Biocontrol Potential and Mechanisms of Action of Plant Associated Yeast

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Review Article

ABSTRACT

Yeasts, single-celled fungi, have established themselves as effective biocontrol agents against various plant pathogens, demonstrating mechanisms such as nutrient and space competition, secretion of antimicrobial compounds, and induction of plant resistance. Their genetic stability, resilience to harsh conditions, and ease of cultivation on low-cost media enhance their applicability in agriculture. This review explores the diverse modes of action employed by yeasts, including biofilm formation, production of lytic enzymes, volatile organic compounds, and mycoparasitism, all contributing to their biocontrol efficacy. Notable registered yeast species like *Candida oleophila*, *Aureobasidium pullulans*, *Metschnikowia fructicola*, and *Saccharomyces cerevisiae* showcase commercial potential in managing plant diseases, with various products already available in the market. Additionally, yeast strains have been shown to enhance plant growth, improving crop vigour and yield. The commercial applications of yeast-based bioproducts highlight their potential as sustainable alternatives to chemical pesticides in agriculture, emphasizing their importance in integrated disease management strategies.

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1. INTRODUCTION

Yeasts are single-celled microorganisms classified under the kingdom Fungi. They can be either facultative anaerobes or obligate aerobes, and typically reproduce by budding. Yeasts have long been utilized in industries such as baking and brewing, and are also involved in the preparation of various foods like idli and dosa. Additionally, some yeasts exhibit medicinal properties, making them useful as alternative treatments for human ailments such as diarrhea, common flu, and respiratory disorders. Certain yeasts, such as *Saccharomyces boulardii*, are probiotic in nature and contribute to lowering cholesterol levels and enhancing immune function in humans. In the plant environment, yeasts are predominantly found in the phylloplane, rhizosphere, and soil.

1.1 Why Yeast?

Yeasts as biological control agents (BCAs) have distinct advantages over bacterial or fungal antagonists, particularly in terms of safety and versatility in mechanisms of action. Yeasts provide pathogen inhibition through mechanisms like competition for nutrients and space, secretion of lytic enzymes, and the production of volatile organic compounds rather than antibiotics or allergenic spores. This makes yeasts suitable for applications directly on consumable plant parts, aligning with the increasing demand for eco-friendly, residue-free crop protection strategies, especially for food crops like leafy greens where chemical residues are a major concern [1,2].

Furthermore, certain yeasts can promote plant growth by modulating plant defenses and enhancing stress tolerance, effectively offering dual benefits as both biocontrol agents and plant growth promoters. This makes them a highly valued option for integrated pest management (IPM) and sustainable agriculture approaches [3,4].

Yeasts possess several beneficial characteristics that make them excellent candidates for use as biocontrol agents Agirman et al., [5] are listed below:

I. Yeasts are genetically stable and effective even at low concentrations.
II. They can endure harsh environmental conditions and are resistant to postharvest chemical treatments such as fungicides, pesticides, and phytohormones.
III. Yeasts are effective against a wide range of pathogens affecting various crops.
IV. They are easy to culture on low-cost media and are not highly demanding in terms of nutrient requirements.
V. Yeasts can be formulated into stable products with extended shelf life.
VI. They are simple to store and distribute.
VII. Yeasts do not produce toxic metabolites harmful to humans.

Their unicellular morphology offers numerous practical applications, including ease of cultivation in fermenters, adhesion properties, and biofilm formation [6].

According to Pantelides et al. (2015), yeasts possess several traits that make them effective antagonistic agents. They have minimal nutritional requirements, can thrive in diverse environmental conditions, grow rapidly, and effectively colonize both healthy and damaged surfaces of fruits, vegetables, and plants, even under dry conditions. Yeasts are non-toxic to humans, resistant to most agrochemicals, and can withstand extreme environmental factors such as temperature variations, desiccation, UV radiation, fluctuating humidity levels, pH changes, and low oxygen levels. Their ability to adapt to fruit microenvironments—characterized by low pH, high sugar content, and osmotic pressure—further enhances their suitability [7]. Yeasts can be easily isolated from a variety of sources, including surfaces of fruits and vegetables, leaves, soil, seawater, and plant roots [8].

In the last three decades, the exploration of yeast isolates as biological control agents (BCAs) has gained significant momentum among researchers, driven by their numerous advantageous traits, such as rapid growth and resistance to pathogens. The interest in utilizing yeasts for biological control has notably surged in the past ten years, reflecting a broader trend toward sustainable agriculture and environmentally friendly disease management strategies [9-14].

Recent studies have demonstrated the efficacy of various yeast species in suppressing plant

diseases, enhancing plant growth, and improving soil health. For instance, yeasts like *Candida* and *Pichia* species have shown promising results in reducing fungal pathogens such as *Botrytis cinerea* and *Fusarium spp.* Elkhairy et al., [15], Ali et al., [16]. Additionally, advancements in molecular techniques have facilitated the identification and characterization of novel yeast strains with potent biocontrol capabilities, paving the way for their application in integrated pest management systems [17]. As the demand for sustainable agricultural practices grows, the potential role of yeasts as effective BCAs continues to be a focal point of ongoing research, emphasizing their contribution to crop protection and yield improvement while minimizing chemical inputs.

2. MODES OF ACTION OF YEASTS AGAINST PLANT PATHOGENS

Yeasts employ several mechanisms to combat plant pathogens, including competition for nutrients and space, secretion of antimicrobial compounds, production of lytic enzymes and volatile organic compounds, direct parasitism, and the induction of plant resistance [18].

2.1 Competition for Nutrients and Space

Nutrient and space competition is a critical factor in microbial ecology and is considered one of the primary modes of action for biocontrol yeasts. Iron is a particularly important nutrient for biocontrol yeasts, and competition for iron plays a key role in their antagonistic activity [7]. In *Aureobasidium pullulans*, the siderophore fusarinine C (fusigen) has been identified as a compound with antibacterial activity, suggesting its role in iron competition within ecological niches [19]. Gore-Lloyd and colleagues [20] found that wild-type *Metschnikowia pulcherrima* colonies exhibited a distinctive red pigmentation due to the production of pulcherriminic acid, which complexes with iron. Mutant *M. pulcherrima* colonies, lacking this pigment, showed reduced iron competition and lower antifungal activity, as demonstrated by decreased inhibition (80%) of *Botrytis caroliniana* mycelium compared to the 98% inhibition observed with the wild-type strain. This iron-deprivation mechanism is thought to be a key factor in *M. pulcherrima*'s antagonism of fungal plant pathogens.

Research has identified *Pichia kluyveri* as a yeast capable of producing siderophores,

thereby sequestering iron and limiting its availability to competing microorganisms. This property makes it a promising candidate for biocontrol applications, particularly in food safety and spoilage prevention [21]. Additionally, other yeasts like *Candida sake* and *Aureobasidium pullulans* have shown similar iron-binding capabilities, particularly on fruit surfaces where iron availability can be controlled to discourage pathogenic fungi [1,22]. Recent investigations have highlighted the role of *Debaryomyces hansenii* in iron metabolism and its ability to enhance plant health by mobilizing iron. This yeast can produce specific metabolites that facilitate the solubilization of iron from complexed forms in the soil, making it more bioavailable for plants. This capability not only supports plant growth but also aids in suppressing pathogenic fungi by altering the microbial community dynamics in the rhizosphere [23]. Furthermore, *Saccharomycopsis schoenii* exhibits a unique ecological adaptation by lacking components of the sulfur assimilation pathway, including enzymes required for synthesizing methionine from inorganic sulfur sources. This metabolic dependency suggests that *S. schoenii* compensates by extracting methionine from other organisms, particularly those it preys upon. Such adaptations allow it to survive in competitive microbial communities and maintain its role as a mycoparasite, where it relies on its hosts or prey for essential nutrients [24]. Additionally, Bencheqroun et al. [25] demonstrated that apple amino acids were significantly depleted at wound sites treated with *Aureobasidium pullulans* strain Ach1-1, particularly in combination with *Penicillium expansum*, compared to untreated wounds. The addition of exogenous amino acids reduced the efficacy of strain Ach1-1 in controlling *P. expansum*, suggesting that amino acid competition plays a role in biocontrol efficacy.

2.2 Biofilm Formation

Biofilm formation is an effective and intricate strategy employed by microbial communities to compete for space on various surfaces. These biofilms can consist of a single microbial species or multiple species coexisting in consortia [26]. The process begins with microbial cells adhering to a surface, followed by modifications in the cell wall, secretion of an extracellular matrix, and, in certain cases, the development of hyphae or pseudohyphae [27]. In *Saccharomyces cerevisiae*, biofilm-forming cells have been shown to be significantly more effective than their

planktonic counterparts in colonizing apple wound surfaces, which in turn enhances their ability to control the spread of blue mold caused by *Penicillium expansum* [28]. Studies by Spadaro and Droby [7] indicate that *P. guilliermondii* has the capacity to form biofilms which can produce extracellular enzymes and antimicrobial peptides within the matrix, providing an additional mechanism of pathogen inhibition. *Meyerozyma guilliermondii* is known for its strong biofilm-forming ability, achieving an optical density (OD) of 0.93 ± 0.01, indicating dense biofilm production. This characteristic is valuable in biocontrol applications, as robust biofilm formation helps the yeast adhere effectively to surfaces and compete with pathogens. Biofilms offer a physical barrier and contribute to the persistence of *M. guilliermondii* on plant surfaces, enhancing its effectiveness against pathogens by limiting their access to nutrients and space [29]. The role of cell-to-cell communication through QS products like N-acyl homoserine lactone is crucial for the coordination of microbial behaviors, including biofilm development. This process enables yeasts and bacteria to establish communities on surfaces, enhancing their survival and interaction with the environment [30].

2.3 Secretion of Lytic Enzymes

The secretion of enzymes that degrade cellular components is indeed a common feature in various host-pathogen interactions. This process is often a key virulence strategy employed by pathogens to gain access to nutrients, evade host immune responses, and cause damage to host tissues. Some of the lytic enzymes produced by yeast are:

2.3.1 Chitinases

The secretion of chitinolytic enzymes is a valuable strategy for biocontrol agents, particularly in fighting fungal pathogens. These enzymes degrade chitin, a key component of fungal cell walls, enhancing the effectiveness of biocontrol agents against plant diseases [31]. Various yeast genera, including *Aureobasidium*, *Candida*, *Debaryomyces*, *Metschnikowia*, *Meyerozyma*, *Pichia*, *Saccharomyces*, *Tilletiopsis*, and *Wickerhamomyces*, exhibit chitin-degrading activity, allowing them to target and dismantle fungal cell walls [32]. Furthermore, the breakdown of chitin produces chito-oligosaccharides (CHOS), which can stimulate plant immune responses, thereby enhancing plant defenses against fungal infections [33].

2.3.2 Glucanases

Glucans are key components of fungal cell walls, and exoglucanases are enzymes that cleave the bonds between 1,3-glucan and 1,6-glucans, playing a crucial role in cell wall modification and adhesion [34]. In *Pichia anomala*, two exo-β-glucanase genes, PaEXG1 and PaEXG2, have been linked to biocontrol activity against *Botrytis cinerea* in fruits, with the deletion of these genes resulting in a marked decrease in biocontrol efficacy [35]. Additionally, Lopes et al. [36] found that six isolates of *Saccharomyces cerevisiae* exhibited antifungal properties against *Colletotrichum acutatum* by secreting exoglucanases in citrus.
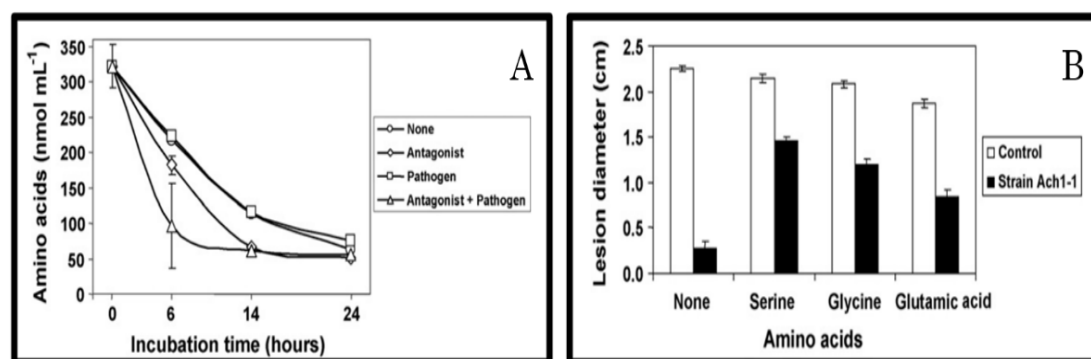

Fig. 1. A. Concentration (nmol mL) of total amino acids in apple wounds during the first 24 h of incubation. Wounds were either intact (none), treated with A. pullulans strain Ach1-1 alone (antagonist), inoculated with P, expansum alone (pathogen) or treated with strain Ach1-1 and then inoculated with P. expansum (antagonist + pathogen). B. Effect of exogenous application of serine, glycine or glutamic acid in apple wounds on lesion diameters (cm) induced by P. expansum in the absence (control) or in the presence of A. pullulans (strain Ach1-1) after 5 days of incubation [25]
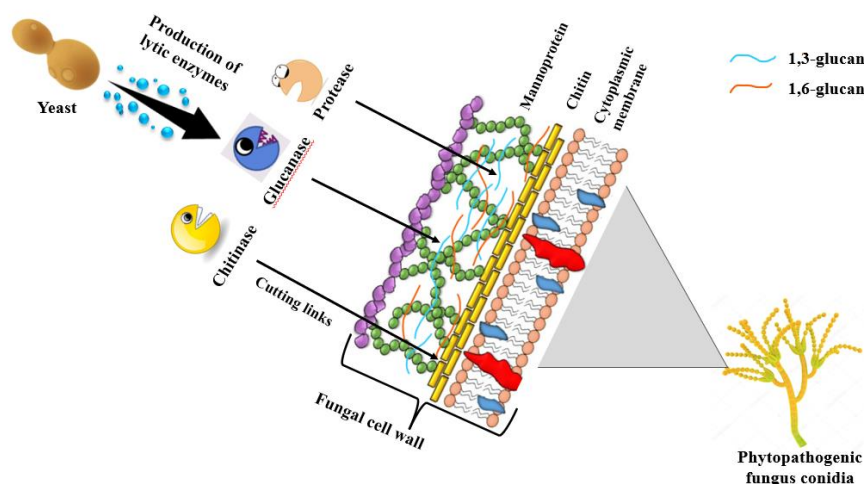
Fig. 2. Illustration of the mechanism by which antagonistic yeasts inhibit the growth of phytopathogenic fungi through the production of lytic enzymes, such as chitinase, glucanase, and protease, which act on the fungal cell wall

2.3.3 Lipases

Lipases are enzymes that catalyze the hydrolysis of lipids, converting them into free fatty acids, glycerol, and other alcohols. Lipolytic activity is commonly observed during the screening of extracellular enzymatic activity in yeast and yeast-like strains [37] Additionally, lipase activity has been identified in various pathogenic yeasts, including species of *Candida*, *Cryptococcus*, and *Malassezia* [38].

2.3.4 Proteases

Proteases are enzymes that cleave alpha peptide bonds between naturally occurring amino acids. In biocontrol yeasts, protease activity may be detected at later stages of biocontrol, suggesting its limited role in this process [39]. The alkaline serine protease Alp5 from *Aureobasidium pullulans* has been shown to inhibit spore germination and reduce germ tube length in several fungal species, including *Penicillium expansum*, *Botrytis cinerea*, *Monilinia fructicola*, and *Alternaria alternata* [40]. Furthermore, protease activity has also been observed in the genera *Metschnikowia*, *Pichia*, and *Wickerhamomyces* [41].

3. TOXIN PRODUCTION

Toxins are proteins, often glycosylated, that bind to specific receptors on the surface of target microorganisms, leading to their destruction through a targeted mechanism. An example of a bioactive compound produced by the biocontrol yeast *Pseudozyma flocculosa* is flocculosin, a low molecular weight cellobiose lipid with the potential to inhibit plant pathogenic fungi [42]. Notable yeast strains that produce toxins against pathogens include *Saccharomyces cerevisiae* (K1, K2, K28, Klus, KHR, KHS), *Pseudozyma flocculosa* (flocculosin), and *Candida nodaensis* (PYCC 3198) [43,42,44]. A significant agricultural breakthrough was the use of killer yeast strains, specifically *Pichia fermentans* strains 27, 28, and 56, to control the postharvest pathogen *Penicillium digitatum* on citrus, with strain 27 demonstrating a protection efficiency of 93.6% [45]. Certain strains of *Saccharomyces cerevisiae* produce killer toxins that target the molds by disrupt cell membranes and inhibit growth in turn reduce spoilage in food products [46].

3.1 Volatile Organic Compounds (VOCS)

Volatile organic compounds (VOCs) are low-water-solubility compounds, typically under 300 Da, produced during the metabolism of organisms. They encompass a variety of chemical types, including hydrocarbons, alcohols, and phenols [47]. Aureobasidium pullulans effectively inhibits several fungal pathogens like Botrytis cinerea, both in vitro and in planta [48]. The biocontrol of table grapes by food yeasts such as Wickerhamomyces anomalus and Saccharomyces cerevisiae is primarily due to VOC production [4]. Inhibition of B. cinerea germination has been associated with

2-ethyl-1-hexanol [49]. In a 2022 study by Natarajan et al., 12 of 45 yeast isolates showed effective inhibition of Aspergillus flavus growth, with S. cerevisiae strain YKK1 achieving up to 92.1% inhibition of mycelial growth and 98.1% reduction in aflatoxin B1 production. Gas chromatography-mass spectrometry analysis identified several antimicrobial compounds, including 1-pentanol and benzothiazole, which significantly reduced fungal growth and aflatoxin synthesis (Figs. 3-4). A study by Konsue et al. [50] reported that *Torulaspora indica* DMKU-RP31, *T. indica* DMKU-RP35, and *Pseudozyma hubeiensis* YE-21 inhibited *Lasidiploidia*

theobromae, while only *Papiliotrema aspenensis* DMKU-SP67 inhibited *Colletotrichum gloeosporioides.* Their antagonistic mechanisms included antifungal volatile organic compounds (VOCs). *T. indica* DMKU-RP35 reduced fruit rot severity by 82.4%, similar to the 87.5% reduction by benomyl, while *P. aspenensis* DMKU-SP67 reduced anthracnose severity by 94.1%, comparable to benomyl's 93.9% reduction. The VOCs also reduced disease severity but were less effective than yeast cells. These yeasts show potential as biological control agents for fruit rot and anthracnose (Fig. 5).
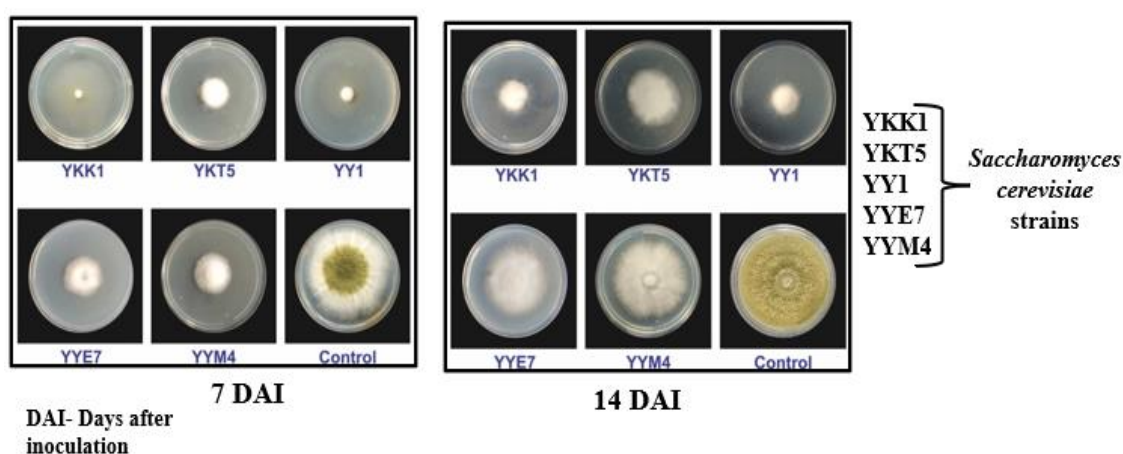

Fig. 3. The inhibitory efficiency of VOCs produced by *S. cerevisiae* strains (YKK1, YKT5, YY1, YYE7 and YYM4) on *A. flavus* at 14 days after inoculation [51]
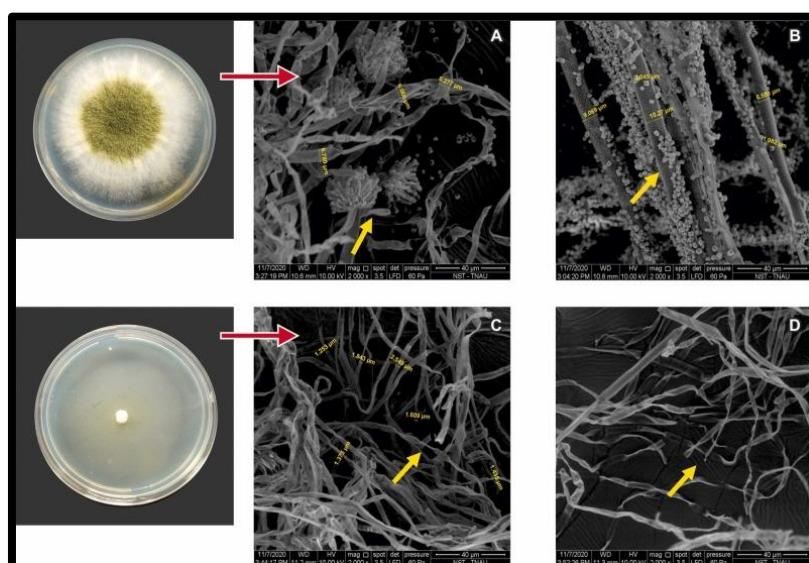

Fig. 4. Scanning electron microscopic view of hyphal and conidial morphology of *A. flavus*. (A and B): Untreated *A. flavus* with normal hyphae, conidiophores and conidia. (C and D): Volatiles treated *A. flavus* with deformed hyphae and the absence of conidia and conidiophores [51]
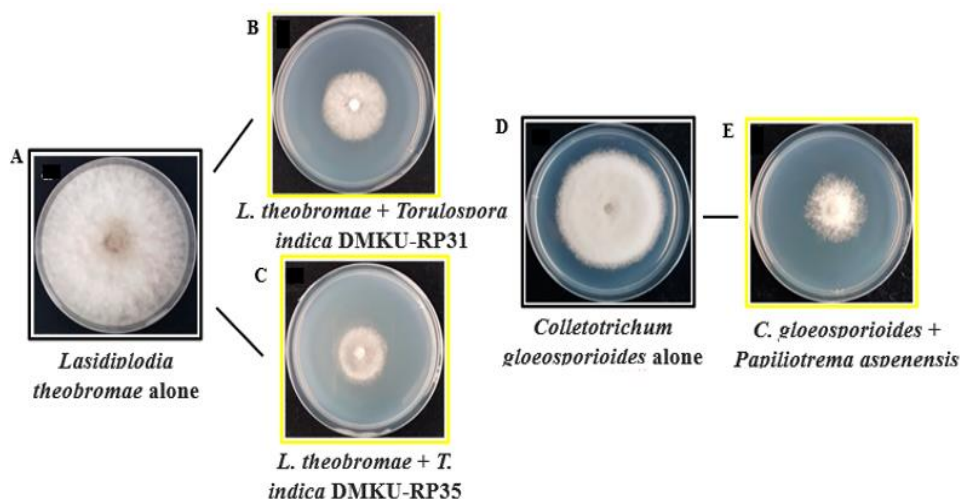
Fig. 5. (A). Growth on PDA at 25 °C of *L. theobromae* alone and (B) with *T. indica* DMKU-RP31; (C) *T. indica* DMKU-RP35; (D) *Ps. hubeiensis* YE-21 for three days; (E) growth of *C. gloeosporiodes* alone and (F) dual cultivation with *P. aspenensis* DMKU-SP67 for 14 days by double plates cultivation to determine antifungal volatile organic compounds (VOCs) production [50]
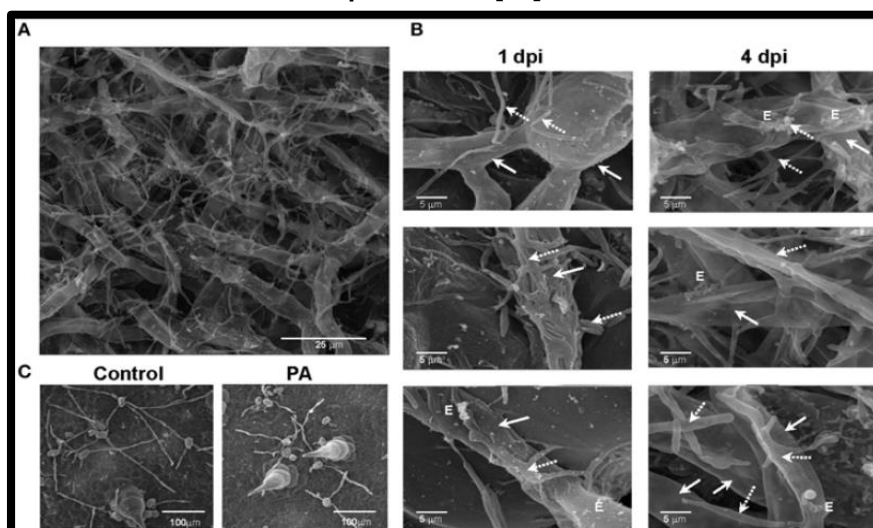

Fig. 6. *Pseudozyma aphidis* –powdery mildew interactions on cucumber cotyledons. SEM microscopy of cucumber cotyledons treated with P. aphidis and infected with Podosphaera xanthii. (A) Cucumber cotyledons treated with *P. aphidis* 4 days post-infection with P. xanthii. (B) Closer look at the interaction *of P. aphidis* and *P. xanthii* mycelium and spores 1- and 4-days post-infection with *P. xanthii*. Cucumber cotyledons treated with *P. aphidis* (PA) or with water (Control) 1-day post-inoculation (C) and 10 days post-inoculation with P*. xanthii* [53]

4. MYCOPARASITISM

Mycoparasitism is a lifestyle in which one fungus engages in parasitic interactions with another. *Pichia guilliermondii* has been observed to exhibit mycoparasitic behavior towards the hyphae of the plant pathogenic fungus *Botrytis cinerea* [52]. *Pseudozyma aphidis* parasitizes the powdery mildew pathogen *Podosphaera xanthii* and *B. cinerea* (Fig. 6) [53]. The genus

Saccharomycopsis comprises predatory yeasts that directly feed on other microorganisms, including their prey, and has been studied for its biocontrol activities against various *Penicillium* species [24].

5. INDUCTION OF RESISTANCE

Biocontrol yeasts can induce systemic resistance in plants against various pathogens [54].

Saccharomyces cerevisiae, *Rhodosporidium paludigenum*, *Candida saitoana*, *C. oleophila*, and *Metschnikowia* species trigger an innate immune response, enhancing resistance against phyllosphere pathogens in fruits. The innate immune response and systemic resistance induced by *Candida oleophila* are linked to several factors, including the overproduction of reactive oxygen species (ROS) in the plant [55]. Additionally, yeasts like *Candida laurentii*, *Cryptococcus flavescens*, and *Rhodotorula glutinis* can be combined with resistance inducers such as salicylic acid or rhamnolipids in integrated disease management strategies [56]. The application of *Candida oleophila* cell suspensions to grapefruit peel tissue boosts ethylene biosynthesis, phenylalanine ammonia lyase activity, and phytoalexin accumulation, thereby stimulating host defence (Fig. 7) [57].

5.1 Plant Growth Promotion by Yeast

Yeasts are promising agents for promoting plant growth. In vitro studies have demonstrated that co-culturing *Nicotiana benthamiana* seedlings with specific yeast strains enhances their growth, leading to healthier and more robust development, which is crucial in the early stages of plant growth. Inoculating tobacco and lettuce seedlings with particular yeast strains also boosts their vigor, increasing the likelihood of successful establishment when transplanted, ultimately improving crop yields (Fig. 8) [58]. Moreover, introducing yeasts such as *S. cerevisiae*, *Debaryomyces hansenii*, and *Lachancea thermotolerans* into maize-growing soil has shown to promote the vigor of maize plants, indicating their positive effect on growth and development [58]. Chen et al. [59] reported that Rhodosporidium paludigenum JYC100 effectively solubilizes calcium phosphate, promoting plant growth under phosphorus-deficient conditions with insoluble phosphate. Co-cultivation with R. paludigenum JYC100 increased biomass, shoot height, and cellular inorganic phosphorus content in plants compared to controls. Additionally, histochemical GUS and qRT-PCR assays revealed decreased transcript levels of phosphate starvation-induced (PSI) genes in these plants. These findings highlight *R. paludigenum* JYC100's capacity to convert insoluble phosphorus into plant-available forms, suggesting its potential as a sustainable alternative to inorganic phosphate fertilizers for enhancing crop yields.

6. REGISTERED BIOCONTROL YEAST SPECIES

6.1 *Candidaole ophila*

C. oleophila was the first yeast developed as a commercial plant protection agent, leveraging its competitive advantages for nutrients and space. This marked a significant advancement in yeast-based biocontrol in agriculture. Its efficacy against plant pathogens stems from mechanisms such as producing hydrolytic enzymes, volatile compounds, biofilm formation, and inducing plant resistance [60,61]. Strains I-182 and O have been commercialized as Aspire and Nexy, respectively, with strain O gaining approval in Europe in 2013 Wisniewski et al., [61]: European Food Safety Authority, [62].

6.2 *Aureobasidium pullulans*

The saprophytic fungus *A. pullulans*, found in various environments, shows biocontrol activity, particularly two registered strains: DSM 14940 (CF 10) and DSM 14941 (CF 40). These strains are effective against fireblight and postharvest diseases, formulated into the wettable powder product Blossom-Protect [63]. They are also registered for controlling postharvest diseases in apples (Boni-Protect) and are under study for use against storage diseases in strawberries and plums [64,65].

6.3 *Metschnikowia fructicola*

Metschnikowia species, particularly *M. fructicola* and *M. pulcherrima*, are primarily found in the phyllosphere and exhibit significant biocontrol potential against postharvest diseases. Their antifungal mechanisms include nutrient competition and the secretion of glucanases and chitinases [20]. *M. fructicola* isolate NRRL Y-30752, discovered in Israel, has been registered to prevent postharvest diseases in crops like sweet potatoes and carrots, with EFSA approval for use in stone fruits, strawberries, and grapes [66,67].

6.4 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae, known for its applications in biotechnology, has demonstrated antifungal activity, especially strains like BY4741 and others that inhibit plant pathogens such as *Aspergillus* and *Fusarium* [68,69]. These strains

employ mechanisms like secreting hydrolytic enzymes and volatile compounds. The commercial product Romeo, containing *S. cerevisiae*, is used to induce systemic resistance against downy mildew in crops [62,18].
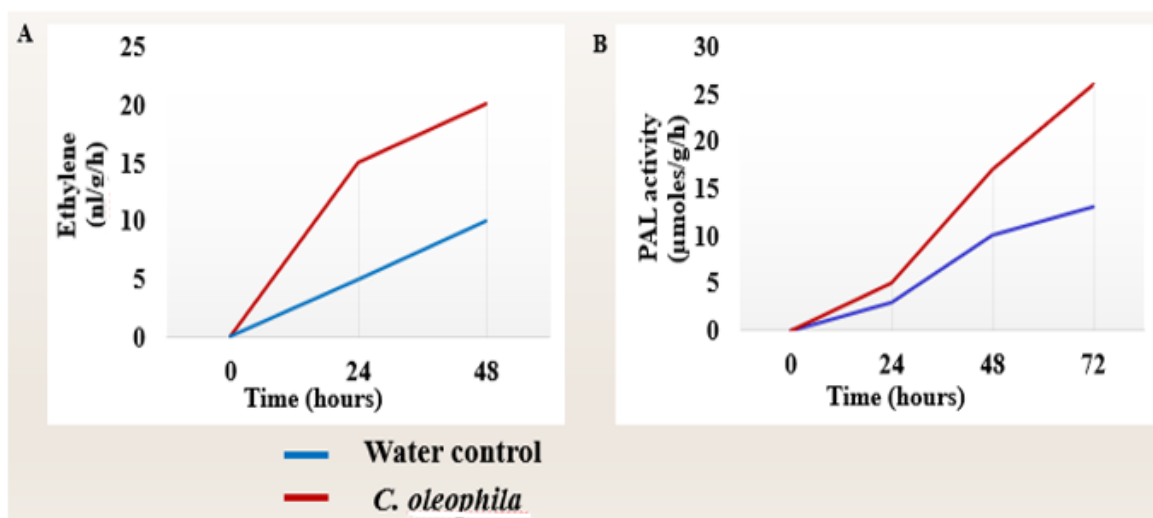

Fig. 7. A. Effects of *Candida oleophila* treatment on ethylene production in grapefruit peel disks. B. Effects of *Candida oleophila* treatment on phenylalanine ammonialyase (PAL) activity in grapefruit peel tissue [57]
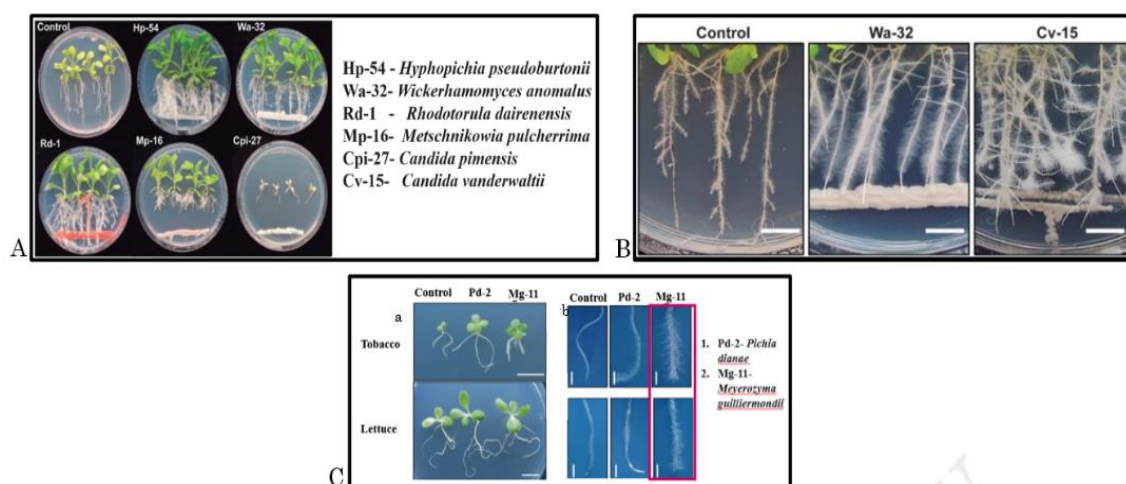

Fig. 8. Effects of plant growth-promoting yeasts on growth and development of *Nicotiana benthamiana* seedlings. A- *N. benthamiana* seedlings (7 days old) were grown on quarter-strength MS agar plates inoculated with yeasts at the opposite ends. The plates were placed vertically and seedlings were co-cultured with yeasts for a further 21 days. Plate samples without yeast served as the control. Several plates are shown as examples of different phenotypes: Hp-54, Wa-32 and Rd-1 enhanced shoot and root weight while Mp-16 only enhanced shoot growth. Cpi-27 is an example of a yeast strain that was deleterious in co-culture with *N. benthamiana* seedlings. B- Detail of *N. benthamiana* roots developed in the presence of yeasts Wa-32 and Cv-15. Greater branching and the presence of longer root hairs can be observed in comparison to the control seedlings roots. C (a)-Yeast inoculated seedlings showed greater shoot and root development than control plants. Scale bars indicate 1 cm. C (b)-Detail of tobacco and lettuce seedling root tips where a large number of root hairs can be observed in the presence of yeast strain Mg-11 [58]

7. COMMERCIAL APPLICATIONS

Several yeast-based bioproducts are currently registered, including Blossom Protect, Biotector, and BoniProtect, all containing *A. pullulans* strains for various plant diseases. Blossom Protect targets fire blight and other rots in fruits, while Biotector is effective against gray mold in grapes and strawberries. BoniProtect prevents fungal diseases in orchards. Julietta is a fungicide with *S. cerevisiae* designed for strawberries and tomatoes, while Nexy, containing *C. oleophila*, is used against mold in apples and pears. Noli features *M. fructicola* for postharvest decay, and Romeo, made from *S. cerevisiae* cell walls, prevents powdery mildew in multiple crops [1].

7.1 Patents on Yeast-Based Products for Plant and Fruit Disease Biocontrol

Germany leads in the number of patents for yeast-based products, followed by the USA and Australia, while India holds 3% of these patents. From 2009 to 2021, the Derwent Innovation database reported 163 patents related to yeasts as biological control agents, highlighting their growing recognition in sustainable agricultural disease management. Together, Germany, the USA, Australia, and China account for 53% of all yeast-related patents worldwide. Of the 163 patents, 73.68% specified the genus or species of the yeast, while 26.31% simply noted "yeast" among the components. These products often contain multiple microorganisms, with 32.89% featuring *M. fructicola*, 11.18% containing *Candida* species, and a mix of *C. oleophila*, *M. fructicola*, and *P. anomala*. Other included yeasts are *Pichia* (9.86%), *Rhodotorula* (7.89%), and *Cryptococcus* (5.92%), either alone or with *Rhodotorula*. Only 1.97% involved *Debaryomyces* [70].

8. CASE STUDY 1

In a 2016 study by Zhimo et al. explored the use of antagonistic yeasts for the biological control of post-harvest fruit diseases in India. They isolated 29 yeast strains from diverse ecological sources and identified three—*Candida tropicalis* YZ1 (CtYZ1), *Saccharomyces cerevisiae* YZ7 (ScYZ7), and *Candida tropicalis* YZ27 (CtYZ27)—that demonstrated notable *in vitro* antagonistic activity against several fungal pathogens. The yeast strains were applied at concentrations ranging from 1 to 4 x 10^8 colony-forming units per milliliter (CFU/ml) to artificially inoculated wounds on banana fruits infected with Colletotrichum musae. The results showed that CtYZ1, ScYZ7, and CtYZ27 reduced the mean lesion diameter by 85.5%, 88.7%, and 91.9%, respectively, while the synthetic fungicide Carbendazim (1.0 g/L) resulted in a 75.8% reduction in lesion size (Fig. 11). These findings highlight the effectiveness of the identified yeast strains as biocontrol agents for fungal pathogens on fruit, indicating their potential for use in sustainable agricultural practices and integrated pest management strategies for post-harvest disease control.

9. CASE STUDY 2

The 2020 study by Into et al. assessed the antagonistic activities of 83 yeast strains against various rice pathogenic fungi, including *Pyricularia oryzae*, *Rhizoctonia solani*, *Fusarium moniliforme*, *Helminthosporium oryzae*, and *Curvularia lunata*. Fourteen strains demonstrated inhibitory effects, with *Torulaspora indica* DMKU-RP31, DMKU-RP35, and *Wickerhamomyces anomalus* DMKU-RP25 effectively inhibiting all tested pathogens. The researchers explored mechanisms behind these antagonistic effects, including the production of volatile organic compounds (VOCs), fungal cell wall-degrading enzymes, and biofilm formation. Additionally, in greenhouse tests, these yeast strains suppressed rice sheath blight caused by *R. solani* by 60.0% to 70.3%, compared to an 83.8% suppression with 3% validamycin (Table 1). These findings suggest the potential of these yeast strains as biocontrol agents for rice sheath blight.

In 2017, Lee et al. studied the isolation of leaf-colonizing yeasts from pepper plants in a major pepper production area of South Korea and their potential for controlling *Xanthomonas axonopodis* infections [73,74]. Using a semi-selective medium with rifampicin to inhibit bacterial growth, they isolated 838 yeast strains from the leaves [75]. Notably, *Pseudozyma churashimaensis* strain RGJ1, applied as a foliar spray at a concentration of 10^8 cfu/ml, significantly protected pepper plants against *X. axonopodis* in greenhouse trials, highlighting its potential as a biocontrol agent against bacterial diseases [76,77]. Additionally, strain RGJ1 unexpectedly conferred protection against several viruses, including Cucumber mosaic virus and Pepper mottle virus, suggesting broad-spectrum disease control capabilities [78]. Co-

culture assays showed no direct antagonism between strain RGJ1 and *X. axonopodis*, indicating that disease suppression likely results from induced resistance in the pepper plants [79,80]. Molecular analyses revealed the expression of induced resistance marker genes, specifically *Capsicum annuum* Pathogenesis-Related proteins CaPR4 and CaPR5, associated with enhanced plant defense responses, suggesting that strain RGJ1 primes the plants for better pathogen resistance (Fig. 13) [81-83].

9.1 Reasons for Non-Popularity for Yeast-Based Plant Protectants

The development and commercialization of yeast-based plant protection products face several challenges, including:

1. **Lack of Mechanistic Understanding**: Insufficient knowledge of yeast interactions with plant pathogens hinders product development.
2. **Registration Hurdles**: The complex and costly registration process for biocontrol products poses challenges for smaller companies.
3. **Collaboration Gaps**: The need for expertise from various fields often leads to difficulties in forming necessary partnerships.
4. **Limited Commercial Potential**: Yeast products may lack the appeal of conventional pesticides, making adoption difficult in the agricultural sector.
5. **Market Acceptance**: Farmers and consumers may be resistant to adopting biocontrol methods.
6. **Long Development Timelines**: Extended product development periods can deter investment and commercial interest.

Table 1. Efficacy of the antagonistic yeasts in suppressing of rice sheath blight disease caused by *R. solani* DOAC 1406 in rice plants grown in pots in the greenhouse [72]

Treatment	Lesion Height (cm)	Disease Incidence (%)	Disease Suppression (%)
Control (negative control)	0	0	0
Rhizoctonia solani (positive control)	23.8 ± 1.6	25.9 ± 2.3	0
R. solani + *Torulaspora indica* DMKU-RP31	7.2 ± 1.4	7.7 ± 1.4	70.3
R. solani + *T. indica* DMKU-RP35	8.3 ± 1.5	8.8 ± 1.4	66.0
R. solani + *W. anomalus* DMKU-RP25	8.1 ± 1.0	8.7 ± 0.8	66.4
R. solani + 3% Validamycin	3.9 ± 0.5	4.2 ± 0.6	83.8

Blossom protect   **Boni protect**   **Nexy**

Noli   **Romeo**

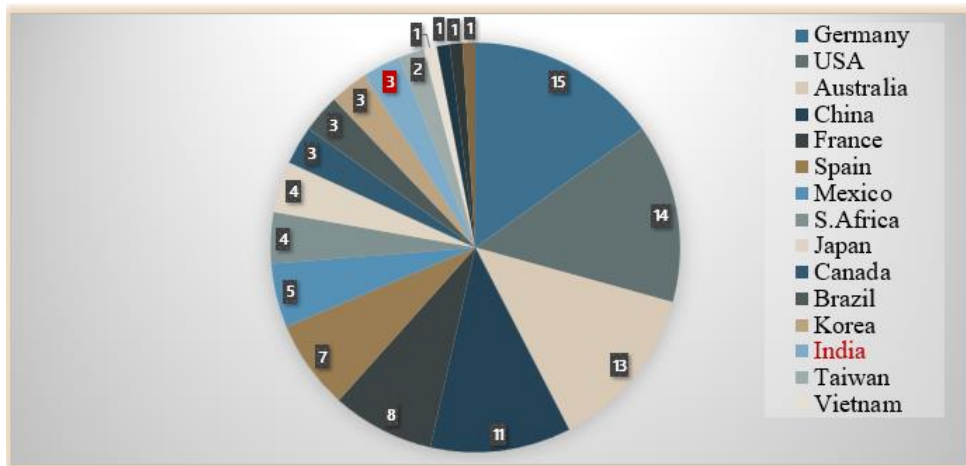**Fig. 9. Commercial yeast-based plant protectants**

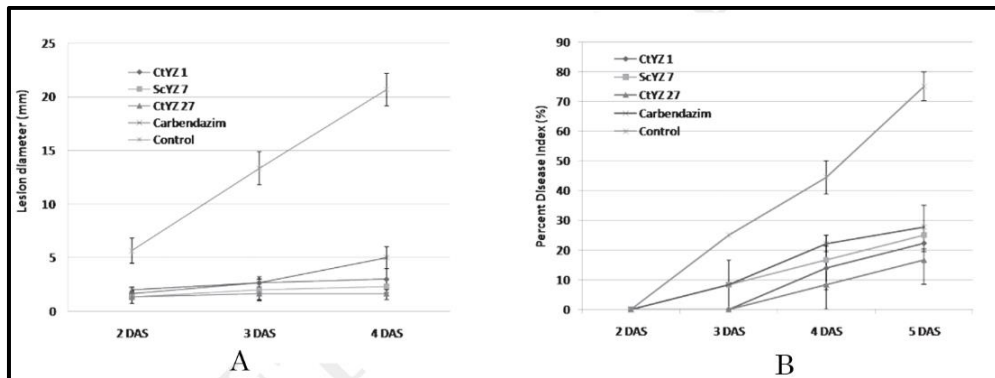**Fig. 10. Patents on yeast-based products for plant and fruit disease biocontrol**

Fig. 11. (A). Changes in anthracnose lesion diameter (mm) of artificially inoculated banana fruits (C. musae at 104 spores ml−1) by CtYZ1, ScYZ7 and CtYZ27 application (1×108 CFU ml−1) at different days of storage as compared to Carbendazim treated and untreated fruits (control) at 28±1°C. (B) Percent Disease Index (%) of latent infection of anthracnose on banana fruits after application of antagonistic yeast strains CtYZ1, ScYZ7 and CtYZ27 (1-4×108CFU ml−1) after different days of storage (DAS) as compared to Carbendazim treated and untreated fruits (control) at 28±1°C [71]
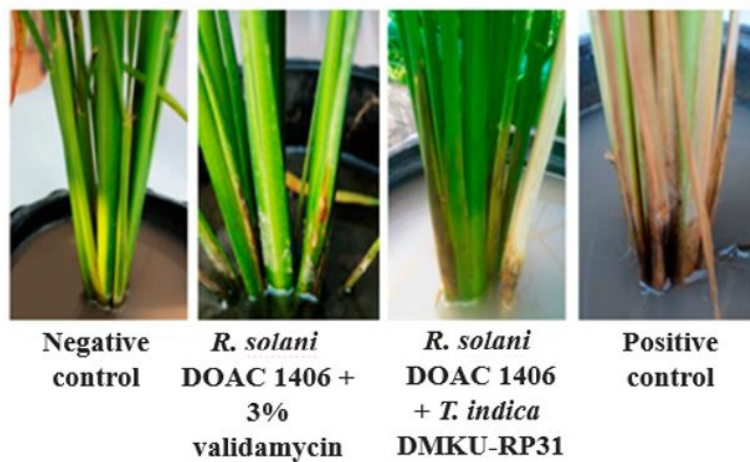

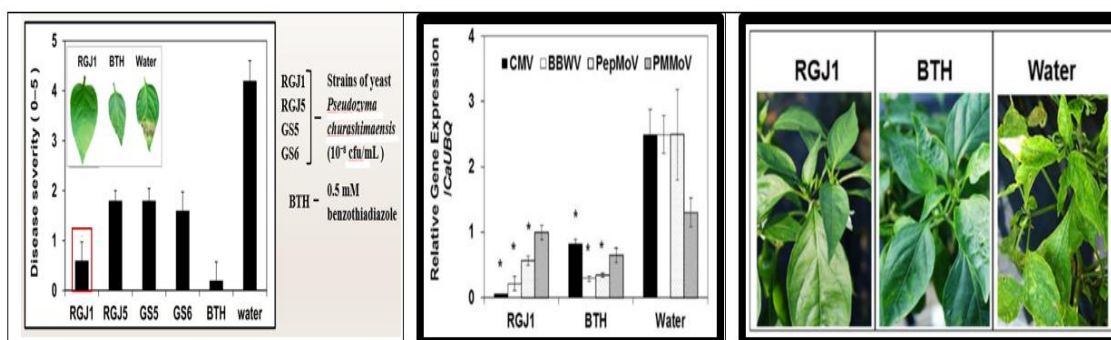**Fig. 12. Rice sheath blight disease lesions 15 days after *R. solani* DOAC 1406 inoculation [72]**

Fig. 13. Biological control of *Xanthomonas axonopodis* pv. vesicatoria by *Pseudozyma spp*. in the greenhouse. (A) The yeast isolates RGJ1, RGJ5, GS5, and GS6 were sprayinoculated on pepper leaves. Pathogen challenge was conducted 1 week after yeast spraying. Disease severity was measured 1 week after leaf infiltration of *X. axonopodis* pv. vesicatoria at OD=0.001. (B) and (C) Disease suppression of naturally occurring virus by spray application of strain RGJ1

10. CONCLUSION

Yeast serves as a promising biocontrol agent for sustainable pathogen management in agriculture, effectively targeting various pathogens. This versatility allows it to address multiple issues, making it particularly valuable for crops affected by different pathogens. Yeast can outperform traditional fungicides, leading to less frequent chemical applications, which conserves resources and minimizes environmental impact. As a natural alternative to chemical pesticides, yeast-based biocontrol reduces risks of soil and water contamination and protects non-target organisms. Overall, yeast-based strategies offer a resilient and sustainable method for controlling plant pathogens, avoiding pesticide resistance and long-term ecosystem harm.

11. FUTURE PROSPECTS

1. **Understanding Modes of Action**: Improved knowledge of yeast mechanisms can lead to targeted strategies.
2. **Consortium Design**: Combining complementary yeast strains can enhance disease defence.
3. **Large-Scale Production**: Advances in yeast cultivation can increase accessibility and affordability.
4. **Industrial Collaboration**: Partnerships between research institutions and industries can boost product development.
5. **Biological Innovation**: Genetic modifications of yeasts may enhance their biocontrol efficacy for specific applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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