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ISOLATION OF RHIZOSPHERIC BACILLUS SPP. PRODUCING ACC DEAMINASE FROM RAJASTHAN'S ARID ZONE SOIL

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Abstract
The North-western region's arid of Rajasthan and dry climate, which is marked by sparse vegetation and distinct microhabitats inhabited by stress-tolerant microorganisms, is the subject of this study. The properties of the ecosystem are greatly influenced by these microorganisms. Due to a lack of funding and knowledge, the abundant microbial diversity of this megadiverse nation is still largely unexplored. The goal of the study is to find and create super-bioinoculants from native ACC deaminase-active bacteria that can control the production of ethylene under stressful circumstances, therefore modulating plant growth. The research has the potential to identify new microbial strains with biotechnological applications and provides small- and medium-sized farmers with solutions for sustainable agriculture in difficult situations. Screening, characterisation, stress tolerance assessment, molecular analysis, and enzyme activity evaluation of the variety of microbes that produce ACC deaminase in Western Rajasthan.
Keywords- PGPR, ACC deaminase, Abiotic factors, Rhizospheric Bacillus

Introduction
Our nation's arid northwest is distinguished by its sparse vegetation and dry environment, which support unusual microhabitats brimming with microbes. Not only do these hardy microbes survive in these harsh environments, but they also play a major role in defining the unique characteristics of their environments. Being a part of one of the twelve megadiverse nations in the world, the potential of rich microbial diversity is still mostly unrealized because of a lack of resources and knowledge. This uncharted territory presents chances for the discovery of novel bacterial strains with distinctive properties, as well as new genera and species of microorganisms with unrealized biotechnological potential.
Numerous intricate physiological and environmental parameters, such as plant genotype, the physical and chemical properties of the soil, the availability of nutrients, and other variables, all have an impact on plant development and productivity (Schwachtje et al., 2019). Furthermore, a number of biotic and abiotic variables, such as salinity, drought, temperature, mechanical wounding, waterlogging, organic pollutants, heavy metals, and other xenobiotics, might affect crop growth and production (Gupta et al., 2013). Due to these reasons, major crops around the world have seen yield losses of between 35 and 50% thus far (Stallworth et al., 2020). Thus, abiotic

stressors are regarded as the main factor influencing agricultural productivity all over the world. Beneficial microorganisms have recently been utilised to both improve crop growth and lessen the negative consequences of salt stress. A key tactic for enhancing agriculture on salt soils is the application of plant growth-promoting rhizobacteria (PGPR) (Tewari and Arora 2014). According to Sobit et al. (2019), the bacterial genera Acetobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Klebsiella, Pseudomonas, and Serratia have all been classified as PGPR. Because of their ability to survive in a variety of biotic and abiotic stress settings, the Bacillus genera have been identified as the dominant community among the beneficial PGPR (Kang et al. 2015; Radhakrishnan et al. 2017).

The main goal of this work is to find and create super-bioinoculants, which are essential for controlling the ethylene hormone in plants, from native ACC deaminase-active bacterial flora. A crucial component of plant growth and development, ethylene can impede root development and overall plant development when it is produced in excess under stressful conditions including salinity, dehydration, and heavy metals. Rhizospheric bacteria are important for maintaining plant growth in challenging environments because they generate an enzyme called ACC deaminase, which helps reduce stress-induced ethylene synthesis.

The possibility for marketing and commercialising these microbial inoculant products—which would primarily help small and medium-sized farmers—makes the research significant. These super-bioinoculants show potential in addressing environmental and agronomic problems. The study intends to screen and isolate the variety of microbes that produce ACC deaminase in Rajasthan's dry zone. Selected strains will be characterised, stress tolerance will be evaluated, molecular analysis will be carried out, and enzyme activity will be assessed under different circumstances.

Material and Method

In the arid region of Rajasthan, India, Bacillus species were isolated from rhizosphere soil samples collected from fields with extreme saline conditions. The isolation method involved dilution on nutrient Agar medium. These Bacillus species were characterized based on their morphological and physiological traits using Bergeys' Manual of Systematic Bacteriology.

To assess their potential for plant growth promotion, regulation, and biocontrol, each Bacillus strain was individually cultivated in 50 ml flasks with Luria–Bertani (LB) medium under controlled conditions. Various characteristics were evaluated, including phosphate solubilization, indol 3-acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, chitinase activity, hydrogen cyanide (HCN) production, and siderophore production.

Genotypic characterization involved DNA isolation and BOX-polymerase chain reaction (PCR) fingerprint analysis, with identification based on 16S rDNA sequences. Specific primers were used to amplify ACC deaminase genes from the Bacillus strains.

Furthermore, the tolerance of these rhizobacteria to salt, pH, and ACC concentration was studied. This methodology aimed to explore the potential of these Bacillus strains for plant growth enhancement, regulation, and biocontrol, focusing on ACC deaminase activity, salt and pH tolerance, and other key traits.

Result and Discussion

In this investigation, 175 different strains of rhizobacteria were recovered from the rhizosphere of plants growing in high salinity conditions, including H. indicum, H. peruvianum, and Lycopersicon esculentum (Table 1) of the 175 isolates, 14 exhibited physiological or morphological traits unique to Bacillus species, such as a positive Gramme response and other biochemical traits including positive catalase activity (Table 2). The synthesis of IAA, phosphate solubilization, and ACC deaminase activity are the putative plant growth promoting (PGP) activities shared by all 14 Bacillus species isolates (Table 3). In order to better characterise each of the 14 isolates' plant stress homeostasis regulating (PSH) (resistance to abiotic stressors) and biocontrol (PGP) activities, additional biochemical analysis was required. BOX-PCR and acdS gene analysis were used to further characterise the genetic makeup of all 14 isolates. Eight of the 14 isolates had their phylogenetic profiles further refined using their 16S rDNA sequencing. After 48 hours of incubation at 30 degrees Celsius, 14 isolates were found to be capable of producing IAA in a range of 7.2–19.25 µg/ml (Table 3). IAA could be synthesised by Bacillus sp. 10 at the lowest level (7.2 µg/ml) and at the maximum level (19.25 µg/ml) by Bacillus sp. 18 (Table 3). After 48 hours of incubation at 30 degrees Celsius, up to 14 isolates were found to be able to demonstrate phosphate solubilization in a range of 61.5-480 ppm (Table 3). IAA could be synthesised by Bacillus sp. 10 at the lowest level (61.5 ppm), and Bacillus sp. 18 at the greatest level (480 ppm) (Table 3). Based on plate assays, 11 of the 14 isolates of Bacillus species that were chosen were able to generate chitinase, siderophore, and hydrocyanic acid (HCN) at varying levels (Table 3). While the production of Indole Acetic Acid (IAA) and phosphate solubilization (Nautiyal et al., 2000) cause direct plant growth, growth-restricting conditions cause indirect plant growth-promotion, which is caused by the production of antagonistic substances like siderophores (Gupta et al., 2002). lytic enzymes, such as chitinase (Lim and Kim, 1995), and HCN synthesis (Antoun et al., 1998). A bacterium can use one or more of these processes to influence plant growth, and it can also employ distinct strategies for growth promotion at different stages of the plant life cycle when stress conditions are present.

In order to compare the genetic redundancy at the molecular level, three groups of all strains were created using BOX-PCR fingerprinting. Additionally, the acdS gene for ACC deaminase enzymes was amplified for each strain, and the results showed that all of the strains possessed this gene, supporting the ACC deamianse assay results (Table 3). lytic enzymes, such as chitinase (Lim and Kim, 1995), and HCN synthesis (Antoun et al., 1998). A bacterium can use one or more of these processes to influence plant growth, and it can also employ distinct strategies for growth promotion at different stages of the plant life cycle when stress conditions are present.

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supporting the ACC deamianse assay results (Table 3). Therefore, in order to identify and choose the strains resistant to these environmental challenges, we set out to test Bacillus spp. isolates for tolerance to high salt, pH, and temperature. Table 3 presents the results, which indicate that there was a considerable degree of variety among the 14 isolates in their ability to withstand environmental challenges. Three strains were only moderately resistant of up to 5% salt stress, whereas five strains (Bacillus spp. 1, 2, 18, 20, 23) were extremely tolerant of salt concentrations up to 6% NaCl (Table 5). Every strain examined in this investigation demonstrated a significant degree of pH tolerance and adaptation. Bacillus spp. 1, 2, 18, 20, and 23 shown better adaptability at low pH than other strains, and all five of these spp. also demonstrated similar results at high pH (Table 5). High resistance to alkaline conditions was shown by all strains that could withstand salt concentrations ranging from 1% to 4% NaCl (Table 5). The ability of Bacillus strains to adapt to alkaline conditions was found to have a strong positive association. Based on their performance under stress, we selected five potential Bacillus spp. isolates from a total of 14 isolates for more research on the regulation of stress homeostasis in bacteria.
Measurements were made of Bacillus sp. growths at various pH ranges. Maximum pH tolerance of 7, 9, and 10 was reported for Bacillus sp. 20 (Figure 1). Almost all strains of Bacillus were growing at a high concentration of 2 and 4% salt. At high salt concentrations of 2, 3, 4, 5, and 6%, Bacillus sp. 2 grows quickly. Every strain of Bacillus species exhibited a decreasing growth rate at a concentration of 4%, which increased to 5% and 6%. The production of PGP traits and the potential for responsive mechanisms against abiotic stresses in plant stress homeostasis regulating bacteria (PSHB) suggest that these bacteria should be properly applied for environmental stress management in order to support the sustainable development of the agricultural system in arid and semi-arid regions. We suggest that removing such native PSHB from stressed soils could be highly helpful in boosting agricultural output in salinity-affected soils.

Table 1: Details of plant host, source, microorganisms, area, soil property, season and number of isolates of the study

Plant	Source	Microorganism	Area	Soil property	Season	No. of isolates
Heliotropicum indicum	Rhizosphere	*Bacillus* spp.	Sardarshahr Bikaner	Arid soil, pH: 10.0-10.5, High saline soil	Winter; day temp. 25^0C	175
Heliotropicum peruvianum	Rhizosphere	*Bacillus* spp.	Sardarshahr Bikaner	Arid soil, pH: 10.0-	Winter; day temp.	

				10.5, High saline soil	25^0C	
Lycopersicon esculentum	Rhizosphere	*Bacillus* spp.	Sardarshahr Bikaner Sri Ganganagar Hanumangrah	Arid soil, pH: 10.0-10.5, High saline soil	Winter; day temp. 25^0C	

Table 2: Characteristics differentiating isolates are closely related to *Bacillus* species.

Characteristics	***Bacillus* spp. Isolates**													
	1	**2**	**3**	**7**	**10**	**12**	**15**	**18**	**20**	**21**	**23**	**25**	**26**	**27**
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
β- galactosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid production														
D- xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D- glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrolysis of														
Casein	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of														
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+, positive; -, negative

Table 3: Plant growth promoting properties of *Bacillus* spp. Isolates of *H. indicum* and *H. peruvianum* grown under abiotic stress (pH 9 and 6% NaCl)

Strains	**Plant growth properties of *Bacillus* spp.**						
	ACC deaminase activity	**IAA production (µg ml^{-1} protein)**	**Phospahte solubilization (ppm)**	**HCN Production**	**Siderophore production**	**Chitinase activity**	**Antagonism to phytopathogens [*Fusarium***

							***udum, Fusarium oxysporum, Macrophomina phaseolina*]**
1	+++	18.17 ± 1.60	447 ± 4.7	+++	++	+++	+++
2	+++	17.27 ± 1.70	410 ± 2.7	+++	+++	++	+++
3	+	8.5 ± 1.70	61.7 ± 7.7	-	+	-	-
7	+++	19.10 ± 1.70	417 ± 6.2	+++	+++	+++	+++
10	+	7.2 ± 1.30	65.2 ± 6.2	+	+	+	+
12	+	9.2 ± 1.70	68.7 ± 7.7	+	-	-	-
15	+	8.7 ± 2.7	62.7 ± 4.2	+	+	+	+
18	+++	19.27 ± 1.82	480 ± 4.2	++++	++++	+++	++++
20	++	11.27 ± 1.71	87.2 ± 4.2	++	++	++	++
21	+++	16.27 ± 1.61	41.50 ± 4.3	+++	+++	+	+
23	++++	18.21 ± 1.87	417 ± 3.7	+++	++	++	++
25	++++	19.21 ± 1.70	41.57 ± 3.8	++++	++++	+++	++++
26	+	7.7 ± 1.37	68.2 ± 6.7	-	-	-	-
27	+	9.6 ± 1.47	66.7 ± 6.0	-	-	-	-

±, standard error; +, average; ++, good; +++, best; ++++, Excellent; -, negative

Table 4: Identification of PSHB *Bacillus* spp. strains isolated from *H. indicum* and *H. peruvianum* based on partial 16S rDNA sequences and NCBI DNA databases

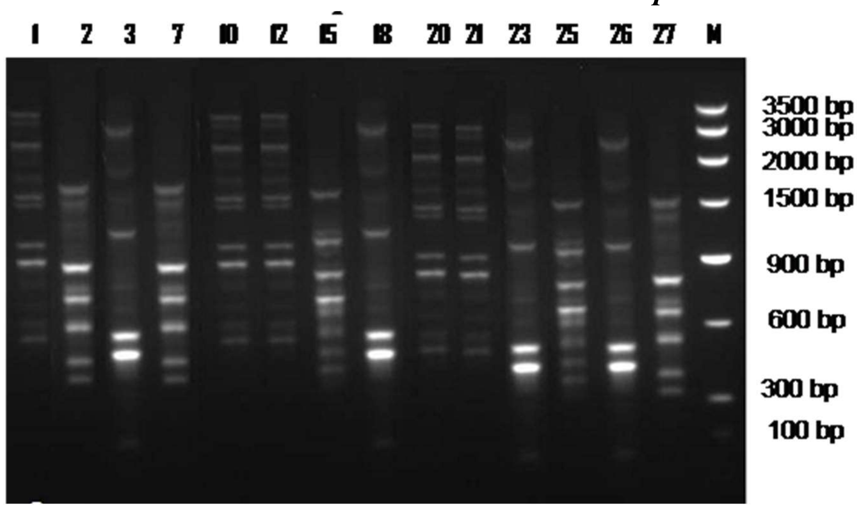
Isolate	Identified as	% Similarity
2	*Bacillus subtilis*	90%

4	*Bacillus amyloliquefaciens*	98%
17	*Bacillus pumilus*	90%
22	*Bacillus subtilis*	90%
27	*Paenibacillus polymyxa*	90%

Table 5: Phenotypic characteristics of *Bacillus* spp. Isolates of *H. indicum* and *H. peruvianum* grown under abiotic stress

Strains	**NaCl % inhibiting the growth**	**Low pH values tolerated**	**High pH values tolerated**	**Maximum temperature tolerated (^{0}C)**
1	5.9	4.3	10.9	41.5
2	5.9	4.3	10.9	41.5
3	4.5	5.5	10.0	41.5
7	6.0	4.5	10.0	40
10	5.0	5.5	9.5	40
12	4.5	5.5	9.0	40
15	4.5	5,5	9.0	40
18	5.9	4.3	10.9	41.5
20	5.9	4.3	10.9	41.5
21	6.0	4.5	10.5	40
23	5.9	4.3	10.9	41.5
25	6.0	4.5	10.5	40
26	4.5	5.5	9.0	38
27	4.5	5.5	9.0	38

Figure 1: BOX PCR pattern (a) and *acds* gene amplification (b) of PSHB *Bacillus* spp. strains isolated from *H. indicum* and *H. peruvianum*



Conclusion

Isolated Bacillus strains from Rajasthan's arid soil display potential for producing beneficial traits under stress, including ACC deaminase, IAA, phosphate solubilization, HCN, siderophore, and chitinase. These bacteria exhibit resilience against abiotic stress and can enhance agricultural sustainability in arid and semi-arid regions. The study offers a valuable gene pool for developing specialized bacterial inoculants, benefiting crop growth. These indigenous strains, following natural selection, can be employed in bioinoculants tailored to local farming conditions. This approach holds potential for cost-effective, eco-friendly resource conservation and promotion among small and medium farmers. Disseminating this knowledge is vital for sustainable agriculture.

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