë festucae* in culture and endophyte-infected *Lolium perenne*. Phytochemistry 75(6):128–139. https://doi.org/10.1016/j.phytochem.2011.11.020

Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions. Nat Rev Microbiol 18:152–163. https://doi.org/10.1038/s41579-019-0284-4

Krause K, Henke C, Asiimwe T et al (2015) Biosynthesis and secretion of indole-3-acetic acid and its morphological effects on *Tricholoma vaccinum*-spruce ectomycorrhiza. Appl Environ Microbiol 81:7003–7011. https://doi.org/10.1128/AEM.01991-15

Kumar A, Verma JP (2018) Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52. https://doi.org/10.1016/j.micres.2017.11.004

Kumar A, Patel JS, Meena VS et al (2019) Recent advances of PGPR based approaches for stress tolerance in plants for sustainable agriculture. Biocatal Agric Biotechnol 20:101271. https://doi.org/10.1016/j.bcab.2019.101271

Kumari S, Kiran S, Kumari S et al (2021) Optimization of siderophore production by *Bacillus subtilis* DR2 and its effect on growth of *Coriandrum sativum*. Res Square. https://doi.org/10.21203/rs.3.rs-567897/v1

Kusale SP, Attar YC, Sayyed RZ et al (2021) Production of plant beneficial and antioxidants metabolites by *Klebsiella variicola* under salinity stress. Molecules 2:1894. https://doi.org/10.3390/molecules26071894

Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. New Phytol 198(3). https://doi.org/10.1111/nph.12235

Lafuente A, Pérez-Palacios P, Doukkali B et al (2015) Unraveling the effect of arsenic on the model *Medicago–Ensifer* interaction: a transcriptomic meta-analysis. New Phytol 205:255–272. https://doi.org/10.1111/nph.13009

Lagier JC, Khelaifia S, Alou MT et al (2017) Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016 1:16203. https://doi.org/10.1038/nmicrobiol.2016.203

Lau CK, Krewulak KD, Vogel HJ (2016) Bacterial ferrous iron transport: the FeO system. FEMS Microbiol Rev 40(2):273–298. https://doi.org/10.1093/femsre/fuv049

Lebrazi S, Niehaus K, Bednarz H et al (2020) Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. J Genet Eng Biotechnol 18:71. https://doi.org/10.1186/s43141-020-00090-2

Lei XG, Weaver JD, Mullaney E et al (2013) Phytase, a new life for an “Old” enzyme. Ann Rev Animal Biosci 1:283–309. https://doi.org/10.1146/annurev-animal-031412-103717

Leontidou K, Genitsaris S, Papadopoulou A et al (2020) Plant growth promoting rhizobacteria isolated from halophytes and drought-tolerant plants: genomic characterisation and exploration of phyto-beneficial traits. Sci Rep 10(1):14857. https://doi.org/10.1038/s41598-020-71652-0

Lerch TZ, Chenu C, Dignac MF et al (2017) Biofilm vs. planktonic lifestyle: consequences for pesticide 2,4-D metabolism by Cupriavidus necator JMP134. Front Microbiol 8:904. https://doi.org/10.3389/fmicb.2017.00904

Li HB, Singh RK, Singh P et al (2017) Genetic diversity of nitrogen-fixing and plant growth promoting *Pseudomonas* species isolated from sugarcane rhizosphere. Front Microbiol 8:1268. https://doi.org/10.3389/fmicb.2017.01268

Li M, Guo R, Yu F et al (2018) Indole-3-acetic acid biosynthesis pathways in the plant-beneficial bacterium *Arthrobacter pascens* ZZ21. Int J Mol Sci 19(2):443. https://doi.org/10.3390/ijms19020443

Liu A, Hamel C, Hamilton R et al (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331–336. https://doi.org/10.1007/s005720050277

Liu Y, Cao L, Tan H et al (2017) Surface display of ACC deaminase on endophytic Enterobacteriaceae strains to increase saline resistance of host rice sprouts by regulating plant ethylene synthesis. Microb Cell Factories 16:214. https://doi.org/10.1186/s12934-017-0831-5

Liu X, Li Q, Li Y et al (2019a) *Paenibacillus* strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. PeerJ 7:e7445. https://doi.org/10.7717/peerj.7445

Liu X, Jiang X, He X et al (2019b) Phosphate-solubilizing *Pseudomonas* sp. strain P34-L promotes wheat growth by colonizing the wheat rhizosphere and improving the wheat root system and soil phosphorus nutritional status. J Plant Growth Regul 38:1314–1324. https://doi.org/10.1007/s00344-019-09935-8

Liu CH, Siew W, Hung YT et al (2021) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase gene in *Pseudomonas azotoformans* is associated with the amelioration of salinity stress in tomato. J Agric Food Chem 69(3):913–921. https://doi.org/10.1021/acs.jafc.0c05628

Loaces I, Ferrando L, Scavino AF (2011) Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microb Ecol 61(3):606–618. https://doi.org/10.1007/s00248-010-9780-9

Lobo CB, Juárez-Tomás MS, Viruel E et al (2019) Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. Microbiol Res 219:12–25. https://doi.org/10.1016/j.micres.2018.10.012

Lu L, Chang M, Han X et al (2021) Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. J Appl Microbiol 131:1919–1931. https://doi.org/10.1111/jam.15082

Luziatelli F, Ficca AG, Bonini P et al (2020) A genetic and metabolomic perspective on the production of indole-3-acetic acid by *Pantoea agglomerans* and use of their metabolites as biostimulants in plant nurseries. Front Microbiol 11:1475. https://doi.org/10.3389/fmicb.2020.01475

Mahanty T, Bhattacharjee S, Goswami M et al (2017) Biofertilizers: a potential approach for sustainable agriculture development. Environ Sci Pollut Res 24:3315–3335. https://doi.org/10.1007/s11356-016-8104-0

Marco DE, Abram F (2019) Editorial: Using genomics, metagenomics and other "omics" to assess valuable microbial ecosystem services and novel biotechnological applications. Front Microbiol 12. https://doi.org/10.3389/fmicb.2019.00151

Martínez-Hidalgo P, Maymon M, Pule-Meulenberg F et al (2019) Engineering root microbiomes for healthier crops and soils using beneficial, environmentally safe bacteria. Can J Microbiol 65(2):91–104. https://doi.org/10.1139/cjm-2018-0315

Mat-Amin N, Rahim M, Redzuan R et al (2016) Acyl homoserine lactonase genes from *Bacillus* species isolated from tomato rhizosphere soil in Malaysia. Res J Appl Sci 11:656–659. https://doi.org/10.3923/rjasci.2016.656.659

Matos A, Gomes ICP, Nietsche S et al (2017) Phosphate solubilization by endophytic bacteria isolated from banana trees. An Acad Bras Cienc 89(4):2945–2954. https://doi.org/10.1590/0001-3765201720160111

Medina-Córdoba LK, Chande AT, Rishishwar L et al (2021) Genomic characterization and computational phenotyping of nitrogen-fixing bacteria isolated from Colombian sugarcane fields. Sci Rep 11:9187. https://doi.org/10.1038/s41598-021-88380-8

Meena VS, Maurya BR, Verma JP (2014) Does a rhizospheric microorganism enhance K^+ availability in agricultural soils? Microbiol Res 169:337–347. https://doi.org/10.1016/j.micres.2013.09.003

Menéndez E, García-Fraile P (2017) Plant probiotic bacteria: solutions to feed the world. AIMS Microbiol 3(4):747–748. https://doi.org/10.3934/microbiol.2017.4.747

Mesa J, Mateos-Naranjo E, Caviedes MA et al (2015) Endophytic cultivable bacteria of the metal bioaccumulator spartina maritima improve plant growth but not metal uptake in polluted marshes soils. Front Microbiol 6:1450. https://doi.org/10.3389/fmicb.2015.01450

Miethke M, Klotz O, Linne U et al (2006) Ferri-bacillibactin uptake and hydrolysis in *Bacillus subtilis*. Mol Microbiol 61:1413–1427. https://doi.org/10.1111/j.1365-2958.2006.05321.x

Misra S, Vijay KD, Mohammad HK et al (2017) Exploitation of agro-climate environment for selection of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing salt tolerant indigenous plant growth promoting rhizobacteria. Microbiol Res 205:25–34. https://doi.org/10.1016/j.micres.2017.08.007

Mitter EK, Tosi M, Obregón D et al (2021) Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies. Front Sustain Food Syst 5:29. https://doi.org/10.3389/fsufs.2021.606815

Molotoks A, Smith P, Dawson TP (2021) Impacts of land use, population, and climate change on global food security. Food Energy Secur 10:e261. https://doi.org/10.1002/fes3.261

Mukherjee S, Sen SK (2015) Exploration of novel rhizospheric yeast isolate as fertilizing soil inoculant for improvement of maize cultivation. J Sci Food Agric 95:1491–1419. https://doi.org/10.1002/jsfa.6848

Myo EM, Ge B, Ma J, Cui H, Liu B, Shi L et al (2019) Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiol 19:155. https://doi.org/10.1186/s12866-019-1528-1

Na X, Xu T, Li M et al (2018) Variations of bacterial community diversity within the rhizosphere of three phylogenetically related perennial shrub plant species across environmental gradients. Front Microbiol 9:709. https://doi.org/10.3389/fmicb.2018.00709

Nabahungu LN, Semoka J, Zaongo C (2007) Limestone, Minjingu phosphate rock and green manure application on improvement of acid soils in Rwanda. In: Bationo A, Waswa B, Kihara J et al (eds) Advances in integrated soil fertility management in sub-Saharan Africa: challenges and opportunities. Springer, Berlin, pp 703–711. https://doi.org/10.1007/978-1-4020-5760-1_65

Nadeem SM, Ahmad M, Zahir ZA et al (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol Adv 32(2):429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Nakamura S, Minamino T (2019) Flagella-driven motility of bacteria. Biomolecules 9:279. https://doi.org/10.3390/biom9070279

Nascimento FX, Rossi MJ, Soares CRFS et al (2014) New insights into 1-aminocyclopropane-1-carbocylate (ACC) deaminase phylogeny, evolution and ecological significance. PLoS One 9: e99168. https://doi.org/10.1371/journal.pone.0099168

Nascimento FX, Tavares MJ, Glick BR et al (2018) Improvement of *Cupriavidus taiwanensis* nodulation and plant growth promoting abilities by the expression of an exogenous ACC deaminase gene. Curr Microbiol 75(8):961–965. https://doi.org/10.1007/s00284-018-1474-4

Nascimento FX, Tavares MJ, Franck J et al (2019) ACC deaminase plays a major role in *Pseudomonas fluorescens* YsS6 ability to promote the nodulation of alpha- and betaproteobacteria rhizobial strains. Arch Microbiol 201:817–822. https://doi.org/10.1007/s00203-019-01649-5

Navarro-Torre S, Mateos-Naranjo E, Caviedes MA et al (2016a) Isolation of plant-growth-promoting and metal-resistant cultivable bacteria from *Arthrocnemum macrostachyum* in the Odiel marshes with potential use in phytoremediation. Mar Pollut Bull 110(1):133–142. https://doi.org/10.1016/j.marpolbul.2016.06.070

Navarro-Torre S, Barcia-Piedras JA, Mateos-Naranjo E et al (2016b) Assessing the role of endophytic bacteria in the halophyte *Arthrocnemum macrostachyum* salt tolerance. Plant Biol 19(2):249–256. https://doi.org/10.1111/plb.12521

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27(2–3):313–339. https://doi.org/10.1016/S0168-6445(03)00048-2

Niu X, Song L, Xiao Y et al (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol 8:2580. https://doi.org/10.3389/fmicb.2017.02580

Nobbe F, Hiltner L (1896) Inoculation of the soil for cultivating leguminous plants. United States Patent Office. Specification Forming Part of Letters. Patent No. 570,813, Dated 3 Nov 1896

Nordstedt NP, Jones ML (2021) Genomic analysis of *Serratia plymuthica* MBSA-MJ1: a plant growth promoting rhizobacteria that improves water stress tolerance in greenhouse ornamentals. Front Microbiol 12:1105. https://doi.org/10.3389/fmicb.2021.653556

Nosheen S, Ajmal I, Song Y (2021) Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability 13:1868. https://doi.org/10.3390/su13041868

Noushina KNA, Ferrante A, Trivellini A et al (2017) Ethylene role in plant growth, development and senescence: interaction with other phytohormones. Front Plant Sci 8:475. https://doi.org/10.3389/fpls.2017.00475

Oliveira ALM, Santos OJAP, Marcelino PRF et al (2017) Maize inoculation with *Azospirillum brasilense* Ab-V5 cells enriched with exopolysaccharides and polyhydroxybutyrate results in high productivity under low N fertilizer input. Front Microbiol 8:1873. https://doi.org/10.3389/fmicb.2017.01873

Orozco-Mosqueda MDC, Glick BR, Santoyo G (2020) ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. Microbiol Res 235:126439. https://doi.org/10.1016/j.micres.2020.126439

Otieno N, Lally R, Kiwanuka S et al (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front Microbiol 6:745. https://doi.org/10.3389/fmicb.2015.00745

Otto BR, Verweij-van Vught AM, MacLaren DM (1992) Transferrins and heme-compounds as iron sources for pathogenic bacteria. Crit Rev Microbiol 18:217–233. https://doi.org/10.3109/10408419209114559

Overvoorde P, Fukaki H, Beeckman T (2010) Auxin control of root development. Cold Spring Harb Perspect Biol 2:a001537. https://doi.org/10.1101/cshperspect.a001537

Pajuelo E, Rodriguez-Llorente ID, Lafuente A et al (2014) Engineering the rhizosphere for the purpose of bioremediation: an overview. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 9. https://doi.org/10.1079/PAVSNNR20149001

Pajuelo E, Arjona S, Rodríguez-Llorente ID et al (2021) Coastal ecosystems as sources of biofertilizers in agriculture: from genomics to application in an urban orchard. Front Mar Sci 8:685076. https://doi.org/10.3389/fmars.2021.685076

Pallai R, Hynes RK, Verma B et al (2012) Phytohormone production and colonization of canola (*Brassica napus* L.) roots by *Pseudomonas fluorescens* 6-8 under gnotobiotic conditions. Can J Microbiol 58:170–178. https://doi.org/10.1139/w11-120

Pandit A, Adholeya A, Cahill D et al (2020) Microbial biofilms in nature: unlocking their potential for agricultural applications. J Appl Microbiol 129:199–211. https://doi.org/10.1111/jam.14609

Paredes-Páliz KI, Caviedes MA, Doukkali B et al (2016a) Screening beneficial rhizobacteria from *Spartina maritima* for phytoremediation of metal polluted salt marshes: comparison of gram-positive and gram-negative strains. Environ Sci Pollut Res Int 23(19):19825–19837. https://doi.org/10.1007/s11356-016-7184-1

Paredes-Páliz KI, Pajuelo E, Doukkali B et al (2016b) Bacterial inoculants for enhanced seed germination of *Spartina densiflora*: implications for restoration of metal polluted areas. Mar Pollut Bull 110(1):396–400. https://doi.org/10.1016/j.marpolbul.2016.06.036

Paredes-Páliz K, Rodríguez-Vázquez R, Duarte B et al (2018) Investigating the mechanisms underlying phytoprotection by plant growth-promoting rhizobacteria in *Spartina densiflora* under metal stress. Plant Biol (Stuttg) 20(3):497–506. https://doi.org/10.1111/plb.12693

Parmar P, Sindhu SS (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. J Microbiol Res 3(1):25–31. https://doi.org/10.5923/j.microbiology.20130301.04

Pattyn J, Vaughan-Hirsch J, Van de Poel B (2021) The regulation of ethylene biosynthesis: a complex multilevel control circuitry. New Phytol 229:770–782. https://doi.org/10.1111/nph.16873

Pedrosa FO, Monteiro RA, Wassem R et al (2011) Genome of *Herbaspirillum seropedicae* strain SmR1, a specialized diazotrophic endophyte of tropical grasses. PLoS Genet 7(5):e1002064. https://doi.org/10.1371/journal.pgen.1002064

Pereira AL (2017) Chapter 2: The unique symbiotic system between a fern and a cyanobacterium, *Azolla-Anabaena azollae*: their potential as biofertilizer, feed, and remediation. In: Rigobelo EC (ed) Symbiosis. IntechOpen. https://doi.org/10.5772/intechopen.70466

Phuc PD, Konradsen F, Phuong PT et al (2006) Practice of using human excreta as fertilizer and implications for health in Nghean Province, Vietnam. Southeast Asian J Trop Med Public Health 37(1):222–229. https://pubmed.ncbi.nlm.nih.gov/16771238

Podile AR, Vukanti R, Ankati S et al (2014) Root colonization and quorum sensing are the driving forces of plant growth promoting rhizobacteria (PGPR) for growth promotion. Proc Indian Natl Sci Acad 80:407. https://doi.org/10.16943/ptinsa/2014/v80i2/55117

Pourbabaee AA, Shoaibi F, Emami S et al (2018) The potential contribution of siderophore producing bacteria on growth and Fe ion concentration of sunflower (*Helianthus annuus* L.) under water stress. J Plant Nutr 41:619–626. https://doi.org/10.1080/01904167.2017.1406112

Prakash J, Arora NK (2019) Phosphate-solubilizing *Bacillus* sp. enhances growth, phosphorus uptake and oil yield of *Mentha arvensis* L. Biotech 9(4):126. https://doi.org/10.1007/s13205-019-1660-5

Qiu Z, Egidi E, Liu H et al (2019) New frontiers in agriculture productivity: optimised microbial inoculants and *in situ* microbiome engineering. Biotechnol Adv 37(6):107371. https://doi.org/10.1016/j.biotechadv.2019.03.010

Quamruzzaman M, Manik SMN, Shabala S et al (2021) Improving performance of salt-grown crops by exogenous application of plant growth regulators. Biomol Ther 11(6):788. https://doi.org/10.3390/biom11060788

Raklami A, Oufdou K, Tahiri AI et al (2019) Safe cultivation of *Medicago sativa* in metal-polluted soils from semi-arid regions assisted by heat- and metallo-resistant PGPR. Microorganisms 7(7):212. https://doi.org/10.3390/microorganisms7070212

Raklami A, Tahiri A, Bechtaoui N (2021) Restoring the plant productivity of heavy metal-contaminated soil using phosphate sludge, marble waste, and beneficial microorganisms. J Environ Sci 99:210–221. https://doi.org/10.1016/j.jes.2020.06.032

Reitz ZL, Sandy M, Butler A (2017) Biosynthetic considerations of triscatechol siderophores framed on serine and threonine macrolactone scaffolds. Metallomics 9:824–839. https://doi.org/10.1039/c7mt00111h

Rijavec T, Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front Microbiol 7:1785. https://doi.org/10.3389/fmicb.2016.01785

Rillig MC (2004) Arbuscular mycorrhizae, glomalin, and soil aggregation. Can J Soil Sci 84(4): 355–363. https://doi.org/10.4141/S04-003

Rivas R, García-Fraile P, Velázquez E (2009) Taxonomy of bacteria nodulating legumes. Microbiol Insights 2009:2. https://doi.org/10.4137/MBI.S3137

Roba T (2018) Review on: The effect of mixing organic and inorganic fertilizer on productivity and soil fertility. Open Access Library J 5:1–11. https://doi.org/10.4236/oalib.1104618

Rodrigue A, Effantin G, Mandrand-Berthelot MA (2005) Identification of *rcnA* (yohM), a nickel and cobalt resistance gene in *Escherichia coli*. J Bacteriol 187:2912–2916. https://doi.org/10.1128/JB.187.8.2912-2916.2005

Rodríguez M, Torres M, Blanco L et al (2020) Plant growth-promoting activity and quorum quenching-mediated biocontrol of bacterial phytopathogens by *Pseudomonas segetis* strain P6. Sci Rep 10:4121. https://doi.org/10.1038/s41598-020-61084-1

Rosier A, Beauregard PB, Bais HP (2021) *Quorum Quenching* Activity of the PGPR *Bacillus subtilis* UD1022 Alters Nodulation Efficiency of *Sinorhizobium meliloti* on *Medicago truncatula*. Front Microbiol 11:3476. https://doi.org/10.3389/fmicb.2020.596299

Salomon MV, Bottini R, de Souza Filho GA et al (2014) Bacteria isolated from roots and rhizosphere of Vitis vinifera retard water losses, induce abscisic acid accumulation and synthesis of defenserelated terpenes in in vitro cultured grapevine. Physiol Plant 51(4):359–374. https://doi.org/10.1111/ppl.12117

Sammauria R, Kumawat S, Kumawat P et al (2020) Microbial inoculants: potential tool for sustainability of agricultural production systems. Arch Microbiol 202(4):677–693. https://doi.org/10.1007/s00203-019-01795-w

Sangeeth KP, Bhai RS, Srinivasan V (2012) *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. J Spices Aromat Crops 21(2):118–124

Santos MS, Nogueira MA, Hungria M (2019) Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. AMB Express 9(1):205. https://doi.org/10.1186/s13568-019-0932-0

Santoyo G, Urtis-Flores CA, Loeza-Lara PD et al (2021) Rhizosphere colonization determinants by plant growth-promoting rhizobacteria (PGPR). Biology 10:475. https://doi.org/10.3390/biology10060475

Sarhan MS, Hamza MA, Youssef HH et al (2019) Culturomics of the plant prokaryotic microbiome and the dawn of plant-based culture media – a review. J Adv Res 19:15–27. https://doi.org/10.1016/j.jare.2019.04.002

Sauer M, Hantke K, Braun V (1987) Ferric-coprogen receptor FhuE of *Escherichia coli*: processing and sequence common to all TonB-dependent outer membrane receptor proteins. J Bacteriol 169:2044–2049. https://doi.org/10.1128/jb.169.5.2044-2049.1987

Sayyed RZ, Chincholkar SB, Reddy MS et al (2013) Siderophore producing PGPR for crop nutrition and phytopathogen suppression. In: Maheshwari D (ed) Bacteria in agrobiology: disease management. Springer, Berlin. https://doi.org/10.1007/978-3-642-33639-3_17

Schuhegger R, Ihring A, Gantner S et al (2006) Induction of systemic resistance in tomato by N-acyl-L-homoserine lactone-producing rhizosphere bacteria. Plant Cell Environ 29:909–918. https://doi.org/10.1111/j.1365-3040.2005.01471.x

Schütz L, Gattinger A, Meier M et al (2018) Improving crop yield and nutrient use efficiency via biofertilization—a global meta-analysis. Front Plant Sci 12. https://doi.org/10.3389/fpls.2017.02204

Schwibbert K, Marin-Sanguino A, Bagyan I et al (2011) A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581 T. Environ Microbiol 13:1973–1994. https://doi.org/10.1111/j.1462-2920.2010.02336.x

Seefeldt LC, Hoffman BM, Dean DR (2009) Mechanism of Mo-dependent nitrogenase. Annu Rev Biochem 78:701–722. https://doi.org/10.1146/annurev.biochem.78.070907.103812

Shahid M, Hameed S, Imran A et al (2012) Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11. World J Microbiol Biotechnol 28(8):2749–2758. https://doi.org/10.1007/s11274-012-1086-2

Shahid M, Ameen F, Maheshwari HS et al (2021) Colonization of *Vigna radiata* by a halotolerant bacterium *Kosakonia sacchari* improves the ionic balance, stressor metabolites, antioxidant status and yield under NaCl stress. Appl Soil Ecol 158:103809. https://doi.org/10.1016/j.apsoil.2020.103809

Shahzad R, Waqas M, Khan AL et al (2016) Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. Plant Physiol Biochem 106:236–243. https://doi.org/10.1016/j.plaphy.2016.05.006

Shariati JV, Malboobi MA, Tabrizi Z et al (2017) Comprehensive genomic analysis of a plant growth-promoting rhizobacterium Pantoea agglomerans strain P5. Sci Rep 7:15610. https://doi.org/10.1038/s41598-017-15820-9

Sharma L, Priya M, Kaushal N et al (2020) Plant growth-regulating molecules as thermoprotectants: functional relevance and prospects for improving heat tolerance in food crops. J Exp Bot 71(2):569–594. https://doi.org/10.1093/jxb/erz333

Sheldrick W, Keith Syers J, Lingard J (2003) Contribution of livestock excreta to nutrient balances. Nutr Cycl Agroecosyst 66:119–131. https://doi.org/10.1023/A:1023944131188

Shiba T, Tsutsumi K, Ishige K et al (2000) Inorganic polyphosphate and polyphosphate kinase: their novel biological functions and applications. Biochemistry 65:315–323

Siddikee MA, Sundaram S, Chandrasekaran M et al (2015) Halotolerant bacteria with ACC deaminase activity alleviate salt stress effect in canola seed germination. J Korean Soc Appl Biol Chem 58:237–241. https://doi.org/10.1007/s13765-015-0025-y

Sindhu S, Jangra M, Dahiya A et al (2019) Exploration of chickpea rhizobia for solubilization potential of phosphorus, zinc and potassium. Pharma Innov J 8(9):426–429. https://www.thepharmajournal.com/archives/2019/vol8issue9/PartH/8-9-45-232.pdf

Singh M (2018) Isolation and characterization of insoluble inorganic phosphate solubilizer rice rhizosphere strain *Enterobacter cloacae* BAU3. J Appl Natl Sci 10. https://doi.org/10.31018/jans.v10i4.1929

Singh B, Satyanarayana T (2011) Microbial phytases in phosphorus acquisition and plant growth promotion. Physiol Mol Biol Plants 17(2):93–103. https://doi.org/10.1007/s12298-011-0062-x

Singh HB, Keswani C, Singh SP (2019) Intellectual property issues in microbiology. Springer, Singapore. https://doi.org/10.1007/978-981-13-7466-1

Singh RK, Singh P, Li HB et al (2020) Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp. BMC Plant Biol 20:220. https://doi.org/10.1186/s12870-020-02400-9

Soumare A, Diedhiou AG, Thuita M et al (2020) Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. Plants (Basel) 9(8):1011. https://doi.org/10.3390/plants9081011

Sridevi M, Mallaiah KV (2009) Phosphate solubilization by *Rhizobium* strains. Indian J Microbiol 49(1):98–102. https://doi.org/10.1007/s12088-009-0005-1

Srivastav AL (2020) Chapter 6: Chemical fertilizers and pesticides: role in groundwater contamination. In: Prasad MNV (ed) Agrochemicals detection, treatment and remediation. Pesticides and chemical fertilizers. Elsevier, San Diego, pp 143–159. https://doi.org/10.1016/B978-0-08-103017-2.00006-4

Stasi R, Neves HI, Spira B (2019) Phosphate uptake by the phosphonate transport system *PhnCDE*. BMC Microbiol 19:79. https://doi.org/10.1186/s12866-019-1445-3

Steenhoudt O, Vanderleyden J (2000) *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24(4):487–506. https://doi.org/10.1111/j.1574-6976.2000.tb00552.x

Subramanian S, Smith DL (2015) Bacteriocins from the rhizosphere microbiome—from an agriculture perspective. Front Plant Sci 6:909. https://doi.org/10.3389/fpls.2015.00909

Subramanian P, Krishnamoorthy R, Chanratana M et al (2015) Expression of an exogenous 1-aminocyclopropane-1-carboxylate deaminase gene in psychrotolerant bacteria modulates ethylene metabolism and cold induced genes in tomato under chilling stress. Plant Physiol Biochem 89:18–23. https://doi.org/10.1016/j.plaphy.2015.02.003

Subramanium N, Sundaram L (2020) Siderophore producing *Pseudomonas* spp. isolated from rhizospheric soil and enhancing iron content in *Arachis hypogaea* L. plant. Int J Agric Technol 16(2):429–442

Tabassum B, Khan A, Tariq M et al (2017) Bottlenecks in commercialisation and future prospects of PGPR. Agric Ecosyst Environ Appl Soil Ecol 121:102–117. https://doi.org/10.1016/j.apsoil.2017.09.030

Taghavi S, Garafola C, Monchy S et al (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75:748–757. https://doi.org/10.1128/AEM.02239-08

Tao J, Wang S, Liao T et al (2021) Horizontal gene transfer of a unique *nif* island drives convergent evolution of free-living N2-fixing *Bradyrhizobium*. bioRxiv. https://doi.org/10.1101/2021.02.03.429501

Tariq M, Hameed S, Malik K et al (2007) Plant root associated bacteria for zinc mobilization in rice. Pak J Bot 39:245–253. http://www.pakbs.org/pjbot/PDFs/39(1)/PJB39(1)245.pdf

Teotia P, Kumar M, Prasad R et al (2017) Mobilization of micronutrients by mycorrhizal fungi. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza—function, diversity, state of the art. Springer, Cham. https://doi.org/10.1007/978-3-319-53064-2_2

Tian H, De Smet I, Ding Z (2014) Shaping a root system: regulating lateral versus primary root growth. Trends Plant Sci 19:426–431. https://doi.org/10.1016/j.tplants.2014.01.007

Timmusk S, Paalme V, Pavlicek T et al (2011) Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. PLoS One 6:e17968. https://doi.org/10.1371/journal.pone.0017968

Torbaghan ME, Lakzian A, Astaraei AR et al (2017) Salt and alkali stresses reduction in wheat by plant growth promoting haloalkaliphilic bacteria. J Soil Sci Plant Nutr 17(4):1058–1087. https://doi.org/10.4067/S0718-95162017000400016

Trivedi P, Leach JE, Tringe SG et al (2020) Plant–microbiome interactions: from community assembly to plant health. Nat Rev Microbiol 18:607–621. https://doi.org/10.1038/s41579-020-0412-1

Tsukanova KA, Chebotar VK, Meyer JJM et al (2017) Effect of plant growth-promoting rhizobacteria on plant hormone homeostasis. S Afr J Bot 113:91–102. https://doi.org/10.1016/j.sajb.2017.07.007

Ustiatik R, Nuraini Y, Suharjono S et al (2021) Siderophore production of the Hg-resistant endophytic bacteria isolated from local grass in the Hg-contaminated soil. J Ecol Eng 22(5):129–138. https://doi.org/10.12911/22998993/135861

Varga T, Hixson KK, Ahkami AH et al (2020) Endophyte-promoted phosphorus solubilization in *Populus*. Front Plant Sci 11:567918. https://doi.org/10.3389/fpls.2020.567918

Verma P, Yadav AN, Khannam KS et al (2017) Chapter 7: Potassium-solubilizing microbes: diversity, distribution, and role in plant growth promotion. In: Panpatte DG, Jhala YK, Vyas RV et al (eds) Microbes for green revolution, vol 1. Springer Nature Singapore Pte Ltd., pp 125–149. https://doi.org/10.1007/978-981-10-6241-4_7

Wagi S, Ahmed A (2019) *Bacillus* spp.: potent microfactories of bacterial IAA. Peer J 7:e7258. https://doi.org/10.7717/peerj.7258

Walitang DI, Kim K, Madhaiyan M et al (2017) Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of rice. BMC Microbiol 17:209. https://doi.org/10.1186/s12866-017-1117-0

Wang D, Lv S, Jiang P et al (2017) Roles, regulation, and agricultural application of plant phosphate transporters. Front Plant Sci 8:817. https://doi.org/10.3389/fpls.2017.00817

Weilharter A, Mitter B, Shin MV et al (2011) Complete genome sequence of the plant growth-promoting endophyte *Burkholderia phytofirmans* strain PsJN. J Bacteriol 193(13):3383–3384. https://doi.org/10.1128/JB.05055-11

Wheatley RM, Poole PS (2018) Mechanisms of bacterial attachment to roots. FEMS Microbiol Rev 42(4):448–461. https://doi.org/10.1093/femsre/fuy014

Win KT, Tanaka F, Okazaki K et al (2018) The ACC deaminase expressing endophyte *Pseudomonas* spp. Enhances NaCl stress tolerance by reducing stress-related ethylene production, resulting in improved growth, photosynthetic performance, and ionic balance in tomato plants. Plant Physiol Biochem 127:599–607. https://doi.org/10.1016/j.plaphy.2018.04.038

Wu SC, Cao ZH, Li ZG et al (2005) Effects of biofertilizer containing N fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166. https://doi.org/10.1016/j.geoderma.2004.07.003

Wu L, Wu H-J, Qiao J, Gao X, Borriss R (2015) Novel routes for improving biocontrol activity of Bacillus based bioinoculants. Front Microbiol 6:1395. https://doi.org/10.3389/fmicb.2015.01395

Wyckoff EE, Mey AR, Leimbach A et al (2006) Characterization of ferric and ferrous iron transport systems in *Vibrio cholerae*. J Bacteriol 188(18):6515–6523. https://doi.org/10.1128/JB.00626-06

Yadav AN, Verma P, Sachan SG et al (2018) Psychrotrophic microbiomes: molecular diversity and beneficial role in plant growth promotion and soil health. In: Panpatte D, Jhala Y, Shelat H et al (eds) Microorganisms for green revolution, Microorganisms for sustainability, vol 7. Springer, Singapore. https://doi.org/10.1007/978-981-10-7146-1_11

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4. https://doi.org/10.1016/j.tplants.2008.10.004

Yang S, Zhang X, Cao Z et al (2014) A growth-promoting bacterium ZJSH1 associated with *Dendrobium officinale*. Microb Biotechnol 7:611–620. https://doi.org/10.1111/1751-7915.12148

Yao H, Jepkorir G, Lovell S et al (2011) Two distinct ferritin-like molecules in *Pseudomonas aeruginosa*: the product of the *bfrA* gene is a bacterial ferritin (FtnA) and not a bacterioferritin (Bfr). Biochemistry 50:5236–5248. https://doi.org/10.1021/bi2004119

Yarzábal LA (2020) Perspectives for using glacial and periglacial microorganisms for plant growth promotion at low temperatures. Appl Microbiol Biotechnol 104(8):3267–3278. https://doi.org/10.1007/s00253-020-10468-4

Yuan ZS, Liu F, Zhang GF (2015) Characteristics and biodiversity of endophytic phosphorus- and potassium-solubilizing bacteria in Moso Bamboo (*Phyllostachys edulis*). Acta Biol Hung 66(4): 449–459. https://doi.org/10.1556/018.66.2015.4.9

Zarei T, Moradi A, Kazemeini SA et al (2020) The role of ACC deaminase producing bacteria in improving sweet corn (*Zea mays* L. var saccharata) productivity under limited availability of irrigation water. Sci Rep 10:20361. https://doi.org/10.1038/s41598-020-77305-6

Zboralski A, Filion M (2020) Genetic factors involved in rhizosphere colonization by phytobeneficial *Pseudomonas* spp. Comput Struct Biotechnol J 18:3539–3554. https://doi.org/10.1016/j.csbj.2020.11.025

Zhang C, Kong F (2014) Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Appl Soil Ecol 82:18–25. https://doi.org/10.1016/j.apsoil.2014.05.002

Zhang HB, Wang LH, Zhang LH (2007) Detection and analysis of quorum-quenching enzymes against acyl homoserine lactone quorum-sensing signals. Curr Protoc Microbiol Chapter 1, Unit 1C.3. https://doi.org/10.1002/9780471729259.mc01c03s05

Zhang N, Yang D, Wang D et al (2015) Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. BMC Genomics 16:685. https://doi.org/10.1186/s12864-015-1825-5

Zheng J, Sinha D, Wayne KJ et al (2016) Physiological roles of the dual phosphate transporter systems in low and high phosphate conditions and in capsule maintenance of Streptococcus pneumoniae D39. Front Cell Infect Microbiol 6:63. https://doi.org/10.3389/fcimb.2016.00063

Zhu L, Guo J, Sun Y et al (2021) Acetic acid-producing endophyte *Lysinibacillus fusiformis* orchestrates jasmonic acid signaling and contributes to repression of cadmium uptake in tomato plants. Front Plant Sci 12:1041. https://doi.org/10.3389/fpls.2021.670216

Part V
Conclusion: A Future Perspective

Chapter 17
Development and Application of Consortia-Based Microbial Bioinoculants for Sustainable Agriculture

Naveen Kumar Arora and Tahmish Fatima

Abstract The co-existence of the biosphere and human civilization depends on sustainability. The interconnections between humans and the environment require holistic thinking and integrated solutions to achieve standard planetary health. For the advancement of planetary health, in 2015, the United Nations (UN) published the 2030 Agenda for Sustainable Development to encourage effective cross-sector action and partnerships so as to ensure policy coherence across the globe. The UN agenda of Sustainable Development Goals (SDGs) attracted wide interest and commitment from organizations, stakeholders, and leaders. However, the Global Sustainable Development Report (GSDR 2019) highlighted that the development model followed was unsustainable and progress made in the last two decades sounds alarming with rising inequalities, climate change, biodiversity loss, and increasing amounts of waste. The achievement of SDGs is dependent on planet health, and this cannot be achieved without agricultural sustainability as the soil is the base of all biological processes, including nutrient cycling, waste decomposition, and various other activities such as symbiotic and asymbiotic nitrogen fixation.

Keywords Microbial consortia · Biofertilizers · Sustainable agriculture · SDG · PGPR

There is a strong link between agriculture, eradication of hunger and poverty, as along with food production and livestock rearing, it provides income, jobs, food, and other goods and services for the majority of people, thereby covering and connecting with almost all the Sustainable Development Goals (SDGs) (Arora and Mishra 2022). The current agricultural stats are not very impressive and show a negative impact on natural resources and the environment. The pressure of feeding 9.7 billion people by 2050 requires food production to increase by 70–100% (Arora and Mishra 2019). Global Assessment of Soil Degradation (GLASOD) report suggests that

---

N. K. Arora (✉) · T. Fatima
Department of Environmental Science, School of Earth and Environmental Sciences,
Babasaheb Bhimrao Ambedkar University, Lucknow, India

D. K. Maheshwari, S. Dheeman (eds.), *Sustainable Agrobiology*, Microorganisms
for Sustainability 43, https://doi.org/10.1007/978-981-19-9570-5_17

during the last 40 years, about one-third of global arable land has been permanently damaged in some way by soil erosion. Food and Agriculture Organization report (FAO 2020) mentions that 33% of the earth's soil is degraded, and more than 90% is expected to deteriorate by 2050. German Environment Agency (UBA 2015) reports that ten million ha of global arable land is rendered unproductive annually. The report further elaborated that approximately 25% of agricultural soil showed a reduced amount of humus and essential nutrients, rendering them unsuitable for crop production. In addition, anthropogenic activities have led to the degradation of one-third of farmland, up to 75% of crop genetic diversity has been lost, and 22% of animal breeds are at risk (United Nations Convention to Combat Desertification 2017). Maintaining the equilibrium between agricultural production and environmental sustainability is a difficult task, which requires an effective, eco-friendly and cost-effective approach.

Beneficial microbes residing in the soil system employ various mechanisms to mitigate biotic and abiotic stresses, nutrient deficiency in plants, elevate soil fertility and disease resistance. Microbes have been documented, researched, and utilized for more than a century; however, their complete-complex relationship with plants is yet to be revealed. To achieve agricultural sustainability through microbial technology, it is necessary to understand their application strategy, involvement in soil conservation, biotic stress management, and most importantly plant–microbe interactions. The microbiome in soil includes bacteria, fungi, archaea, and protozoans. The rhizosphere zone embodies the richest diversity of microbes, such as arbuscular mycorrhizal fungi (AMF), phosphate-solubilizing microorganisms (PSM), nitrogen-fixing bacteria, plant growth-promoting rhizobacteria (PGPR), actinomycetes, and biocontrol strains. The association of phytomicrobiome and host plant is addressed as holobiont; the former can exist as epiphytes (living on plant surfaces), as endophytes (living inside plant cells), in soil, as rhizosphere microbes present on the subsurface of plant organs, and at root–soil interfaces. The bioinoculants designed for the agricultural field can include both symbiotic and asymbiotic microbes, which can be used as biocontrol agents (for controlling pests and diseases), biostimulants (mobilizing locally available nutrients for plant uptake), and biofertilizers (increasing both plant growth and soil fertility). Symbiotic associations form specialized roots or structures (like nodules) in roots housing microbes in them. Examples of symbiotic plant–microbe interactions include rhizobia and legumes, other nitrogen-fixing bacteria and AMF. In lineation to Liebig's law of the minimum, nitrogen (N) is the most limiting factor in the soil followed by phosphorous. Since most of the agricultural lands are under N-deficient conditions, farmers are forced to apply chemical fertilizers, ruining the concept of sustainability. Reducing chemical abuse, rhizobial bioinoculants can be applied to initiate biological N fixation (BNF) in plants under nutrient-deficient conditions. The interaction between rhizobia and legume is guided by plant exudates and rhizosphere microbial composition. Fixation of N depends upon the nutrient status of the soil and accordingly plants send signals to initiate or inhibit the fixation process. Therefore, the application of N fixers and regulators can optimize the nutrient level of the soil and can reduce the chances of eutrophication, which is the major drawback of the usage of chemical fertilizers. Rhizobia have also been found to induce plant resistance against various diseases both in leguminous and non-leguminous plants. Inoculation of seeds with nitrogen-fixing bacteria such as *Rhizobium* or *Bradyrhizobium* before sowing aids better nodule formation in the roots and increases the rate of fixation.

Along with N, phosphorous (P) and potassium (K) are the essential macronutrients required for growth and metabolism. Use of chemical fertilizers to quench the need of nutrients is still the trend, and Food and Agriculture Organization Corporate Statistical Database (FAOSTAT 2021) reported that each year 27 billion tons of NPK is being used in agro-ecosystems around the globe. In order to replace chemicals, bioinoculants have been designed using consortia of microbes exhibiting N-fixing and phosphate- and potassium-solubilizing properties. The application of phosphate-solubilizing microbes (PSMs) increases the availability of P in soil and makes it available for take up by the plants. Fungal inoculants are better candidates for solubilization of P. Fungal hyphae increase the root surface area and are thinner, so they reach deeper soils, chelating nutrients and water for the plants. Some interesting symbiotic fungal root endophytes such as *Serendipita indicia* and *Serendipita vermifera* induce resistance against fungal pathogens and different insects/pests helping in the overall growth of plants (Mahdi et al. 2022). These fungal strains can be included in the bioformulation along with other PGPR as consortia for better results. K is another important macronutrient supporting the growth and development of plants, and its unavailability reduces agricultural productivity. The application of potassium-solubilizing microbes (KSMs) compensates for the deficiency of K and is an appropriate alternative to potash usage. Globally, N-fixing fertilizers dominate the bioinoculant market, followed by phosphate-solubilizing microbial products (Biofertilizer Market 2022). Multinational companies also commercialize potassium- and zinc-solubilizing products along with NPK consortia liquid/solid products. Rhizobia along with other PGPR share a common micro-habitat, and multiple interactions take place between different microbial groups at the root–soil interface, providing complete protection and growth enhancement in plants. The inconsistent performance of single microbe-based inoculants further emphasizes the need for consortia-based bioformulation. Bacteria, actinobacteria, and fungi are the three important moieties of a functional microbiome, each carrying different roles for the ecosystem. Therefore, the synergies between groups like bacteria and fungi can have several advantages by helping in combating various stresses and requirements of the plant. Modern agricultural systems using novel technologies have shown increasing popularity of probiotic bacteria for crop yield and quality enhancement. The integrated product developed using these bacteria along with specific and indigenous strains can help curb the impact of climate change and can increase the productivity of crops through climate-smart agriculture. The market of microbial inoculants is increasing at a compound annual growth rate (CAGR) of 10.50% during the forecast period of 2022–2029, and the value is expected as USD 950.24 million by 2029. According to Statista (2022) as of 2022, approximately 74.9 million hectares of agricultural land equivalent to almost 1.5% of total farming area across the globe is under organic farming practices.

Though the market of bioinoculants utilizing microbial technology is increasing, the scenario is unsatisfactory, especially in developing and poor countries where conservative agricultural practices challenge sustainability. The reason for this gap is the application methods or failure of bio-products at field levels due to climatic variations or quality issues. The success of microbial technology cannot be achieved without understanding the complex interactions among the microbes and between plants and microbes. The nature of the association between the moieties is dependent on the signaling networks, and that has to be clearly elucidated. Interaction studies also hint towards the use of additives or bridging molecules like exopolysaccharides (EPS), lipochitooligosaccharides (LCOs), and flavonoids that could favor a better establishment of microbes in the rhizosphere/rhizoplane zone (Fatima and Arora 2021). Identification and culturing of novel microbes exhibiting multifarious PGP properties could reduce the maintenance of multiple strains in a consortia-based bioformulation. In this era of climate change, the selected microbes for a bioinoculant product should possess stress-tolerant attributes as a "hard fast rule" to minimize the chances of failure under climatic anomalies and to enhance the shelf life of the product. Due to the increasing impact of climate change around the globe, it is also important to develop tailor-made bioinoculants, especially for the stressed agroecosystems. Indigenous strains with diverse characteristics or diverse PGPR with an array of features can be the solutions for such stressed ecosystems. Multifaceted bioinoculants are the need of the future. However, regulatory bodies from around the globe need to work out common frameworks that support novel microbe-based products for agriculture sustainability. It is also important to track the fate and functionality of introduced microbes and elucidate their impact on rhizosphere microbiota so as to ensure a healthy population of keystone species. Environmental DNA barcoding can be the modern approach for studying the impacts of introduced species on soil autochthonous microbial diversity. Polyphasic approaches can be applied to contrast various application methods, optimize the best protocols and doses of microbial products for enhancing the efficacy and practical application of bioinoculants.

References

Arora NK, Mishra I (2019) United Nations sustainable development goals 2030 and environmental sustainability: race against time. Environ Sustain 2:339–342. https://doi.org/10.1007/s42398-019-00092-y

Arora NK, Mishra I (2022) Current scenario and future directions for sustainable development goal 2: a roadmap to zero hunger. Environ Sustain 5:129–133. https://doi.org/10.1007/s42398-022-00235-8

Biofertilizer Market (2022) Biofertilizer Market Share Size to [2022–2030] Biofertilizer Market: Information by Product (Nitrogen Fixing, Phosphate Solubilizing), Application (Seed Treatment, Soil Treatment), Crop Type, and Region—Forecast till 2030. https://straitsresearch.com/report/biofertilizer-market

FAO (2020) The state of agricultural commodity markets 2020. Agricultural markets and sustainable development: Global value chains, smallholder farmers and digital innovation, Rome. https://doi.org/10.4060/cb0665en

FAOSTAT (2021) In brief the state of food and agriculture 2021. FAO, Rome. https://doi.org/10.4060/cb7351en. Accessed on 10 Nov 2022

Fatima T, Arora NK (2021) *Pseudomonas entomophila* PE3 and its exopolysaccharides as biostimulants for enhancing growth, yield and tolerance responses of sunflower under saline conditions. Microbiol Res 244:Article 126671

Mahdi LK, Miyauchi S, Uhlmann C, Garrido-Oter R, Langen G et al (2022) The fungal root endophyte *Serendipita vermifera* displays inter-kingdom synergistic beneficial effects with the microbiota in *Arabidopsis thaliana* and barley. ISME J 16(3):876–889. https://doi.org/10.1038/s41396-021-01138-y

Statista (2022) Area of organic farming worldwide 2000–2020. https://www.statista.com/statistics/268763/organic-farming-area-worldwide-since-2000/

UBA (German Environment Agency) (2015) Ten million hectares of arable land worldwide are 'lost' every year. Joint press release by the German Environment Agency and the FederalMinistry for Economic Cooperation and Development. Wörlitzer Platz 1, 06844 Dessau-Roßlau, Germany. https://www.umweltbundesamt.de/en/press/pressinformation/ten-million-hectares-of-arable-land-worldwide-are. Accessed 10 Nov 2022