



## Protecting Plants from Disease and Increasing Their Yields Through the Use of Yeasts as a Biological Agent

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### Abstract

Global demand for biocontrol products is forecast to rise, and their use will likely make it easier to adopt sustainable agriculture practices. In sustainable agriculture, the use of new biocontrol agents is essential for developing an efficient crop-protection plan. Many plant diseases have natural enemies among the yeasts that inhabit a wide variety of environmental niches. Yeasts can swiftly colonize plant surfaces, use a wide variety of nutrients, tolerate a broad temperature range, and create no toxic metabolites, all without negatively impacting the final food products. This means they have the potential to serve as an effective biocontrol agent. This document provides a concise overview of yeast's biological properties and capabilities. The protective strategies yeasts use against plants are also discussed. Some of these mechanisms include the release of volatile organic chemicals, the synthesis of lethal poisons, the battle for limited resources, the synthesis of lytic enzymes, the development of plant immunity, and mycoparasitism. Additionally, examples of yeasts employed for pre- and post-harvest biocontrol are offered, and the underlying processes by which yeasts interact with their plant hosts are outlined. The benefits and drawbacks of yeast-based goods are outlined, as well as a list of commercially accessible yeast-based products.

**Keywords:** biological agent of protection, microbial antagonism, enzyme secretion, organic agriculture

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### INTRODUCTION

Biopesticides and biofertilizers, often known as "microbial-based" pesticides or fertilizers, are projected to play a larger role in agricultural operations throughout the globe as part of a drive to adopt sustainable agriculture policy. By improving crop nutrition and acting as biopesticides, several microbial strains have the potential to increase plant production [1]. Yeasts are included because of their direct and indirect effects on pathogenic bacteria. Pathogenic bacteria may have an adverse effect on crops at any time, including before, during, and after harvest. As a result of plant infections, yields and quality of crops are drastically reduced, and they may no longer be suitable for human use. A health danger may also be posed by

infected goods. Chemical plant-protection products are often utilized in modern, intensive farming. However, organic farmers are not allowed to utilize any methods that include the application of chemicals to their crops. There are also potential drawbacks to using pesticides, such as the development of resistance in pests to the active ingredients.

For both ethical and environmental reasons, today's conscientious shoppers are more likely than ever to choose organically cultivated foods. Yeasts and other microorganisms used in biopesticides may contribute to the production of such goods. The introduction of novel biocontrol agents into the biopesticide market is still necessary to provide an efficient crop-protection strategy in sustainable agriculture. We still need to learn more about and implement hostile yeasts into

practice as a possible biocontrol agent. Yeast is a promising microorganism for use as an antagonist against plant pathogens because it satisfies all the requirements for such a role, including rapid colonization of the plant surface, component utilization, temperature tolerance, metabolites production which are no harmful, and effects on the final food product detrimental absence. It's clear that they're actively metabolizing. They are successful in many environments because of the ways in which they influence other microorganisms, such as by lowering the number of phytopathogens. To determine which yeast species and strains have the potential to be utilized as biofungicides, it is sufficient to cultivate them and collect their metabolites. Yeasts ferment sugars into carbon dioxide and water in an aerobic environment, whereas in an anaerobic environment, alcohol is produced. Their colonies are made up of individual cells that might be spherical, elliptical, oval, or cylindrical. The species, cultural context, and colony's age all have a role in determining their size and form. The length and breadth of the cells normally fall between 3 and 10  $\mu$ m and 2 and 7  $\mu$ m, respectively.

They have both asexual (vegetative) and sexual modes of reproduction (sexually). In order for yeast to initiate its initial form of asexual reproduction—budding—specific parameters must be met, such as a warm temperature and an abundance of food. *Candida*, *Saccharomyces*, *Pichia*, and *Rhodotorula* are all fungi that reproduce in this manner. The bud cells are miniature versions of the parent cells. The cells may break out from the parent cell to produce a new organism or merge with it to form a pseudomycelium, as seen in the genus *Candida*. Fission is the second kind of asexual reproduction. The cell develops by extending itself in one direction, and the offspring are exact replicas of the parent. *Schizosaccharomyces* is known for reproducing in this manner. Yeasts go through sporulation when they are under stress, as when they don't have enough food. In each species of yeast, the spores take on a distinct form. Asexual reproduction results in the formation of diploids when haploid spores successfully mate.

A lower rate of horizontal gene transfer is seen in these organisms due to the complexity of their genome architecture when compared to other fungus. In addition, the absence of plasmids in most yeast species (with the exception of several *S. cerevisiae* strains) eliminates the potential danger posed by plasmid-based pathogenicity and toxin production genes.

## **Yeasts Bioactivity Mechanisms**

Unlike bacteria and filamentous fungus, yeasts' impacts on plants and their diseases are not as well documented. They aid in the development and defense

of agricultural plants in both direct and indirect ways. Both as biostimulants to promote growth and as biopesticides to control the spread of disease, they are beneficial to plants. For yeasts to be effectively used as plant-protection agents, it is necessary to have a firm grasp of the methods through which they communicate with plants and plant diseases.

VOCs are byproducts of the main and secondary metabolism of microorganisms such fungus, bacteria, and yeast [2]. They help other microbes develop or keep them in check, depending on the species [3], and they communicate between cells [4]. These have a high vapor pressure at ambient temperature and are insoluble in water despite their tiny size (often < 300 Da). No direct interaction between the biocontrol agent and the pathogen is required. In recent research, the volatolome's function has been outlined. *Sporidiobolus pararoseus* Fell & Tallman [5], *Candida sake* [6], *Hanseniaspora* [7], *Wickerhamomyces anomalus* (E.C. Hansen) Kurtzman, *Mucor pulcherrima*, *Aureobasidium pullulans*, and *Saccharomyces cerevisiae* [8,9] are only few of the species that create volatile organic compounds. They have been shown to be effective in inhibiting the development of harmful microorganisms including *Botrytis cinerea* and *Colletotrichum acutatum*.

All bacteria, including plant diseases, compete with yeasts for food and habitat [21,22]. As its principal method of action, this mechanism is crucial for safeguarding plant goods in storage, such as fruit storage [21], and in the natural environment, where supplies may be scarce. Yeasts are able to block the pathogen's mycelial development and spore formation because they grow quickly and intensely, producing a biofilm on the plants surface. This biofilm is a membrane of linked microorganisms that may be considered as a consortium or a single organism. Yeasts can colonize plant surfaces, particularly in damaged places where diseases may more easily get access to released nutritional substrates [23]. In order to increase their biomass, yeasts consume available nutrients, leaving less for disease-causing microorganisms to use. Individual yeast cells adhere to the surface of the plant during biofilm development, creating a network of intercellular bridges and hyphae or pseudohyphae [24,25].

Another well-studied process is the synthesis of lytic enzymes by the yeast upon coming into contact with the pathogen. Necrotrophs are an especially good match for this mechanism [33]. Enzymes including chitinases, glucanases, lipases, and proteases may all be secreted by yeasts. Secreted chitinases are beneficial for biocontrol agents because they facilitate the effective breakdown of the cell wall of plant diseases. *Candida*, *Metschnikowia*, *Meyerozyma*, *Pichia*, and

Saccharomyces are only few of the yeast genera where this action has been shown [34–37]. Furthermore, by digesting chitin and generating chitooligosaccharides, chitinases may promote natural plant immunological responses [38]. Lipases are enzymes that function on substrates that are insoluble in water. The yeasts *Candida* and *Cryptococcus* have been shown to have them [39,40]. Fungal cells rely on beta-glucans for adhesion and toxin tolerance in the cell wall.

Proteases play a crucial role in competitive processes, although their synthesis by yeast has not been well researched. *Candida oleophila* Montrocher's protease secretion was characterized by Bar-Shimon et al. [34], while that of *Metschnikowia*, *Pichia*, and *Wickerhamomyces* was described by Pretschner et al. [45]. Yeasts may also boost a plant's defense mechanism naturally [46]. The presence of microbes, including diseases, may be detected and dealt with by the plant's own immune system. This resistance is produced everywhere throughout the body. By increasing the synthesis and activity of molecules such as phytoalexins [47], chitinase and -1,3-glucanase [48], and peroxidase [49], yeasts may trigger the systemic defense of plants against a wide variety of diseases. The vital plant defense mechanism of mycoparasitism is very seldom discussed. Yeast's ability to cling to and perforate the fungus's cell wall is a key aspect of this process because it causes the cell cycle to be arrested, which in turn alters the fungus's shape and reduces its turgor. Glucanase and other enzyme secretion are associated with this process (described above).

## **Plant Hosts and Yeasts Interaction with each other**

Yeasts in the soil are beneficial to plant root development [57-59] and are concentrated in the rhizosphere [54-56]. Colonizing yeasts, especially those on the surface of leaves [62-64], boost plant development [60,61]. Making plant nutrients (such as nitrogen, phosphorus, and potassium) accessible for plants may be the mechanism for promoting plant development [65]. They also play a crucial role in regulating plant development and physiology by secreting hormones including auxins and cytokinins [66]. Yeasts also help plants endure physiological stress better [67].

Plants' access to nutrients is improved with the help of yeasts. Reduced plant output may be attributed, in part, to insufficient nitrogen (N) availability [68]. Bacteria are one kind of microbe that plants often employ to get nitrogen [67]. However, this capacity is shared by certain yeast species. Significant contributions are made by the yeast-produced enzyme 1-aminocyclopropane-1-carboxylase (ACC), which catalyzes the release of significant quantities of

ammonia and so sets in motion a microbe-mediated nitrogen-acquisition mechanism in plants [70]. Deaminases may be produced by a number of yeasts, including *Candida tropicalis* and several species of *Cryptococcus* [71]. Denitrification, the process by which nitrate is reduced to nitrogen or nitrite in between anaerobic respiration and converted to physiologically usable forms for plants [72-74], is facilitated by other yeasts. Phosphorus (P) is second only to nitrogen as a vital plant nutrient [75]. Crop yields might be drastically impacted by its inadequacies. Microbes may use either organic or inorganic forms of this element to make it accessible to plants [76]. The *Rhodotorula* genus, for example, offers dissolved phosphorus by reducing the pH of the water [77-79]. Other examples are the *Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>*-dissolving bacteria *C. tropicalis* and *Lachancea thermotolerans* (Filippov) Kurtzman. Also, some soil-dwelling organisms produce citric acid to break down inorganic phosphorus complexes [69]. Potassium (K), the third most important macronutrient for plants, is essential for a variety of functions, including plant development [70]. Most of this element in soil is bound up in insoluble mineral complexes, therefore the microorganism's involvement in unlocking it is crucial [80].

The presence of microbes may improve the availability of other nutrients for plants. The formation of organic acids in the rhizosphere is often to blame for this phenomenon. Some yeasts, including *S. cerevisiae* and *Williopsis californica* (Lodder) Kurtzman, Robnett, and Basehoar-Power, have been found to oxidize sulfur and other nutrients [83–85].

Phytohormones are plant growth hormones that yeast may produce. Auxins, which include the heterocyclic chemical component indole [86] (conjugated benzene and pyrrole rings) govern several critical plant activities [87]. According to X.Z. Liu, F.Y. Bai, M. Groenew, and B. Boekhout, *Rhodosporidiobolus fluvialis* (Fell, Kurtzman, Tallman & J.D. Buck) Wang, Q.M., Bai, F.Y., Groenew, M., and Boekhout, B. Yeasts such as *Candida maltosa* and *P. kudriavzevii* Komag., Nakase & Katsuya [58,66,70,88]. The cytokinins are a class of phytohormones that may be produced by yeast. There is significant evidence that they influence plant cell division. *Sporobolomyces roseus* Kluyver & C. B. Niel, *Mucor pulcherrima*, and *Acremonium pullulans* are all examples of such organisms [89]. Also present are yeasts that generate gibberellic acid, a plant growth stimulator that has been shown to hasten germination [90].

Reduced crop yields, even by more than half, may be attributed to abiotic stress [91]. Fortunately, microorganisms like yeasts help alleviate this stress by preventing the negative consequences of high

temperatures [92,93], prolonged periods of drought [94,95], high salinity [96,97], and the presence of heavy metals [84]. In response to abiotic stress, plants often release the hormone ethylene [98-100]. This plant regulator is quite powerful, with a wide range of effective concentrations. When a plant is being grown, its output affects every step of growth [101]. Extremely high concentrations of ethylene, however, have been shown to be deleterious to plant growth [102,103]. Plant development is stimulated by ethylene, although a deaminase enzyme found in bacterial cytoplasm helps keep levels low [104]. It has been reported that certain yeasts can inhibit ethylene production and stimulate plant growth. These yeasts include *Candida tropicalis*, *Pichia rugulosa*, *Pichia antarctica*, *Aspergillus pullulans*, *Dothideomycetes* sp., *Cryptococcus* sp., *Rhizophagus paludigenum*, and *Trichoderma globosus* [58,67,105,106].

### Use of Yeast for Postharvest Protection

Products now on the market provide for biological management of postharvest illnesses. Products based on beneficial organisms, the efficacy of which is very context-dependent, are still few. Some 30%-50% of the fruit never reaches the customer due to spoilage during storage [113]. Fungal infections include *Alternaria*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Monilia*, *Penicillium*, and *Rhizopus* are responsible for a significant portion of crop losses. In the modern period, with its expanding population and limited natural resources, reducing these losses is crucial [114]. Although microorganism-based agents, such as yeast, show promise for avoiding such losses, there is currently a dearth of them on the market. Research into such biological agents seems to be crucial in light of the rising awareness and desire among consumers for high-quality items preserved by natural means.

Strawberries, tomatoes, grapes, kiwis, mangoes, pears, and apples have all been named as examples of foods that may be preserved using yeast-based treatments. Kowalska et al. [115] conducted two tests to assess the efficacy of the yeast species *Cryptococcus albidus* in preventing *Botrytis cinerea* infection in strawberries after harvest. After 10 days of storage, the proportion of damaged fruit rose. *B. cinerea* is a major fruit-storage pathogen. *R. glutinis* [116], *Hanseniaspora opuntiae* Cade, Poot, Raspor, & M.T. Sm [117], *A. pullulans* [118,119], and *L. thermotolerans* and *M. pulcherrima* [120] have all been shown to be effective against grey mold. Additional research showed that chitin extracted from *S. cerevisiae* cell walls was efficient in preventing apple decay during storage [121]. *Scheffersomyces spartinae* (Ahearn, Yarrow, & Meyers) Kurtzman & M. Suzuki and *Candida pseudolambica* M.T. Sm. & Poot have both been

shown to be beneficial in apple protection via studies on the impacts of yeasts recovered from sea sediments [122].

Losses in fruit storage may also be brought on by the fungus species *Aspergillus*. Tryfinopoulou et al. [123] investigated and found that *Rhodotorula*, *Metschnikowia*, *Saccharomyces*, and *Pichia* yeasts were all efficient against *Aspergillus*. The antagonistic action of *S. pararoseus* against *Aspergillus niger* Tiegh was reported by Li et al. *Candida nivariensis* (Alcoba-Flórez, Méndez-lv., Cano, Guarro, Pérez-Roth & Arévalo) was reported to be effective against *Aspergillus flavus* Link by Jaibangyang et al. [125]. In addition to molds and mildews, penicillium is another potential problem in storage. *M. pulcherrima* was shown by Assaf et al. [126] to be efficacious against four different strains of *P. expansum*, reducing illness symptoms in humans. *Candida sake* (Saito & Oda) van Uden & H.R. Buckley, isolated from the Arctic environment, was shown to be effective against *P. expansum* by Alvarez et al. [127]. An increased immune response to *Penicillium digitatum* was reported by Hershkovitz et al. [128], while the cell wall of *Rhodosporidium paludigenum* (Fell & Tallman) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout was shown to do the same by Sun et al. [121].

### Products Available for Crop-Protection as Yeast-Based Worldwide

Currently, there are a number of yeast-based bioproducts and a yeast-cell-wall-derived bioproduct registered on a global scale (Table 3). Germinated cells of *A. pullulans* may be found in fungicides like Blossom Protect (a bactericide and fungicide) and fungicides like Botector and BoniProtect (strains DSM 14940 and DSM 14941). Blossom Protect may be used in cold storage facilities and apple orchards to prevent the spread of fire blight, bitter rot, grey mold, damp and brown rot, and anthracnose. Grapevines, strawberries, and other fruits are protected against grey mold with the help of Botector. To prevent the spread of *Pezizula* sp., *Nectria* sp., *B. cinerea*, *Monilinia fructigena* Honey, and *P. expansum*, orchards utilize BoniProtect. The fungicide Julietta, which contains the LAS02 strain of *S. cerevisiae*, is used to protect strawberries and tomatoes against grey mold in protected environments like greenhouses and cold frames. The yeast *C. oleophila* found in Nexy is effective against grey and blue mold that may develop on apples and pears during storage. Postharvest deterioration in some fruits and berries caused by *Botrytis* and *Monilinia* spp. may be prevented with the use of Noli, which contains the *Metschnikowia fructicola* strain NRRL Y-27328 KM1110 WDG. Plants like grapevines, lettuce, tomatoes, strawberries, and cucumbers may be



protected against powdery mildew and grey mold with the help of Romeo, a product containing cerwisian and whose major constituent is the cell walls of *S. cerevisiae*.

Increased commercial availability of yeast-based products, frequently in combination with other microorganisms and plant extracts, are also accessible as plant growth and development enhancers. In contrast to plant protection products, agricultural fertilizers are not subject to the same level of scrutiny about their fitness for use in agriculture. Likewise, it's not easy to construct a list of such commercial items that are accessible in many nations.

## Prospects

The usage of agrichemicals may be reduced or eliminated entirely and plant quality improved by including microbial agents into plant protection. However, there are stringent standards that must be met by biological plant protection agents. They must be very effective in preventing the spread of disease-causing organisms. Both in vitro and in planta investigations are time-consuming and costly, making development and execution a lengthy process. In theory, they should have a low cost per unit of biomass produced, but in practice, the production process is often tedious and resource-heavy. To enable the microorganisms' survival, a suitable carrier (such lignite dust) must be utilized. Their usefulness and viability depend on correct formulation. Yeasts, like other biocontrol agents, may be made more effective and last longer with the help of the correct carrier, one that is efficient, biodegradable, and nonpolluting. At the moment, solid (peat, powder, and granules) and liquid carriers are employed for biopesticides. It is also essential that, regardless of manufacturing size, the antagonistic qualities shown in the lab be maintained. Keeping their antagonistic characteristics and ensuring consistent performance throughout a wide range of environmental conditions is another challenge. The bacteria' compatibility with the plant is also crucial. The number of yeast strains showing antagonistic action against plant diseases in laboratory trials is far larger than those put into practice for a variety of reasons, including the difficulties already mentioned. Systemic biocontrol techniques that take into account beneficial microorganisms, crops, pests, and agricultural practices are needed to address these challenges [129]. Biocontrol approaches and tactics need adjustments to the present productive structure, which includes technological production systems, regulations, and markets [130].

Despite these obstacles, there is a pressing need for the research, development, and commercialization of biological organic crop protectants and yield

enhancers. As was previously indicated, yeasts are ecologically benign and may take part in the bioremediation method [131]. Moreover, they can be employed as biocontrol agents against plant infections. *Rhodotorula mucilaginosa* was utilized to get rid of neonicotinoid pesticides and thiacloprid [72], while *Rhodotorula glutinis* and *Rhodotorula rubra* were demonstrated to decompose organophosphorus chlorpyrifos [132]. In addition to granulovirus, which enhances larval mortality and ensures improved protection of the apple tree against apple fruit invasion by *Cydia pomonella* [135], genetically engineered yeast strains *M. pulcherrima*, *Cryptococcus tephrensis* and *A. pullulans* may lower pest populations [133,134]. In field trials with cotton, tomato, and maize, a modified *Yarrowia lipolytica* yeast successfully eradicated *Helicoverpa armigera* by producing the pest's sexual pheromone [136-138].

## Conclusions

Effective, long-lasting, and ecologically benign, biological control using microorganisms [139]. Successful use of it may lessen the need for chemical fungicides, which have a major negative impact on human health and the environment [140]. As the need for biological plant-protection agents grows, more study into the topic is warranted; this is especially true when considering the fact that living microorganisms require specific environmental conditions in order to survive after application; as such, the strategy of treatments based on living yeasts or substances produced by them must be developed in tandem with the technology of production for these biological products [141,142].

Yeasts have significant protective capabilities and have been recognized for a while, so the prospects for utilizing them are highly intriguing. The molecular connection between the plant pathogen and the yeast cell that triggers the plant's defensive response is still the subject of active research. In response to abiotic stressors, plants release the hormone abscisic acid (ABA). Numerous gene duplications encoding homologous signaling components are required for the efficient operation of the intricate ABA signaling system. Among the various functions of phytohormones is abscisic acid's (ABA) ability to boost a plant's resilience to a variety of abiotic stressors. The function of this complex and the highly multiplexed main signaling channel are being studied using yeast as a reconstitution system. More research is required to determine whether of the newly developed models from the reconstructed ABA signal transduction pathway in yeast are reflective of the signaling pathways found in plants. As with nanocompounds [143], maybe isolating molecules and

getting to work on them can aid in the development of new protective revolutionary bioproducts. Nanocompounds with a low environmental impact, which are occasionally mixed with bioinoculants, have been shown to be an effective replacement for chemical fertilizers in environmentally friendly farming [144,145]. Creating nanotechnology using yeast compounds for application in plant defense is a new and exciting task.

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