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Microbial biocontrol agents: A boon of combating rice pathogens

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Abstract

Rice is the primary food source for 2.5 to 3.5 billion people worldwide, particularly in developing countries. However, rice production faces threats from various diseases caused by harmful bacteria, fungi, and viruses. These diseases can significantly reduce crop yields and jeopardise global food security. Farmers often rely on chemical pesticides to combat these issues, but over time, these chemicals could harm the environment, pollute soil and water, and lead to pathogen resistance, rendering the chemicals less effective. Additionally, they can eliminate beneficial microbes that promote healthy plant growth. Consequently, scientists and farmers are increasingly turning to microbial biocontrol agents as a safer and more sustainable alternative. Biocontrol microorganisms are one of the alternatives towards agrochemicals as it is environmentally friendly and can act as a growth-promoting organism for rice plants. These beneficial microorganisms such as *Bacillus* spp., *Trichoderma* spp., *Streptomyces* spp., *Pseudomonas* spp., *Burkholderia* spp., certain types of yeast and bacteriophage naturally protect rice plants by inhibiting harmful pathogens. They achieve this through various mechanisms, including producing natural antibiotics, competing for nutrients, enhancing the plant's immune system, and directly attacking the pathogens. This review highlights the potential of these microbes to manage rice diseases effectively while promoting plant growth, reducing chemical usage, and fostering a healthier, more sustainable agricultural system.

Keywords: *rice pathogens, biocontrol agents, rice diseases, microorganisms, food security*

Introduction

Rice is the primary food source for 2.5 – 3.5 billion people, accounting for half of the world's population, primarily in economically developing countries (Asibi et al. 2019; Firdaus et al. 2020). As a dietary staple, rice provides significant energy, with 80% of its composition consisting carbohydrates and starch (Carcea 2021). Over 100 countries produce rice, with 90% of the global rice production occurring in Asia (Fukagawa & Ziska 2019). According to the Food and Agriculture Organisation of the United Nations (FAOSTAT 2021), more than 787 million tonnes of rice were harvested globally in 2021, with Asia producing over than 708 million tonnes. Of this total, Malaysia accounted for 2.418 million tonnes of global rice production.

However, disease affecting rice can significantly reduce production yield, threatening food security (Asibi et al. 2019). Countries reliant on the agriculture are particularly vulnerable to these pathogens. In 2017, Malaysia imported 739 thousand tons of rice, primarily from Thailand and Vietnam (Che et al. 2019) Failure to control rice diseases will increase the country's dependence on rice imports to feed its population. Rice diseases are caused by various pathogens, including bacteria, fungi and viruses all of which can interfere with photosynthesis and

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inhibit plant growth (Azizi & Lau 2022). Among these diseases, bacterial leaf blight (BLB), bacterial panicle blight (BPB), rice bacterial leaf streak, and rice blast disease have a particularly damaging to rice production (Chompa et al. 2022).

The use of agrochemicals to treat rice disease infections can lead to various adverse effects. For instance, overuse of fungicides can catalyse genetic mutation in fungi, resulting in the emergence of resistance strains. Additionally, environmental exposure to bactericides can lead to a substantial increase in antibiotic-resistant bacteria (Miller et al. 2022). These bacteria are likely to be able to infect humans, causing infections that are difficult to treat. Moreover, conventional chemical often lacks of specificity and can harm beneficial microorganisms essential for supporting plant growth (He et al. 2021). Therefore, alternative solutions such as the use of biocontrol agents is critical for effectively managing rice diseases outbreak.

Biocontrol has been employed to manage plant pathogens using living organisms for the past 4,000 years in Egypt. However, advanced research into the application of biocontrol in modern agriculture began in the early 19th century (He et al. 2021). Microorganisms such as bacteria and fungi show promise as biocontrol agents against plant pathogens. The effectiveness biocontrol relies on agonistic and antagonistic interactions between pathogens and microorganisms (Mishra et al. 2015; Pandit et al. 2022). These biocontrol actions are typically host-specific, thereby reducing unintended impacts on non-target organisms and the surrounding environment (Pandit et al. 2022). Additionally, biocontrol is generally less expensive than alternatives such as improving rice varieties through genetic engineering (Jin & Drabik 2022). For example, biocontrol microorganisms such as *Trichoderma* species are cost effective and eco-friendly (Sood et al. 2020).

One of the primary modes of action in biocontrol is antibiosis, commonly observed in microorganisms inhabiting the plant rhizosphere. These microbes produce secondary metabolites such as antibiotics, toxins or hydrolytic enzymes which destroys the pathogen's cell wall and inhibits the their growth (Syed Ab Rahman et al. 2018; Pandit et al. 2022). Additionally, biocontrol agent competes with pathogen for essential nutrients. Plant roots release nutrients such as amino acids, vitamins and polysaccharides which are critical for microbial proliferation, including that of harmful pathogens. Beneficial microorganisms colonise these nutrient-rich zones and outcompeting pathogens and restricting their access to vital resources (Liu et al. 2024). Another key mechanism is mycoparasitism, in which biocontrol organisms parasitise fungal pathogens inhibit growth of mycelium and effectively halting the spread of infection (Poveda 2021).

This review aims to provide an in-depth analysis of the microbial activities used in managing rice diseases. It will synthesise current knowledge on the potential of microbes used as biocontrol agents against various rice pathogens.

Biocontrol organisms

***Bacillus* spp.**

Bacillus are Gram positive, rod-shaped, aerobic bacteria capable of producing endospores. This genus is the most diverse bacteria with 273 species recorded. Notable *Bacillus* species such as *Bacillus cereus, Bacillus subtilis*, and *Bacillus velezensis* reside in plant tissues (Etesami et al., 2023). Additionally, bacteria can be found in soil and plant roots. The isolation of various root-associated bacterial endophytes indicates that *Bacillus* species play a crucial role in managing rice disease infections. For example, *Bacillus altitudinis* exhibit antagonistic action against the *Magnaporthe oryzae* pathogen (Khaskheli et al. 2020). The author stated that related bacteria, such as *Bacillus wiedmannii, Bacillus aryabhattai, Bacillus marisflavi*, and *Bacillus indices* also demonstrate effectiveness against the *Fusarium graminearum* which cause rice seedling infections. Furthermore, various *Bacillus* species have been shown to have actions against other pathogenic fungi such as *Fusarium moniliforme* and *Rhizoctonia solani*.

Bacillus subtilis provides rice plants with high resistance to *R. solani* responsible for rice sheath blight disease. Exposure of rice seeds to *B. subtilis* also reduces rice germination time. In addition to *B. subtilis*, exposure to other *Bacillus* species, such as *B. megaterium* and *B. altitudinis,* enhance plant resistance to the disease compared to rice that is not exposed to any bacteria (Shasmita et al. 2019). Moreover, *B. subtilis* helps rice plants combat against *M. oryzae* infections. Inoculation of rice with the *B. subtilis* strain JN005 (*Table 1*), both in infected and healthy rice reduces disease severity. Exposure to this strain increases in the presence of defense-related enzymes such as peroxidase (POD), phenylalanine ammonialyase (PAL), superoxide dismutase (SOD), and catalase (CAT) (Zhu et al. 2021).

In the presence of high concentrations of *B. cereus* HS24, the entry of Ca^{2+} ions into the conidia *M. oryzae* is block resulting in the absence of these ions within the fungal cell. This blockage occurs when these bacteria inhibit the transcription of genes such as calmodulin-dependent protein kinase II, PMC1, and CCH1 which in turn disrupts the Ca^{2+} signaling pathway (Lianmeng et al. 2020). Ca^{2+} signaling is essential in fungal growth processes including the development of sexual and asexual structures, response to pressure, and pathogenicity through the production of appressorium structures (Roy et al. 2021). Disruption of this signal reduces the growth of the conidial and appressorium structures of the fungus.

Additionally, secreted proteins from the *B. licheniformis* BL06 interfere with the transcription of the chitin synthase gene in *M. oryzae,* a gene essential for fungal cell walls biosynthesis. This interference further impairs the formation of the appressorium, thereby reducing the pathogenicity of the fungus (Liu et al. 2021). In contrast, *B. licheniformis* BC98 secretes surfactin, a lipopeptide induces morphological changes in *M. grisea* resulting in vacuolated cytoplasm and bulbous hyphae, as observed through electron microscopy (Tendulkar et al. 2007). Furthermore, a study by Chengqi et al. (2022) demonstrated that *B. altitudinis* ST15 has antagonistic action against the fungus *Xanthomonas oryzae*. The antimicrobial activity this bacterium has been significantly affect fungal growth, surpassing the effect of chemical compounds such as zinc thiazole and benzothiazolinone. Notably, the metabolites produced by the bacterium remain stable even when exposed to high temperatures and in the presence of protease enzymes and ultraviolet radiation (Chengqi et al. 2022). Genome analysis of the *B. altitudinis* P-10 bacterium, obtained from plant rhizosphere, revealed genes responsible for producing lichenysin or bacilysin like non-ribosomal peptides, terpenes, and bacteriocins with potential in disease biocontrol (Budiharjo et al. 2017).

Furthermore, the species *B. velezensis* strain IBUN 2755 is suggested to inhibit the growth of the pathogenic bacterium called *Burkholderia glumae* through nutrient competition. This occur because both bacteria identical requirement for nicotinic acid, each requiring a carbon source found in plant roots, such as alanine, proline, glucuronic acid, arabinose, and mannose (Perea- Molina et al. 2022). As such, the growth of the pathogenic bacteria will be limited due to the *B. velezensis* consuming the available nutrient.

Streptomyces spp.

Streptomyces is a genus of gram positive, non-acid-fast, non-motile, and catalase-positive bacteria (Flärdh & Buttner, 2008; Hasani et al. 2014; Law et al. 2017). These bacteria can form filamentous structures resemble fungal mycelium and produce spores to aid in their dissemination (Chater 1984; Vurukonda et al. 2018). *Streptomyces* can be found in various soil types, on the water surface, forming colonies in the open or acting as endophytes within plant root structures (Marella, 2014; Vurukonda et al. 2018).

Table 1. Mode of action for *Bacillus* spp., *Streptomyces* spp., *Pseudomonas* spp., *Burkholderia* spp., *Trichoderma* spp. *and yeast*

	Species	Strain	Mode of action
Bacillus	*Bacillus cereus*	JN005	Produce enzyme as peroxidase (POD), phenylalanine ammonialyase (PAL), superoxide dismutase (SOD), and catalase (CAT).
		HS24	Inhibit the transcription of genes which in turn disrupts the Ca^{2+} signaling pathway.
	Bacillus licheniformis	BL06	Inhibit the transcription of the chitin synthase gene.
		BC98	Induces morphological that changes vacuolated cytoplasm and bulbous hyphae.
	Bacillus altitudinis	ST15	Affects fungal growth
		P-10	Inhibit Fungal growth
Streptomyces	*Streptomyces* sp.	*UPMRS4*	Inhibiting the colonization of the pathogenic fungus.
	Streptomyces manipurensis	MBRL 201T	Exhibit antagonistic activity of fungi by break down the cell walls of these fungi.
	Streptomyces roseoverticillatus	63	Exhibits antibacterial activity againt fungi
	Streptomyces araujoniae	HSA312	Inhibit formation of appressoria in the fungus
Pseudomonas	*Pseudomonas* sp.	VsMKU4036	Produce hydrolytic enzymes that lyse the cell walls of pathogens.
	Pseudomonas aeruginosa	BRp3	Penetrates cell membrane of bacteria that reduced pyocyanin.
		CGK-KS-1	Inhibit the growth of fungus
	Pseudomonas mosselii	BS011	Inhibit the growth of the fungus's appressorium
	Pseudomonas putida	RRF3	Produce salicylic acid to regulate plant immunity
Burkholderia	*Burkholderia cepacia*	JP2-270	Produces an antifungal compound.
	Burkholderia gladioli	NGJ1	Induces apoptosis in the fungal cells.
		KJ-34	Inhibit spore germination and hyphae formation.
	Burkholderia vietnamiensis	A-7.3	Inhibit the infection to prevent primary infection of rice seed.
		V-16.5	Strong antagonistic activity toward pathogen.
Trichoderma	*Trichoderma harzianum*	Th3	Inhibit the growth of pathogen.
	Trichoderma asperellum	-	Produce reactive oxygen.
	Trichoderma erinaceum	-	exhibits suppression of soil-borne pathogens
Yeast	*Torulaspora indica*	*DMKU-RP31*	Degrade the fungal cell wall.
		DMKU-RP35	Degrade the fungal cell wall.
	Wickerhamomyces anomalus	*DMKU-RP25*	Degrade the fungal cell wall.
	Lachancea kluyveri	SP132	Enhance the immune system of plant.

Isolation of 112 bacterial species from the rice plant rhizosphere revealed that four strains of *Streptomyces* strain PC 12, D4.1, D4.3, and W1 exhibit suppressive activity against the Pyricularia fungus genus, which is responsible for blast disease. Among these, the P12 strain was identified as *Streptomyces palmae* P12 (Chaiharn et al., 2020). This bacterium was demonstrated plant-growth-promoting rhizobacteria (PGPR) activity in rice with treated plants showing the highest height and the lowest incidence of dry leaves.

A study by Awla et al. (2017) indicate that *Streptomyces* sp. strain UPMRS4 (*Table 1*) can reduce the incidence of *Pyricularia oryzae* disease by 67.9% in tested rice plants while simultaneously enhancing their growth. Biochemical test showed that these bacteria produced ammonia, urea and enzymes such as amylase, protease and lipase. Additionally, the expression of chitinase (*Cht-1*), glucanase (*Gns1*), pathogenesis-related gene (*OsPR1a*) and salicylic acid-responsive gene (*Oswrky45*) increased during the early stages of treatment inhibiting the colonization of the pathogenic fungus.

Moreover, the novel *Streptomyces manipurensis* MBRL 201T has been observed to exhibit antagonistic activity against six important rice pathogens, including *Curvularia oryzae, Fusarium oxysporum, Helminthosporum oryzae, P. oryzae, Rhizoctonia oryzae-sativae* and *R. solani*. This strain produces enzymes such as chitinase, β-1,4-glucanase, lipase and protease which can break down the cell walls of the fungi (Tamreihao et al. 2019). Additionally, it releases volatile compounds like ammonia. Furthermore, the author also mentioned that, this bacterium harbours PKS-III and NRPS genes, suggesting its potential to synthesise antibiotic compounds.

Furthermore, rice plants treated with *S. roseoverticillatus* 63 (Sr-63) demonstrate reduced rice leaf blight infection. This effect is attributed to the production of carbomycin B by the bacteria which exhibit antibacterial activity against the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) pathogen (Shi et al. 2021). *Figure 1* illustrates that carbazomyzin B inhibits the synthesis of bacterial membrane structures and prevents the production of xanthomonadin and extracellular polymeric substances (EPS). Xanthomonadin serve of *Xoo* that facilitating quorum sensing during biofilm production while EPS promote bacterial biofilm attachment in the environment (Jamal et al. 2018). Consequently, reducing the formation of bacterial biofilms decrease the infection rate of this pathogen on rice plants. Additionally, carbomyzin B inhibits malate dehydrogenase activity and bacterial protein expression while altering bacterial cell membrane components that disrupt hydrophobicity (Shi et al. 2021).

Streptomyces araujoniae HSA312 exhibits an inhibitory effect on the formation of appressoria of the *M. oryzae*. The inhibition rate is 73.53% after five days and increases to 79.59% after seven days (Lianmeng et al. 2020). This inhibition is attributed to the bacterium's resistance to UV light and its rapid to colonisation the rice plant's phyllosphere. However, the inhibitory action of this bacterium has not yet been shown to compete with chemical substance.

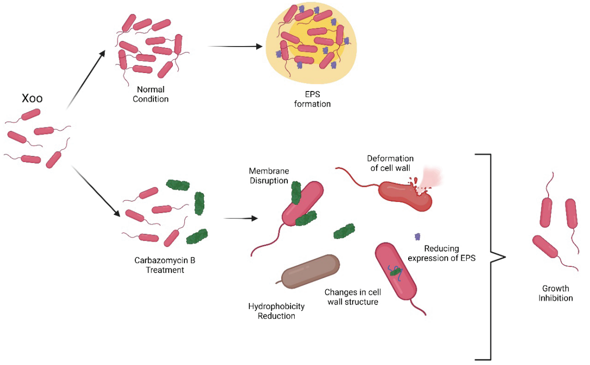

Figure 1. Effects of Carbomyzin B on Xanthomonas oryzae pv. oryzae

***Pseudomonas* spp.**

Pseudomonas is a genus of Gram negative, motile bacteria characterised by polar flagella. Some species within this genus are capable of producing yellow pigments. *Pseudomonas* belongs to the gamma subclass in Proteobacteria, with over 100 identified species (Guzmán-guzmán & Santoyo 2022). Most strain of *Pseudomonas* are fluorescent producing water-soluble fluorescent pigments (Suhana et al. 2020). Within this group, certain species are pathogenic to plants while other exhibit antagonistic properties against plant pathogens.

Of the 30 tested *Pseudomonas* species, 27 demonstrated broad-spectrum antimicrobial activity against pathogens such as *R. solani, Macrophomina phaseolina, Scleotium rolfsii, Helminthusporium solani* and *F. oxysporum* RACE (Varatharaju et al. 2020). The authors also mentioned that *Pseudomonas* sp. VsMKU4036 was selected from its production of hydrogen cyanide, indole acetic acid (IAA) and hydrolytic enzymes that play a role in the biological control of pathogens. Hydrogen cyanide aids in the expression of antibacterial genes (Nandi et al. 2017) while hydrolytic enzymes such as protease, amylase, gelatinase and pectinase work by lysing the cell walls of pathogens (Jadhav et al. 2017).

Furthermore, *Pseudomonas aeruginosa* BRp3 (*Table 1*), isolated from the rhizosphere of rice plants demonstrated a reduction in the infection of leaf blight pathogens including *X. oryzae* pv. *oryzae* and *Fusarium* species (Yasmin et al. 2017). The authors also mentioned, this reduction was likely due to the production of phenazines by the bacterium such as 1-hydroxy-phenazine, pyocyanin and pyochelin, which were identified through mass spectrophotometric analysis. Phenazines are broad-spectrum antibiotics with antibacterial and antifungal properties with one in three phenazines produced by *Pseudomonas* species (Serafim et al. 2023). For instance, pyocyanin is a molecule that rapidly penetrates the bacterial cell membrane and can act with reducing agents. Reduced pyocyanin can function as reactive oxygen species that eliminate pathogens (Biessy & Filion 2018).

Other than that, *Pseudomonas aeruginosa* strain CGK-KS-1 produces the metabolites Chumacin-1 and Chumacin-2, which can inhibit the growth of the *X. oryzae* pv. *oryzae* responsible for bacterial leaf blight (Kanugala et al., 2019). Chumacin-1 and Chumacin-2 restrict the synthesis of the quorum sensing signaling factor, cis-11-methyl-2-dodecenoic acid, by disrupting ion signals. Additionally, these metabolites inhibit the production of xanthan gum thereby affecting biofilm formation of the bacterial pathogen (Kanugala et al. 2019). Quorum sensing activity in bacteria occurs when signals such as xanthan gum and extracellular polysaccharides (EPS) are present. An increase in the bacterial population lead to a higher production of these signaling molecules. Elevated concentrations of these signals activate the quorum sensing pathway and enhance the expression of various genes, including virulence factors and biofilm production (Feng et al. 2023). Disruption of the quorum sensing pathway prevent the production of these genes.

In a study characterising *Pseudomonas mosselii* BS011 for its in controlling rice diseases caused by *M. oryzae*, the bacterium's secondary metabolites were found to inhibit the growth of the fungus's appressorium (Yang et al. 2023). Genetic analysis revel that seven genes are involved in synthesising these secondary metabolites. The removal of the *c-xtl* gene cluster from the bacterium's genome reduced pathogen growth inhibition, indicating that this gene cluster is essential for antifungal activity. Additionally, *P. mosselii* strain 923 produces a molecule called pseudoiodinine, which exhibit antimicrobial activity against the pathogens *Xanthomonas* and *M. oryzae* (Yang et al. 2023).

Pseudomonas putida RRF3 enhances the signaling pathway in the rice plant immune system against pathogenic diseases by producing of salicylic acid (SA)-responsive pathogenesis-related proteins. Those protein play a crucial role in regulating plant immunity, cell wall modification and the production of secondary metabolites (Kandaswamy et al. 2019). In the presence of pathogens like *Xoo, P. putida* also decreases the production of ethylene in the plant (Wang et al. 2021). Ethylene is involved in the growth of roots and shoots. By reducing ethylene level, the rice plant can allocate its resources to express immune genes and combat infection (Ravanbakhsh et al. 2018).

Burkholderia spp.

Burkholderia is a gram-negative, aerobic, rod-shaped and motile. This bacteria is catalase-positive, urease-positive, non-spore forming and does not ferment lactose (Graves et al. 1997; Elshafie and Camele 2021). Most of these bacteria can assist in plant growth by releasing allelochemicals through the rhizosphere, producing antibiotics and siderophores (Compant et al. 2005; Elshafie & Camele 2021). Bacteria from this group also have the potential to be biocontrol agents through the production of active secondary metabolites against pathogens.

The *Burkholderia cepacia* complex (Bcc) group is a ubiquitous bacterium that provides sources of novel antibiotics and bioactive secondary metabolites (Foxfire et al., 2021). For instance, *Burkholderia* sp. strain JP2-270 (Table 1), which belongs to the Bcc group, produces an antifungal compound called occidiofungin which acts against the pathogen *M. oryzae* (Wu et al.,2023). Occidiofungin targets fungal cells by binding actin leading to cell apoptosis (Emrick et al. 2013; Hansanant & Smith 2022). Actin is crucial for various fungal cell processes including endocytosis, hyphae induction, and nuclear segregation (Berepiki et al. 2011; Hansanant & Smith 2022).

Burkholderia gladioli strain NGJ1 exhibits mycophagy towards the fungus *R. solani*, consuming the fungus. *Figure 2* shows *B. gladioli* NGJ1 infected by a bacteriophage and undergoing genomic changes induced by the phage. Under calcium deficient condition, the bacterium utilise a component of the bacteriophage known as the type 3 secretion system (injectisome) of the bacteriophage to inject the Bg_9562 protein into the fungus (Yadav et al. 2020). This process induces fungal cells death responses allowing the bacterium to obtain nutrients from the dead fungal cells (Kumar et al. 2018). The required nutrients include nicotinic acid which is essential for the motility and biofilm synthesis of this bacterium (Das et al. 2023).

Another strain, *B. gladioli* KJ-34, exhibits broad-spectrum antifungal activity, demonstrating a significant suppression ratio of 75% against *M. oryzae*. The fermentation products of KJ-34 inhibit spore germination and hyphae formation. Metabolomics analysis indicate that this bacterium produces various antifungal compounds (Yang et al. 2023). Additionally, two strains of *Burkholderia vietnamiensis*, A-7.3 and V-16.5 which hyphae-colonising soil bacteria (HCSB) demonstrate high antagonistic activity against the pathogen *R. solani* (Cuong et al. 2011; Nicolaisen et al. 2018). Strain A-7.3 can inhibit the infection of *R. solani* AG1-IA by reducing the formation and function of sclerotia thereby preventing primary infection of rice seeds.

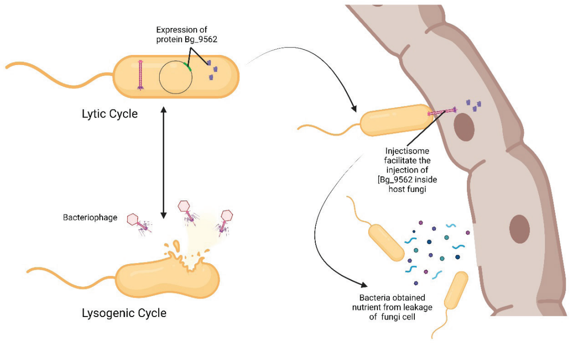

Figure 2. Burkholderia gladioli strain NGJ1of using the mechanism of bacteriophage to lyse host fungi.

***Trichoderma* spp.**

Trichoderma is a genus of filamentous fungi commonly found in soil, where it is saprophytic and non-virulent and lives in symbiosis with plants (Ferreira & Musumeci, 2021). The sexual reproduction of the *Trichoderma* genus is represented within this group which also includes the Hypocrea genus (Mukhopadhyay & Kumar 2020). *Trichoderma* is an Ascomycota fungus that is septate and produces conidiophores. These fungi often exhibit bright green conidial structures (Bissett, 1984). They are typically found in environment rich in decomposing plants material, particularly those containing cellulose (Jaklitsch 2009; Kubicek et al. 2009; Mukhopadhyay & Kumar 2020).

A study was conducted on four *Trichoderma* strains which are *T. paraviridescens* (BDISOF67), *T. erinaceum* (BDISOF91), *T. asperellum* (BDISOF08) and *T. asperellum* (BDISOF09). These strains were tested against the Xoo (Islam et al. 2023). The results showed that *Trichoderma* enhances the expression of key defense-related enzymes, including phenylalanine ammonia-lyase, catalase, polyphenol oxidase and peroxidase. According to *Figure 3*, *Trichoderma* also aids in the synthesis of jasmonic and salicylic acids. Additionally, it upregulates the *WRKY* gene, triggering a cascade that activates other defense genes such as parthogenesis-related genes (PR genes), *OsACS2*, and *OsHI-LOX*, thereby enhancing the plant's immune response against *Xoo*.

Trichoderma harzianum fungus exhibits activity against the fungal rice blast disease, *M. oryzae* Cavara. This effect is observed in the leaves and the neck blast (NB) infection where a reduction in disease severity is noted under high disease pressure conditions (Chou et al., 2020). The growth of *T. harzianum* Th3 (*Table 1*) in conjunction with the bacteria *P. fluorescens* RRb11 can act synergistically inhibit the development of the *M. oryzae* pathogen (Jambhulkar et al. 2018). However, this combination of biocontrol microorganisms demonstrates antagonistic effect in the case of bacterial leaf blight infection. Alginate encapsulation of the *T. harzianum* fungus enables it to inhibit the growth of *Bipolaris oryzae*, the pathogen causing rice brown disease (Anuar et al. 2020).

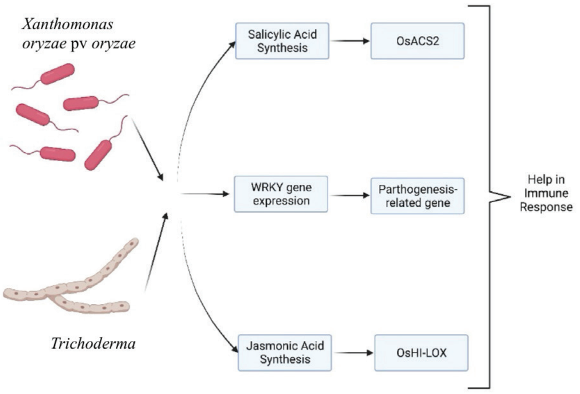

Figure 3. The plant immune response pathway is induced by Trichoderma fungi

Similarly, Trichoderma asperellum also has the potential to control rice blast caused by the *M. oryzae*. This can evident through soil inoculation and leaf spraying both of which reduce disease infection (Sousa et al., 2020). The author also stated that analysis of treated plants indicated a significant increase in the expression of genes J10sPR10 and LOX-RLL with increase of 35% and 474% respectively, compared to the control. The J10sPR10 gene encodes pathogenesis-related protein 10 (PR10), which plays a role in host defense control by producing reactive oxygen species and H2O2 by plant leaf cells (Peng et al. 2017).

The fungus obtained from tree bark identified as Trichoderma erinaceum exhibits suppression against soil-borne pathogens, including *R. solani, S. rolfsii*, and *S. oryzae*. Treatment with this fungus on rice seeds has been shown to increase expression of stress-related enzymes such as catalase, peroxidase, and superoxide dismutase. This enhancement support seed germination and plant growth under biotic and abiotic stress conditions. Additionally, treatment with *T. erinaceum* and *T. hebeiensis* boost the synthesis of indole acetic acid, soluble phosphate, and prussic acid, which promote plant growth and inhibit pathogen development (Swain et al. 2021).

Yeast

Yeast is a unicellular eukaryotic microorganism that belongs to the fungal group which exhibits diverse biological activities making it promising for developing products such as food, beverages and agricultural applications (Segal-Kischinevzky et al. 2022). Yeast can be found in various habitats and possesses antagonistic properties against rice pathogens. There are two types of yeast: Ascomycota and Basidiomycota. Ascomycota yeast reproduces through budding, while Basidiomycota yeast exhibits dimorphic growth, having both yeast and filamentous forms (Ferraz et al. 2019).

A study by Kunyosying (2018) revealed that five out of 63 yeast strains showed inhibition ranging from 50% to 65% against the *P. oryzae*. These yeasts did not completely prevent infection but instead delayed spore germination. Their action hindered the formation of appressoria and germ tubes, thereby reducing the infection caused by *P. oryzae* (Kunyosying et al. 2018). Furthermore, 14 yeast strains isolated from the leaf phylloplane of rice have been found to inhibit the growth of rice pathogens such *as P. oryzae, R. solani, Fusarium moniliforme, Helminthosporium oryzae* and *Curvularia lunata*. Among these strains, *Torulaspora indica* DMKU-RP31, *T. indica* DMKU-RP35, and *Wickerhamomyces anomalus* DMKU-RP25 exhibited activity against all tested pathogens with inhibition against *R. solani* ranging from 60.0% to 70.3% (Into et al. 2020). The author also stated that these three yeasts also produced volatile organic compounds, enzymes that can degrade the fungal cell wall and biofilms which are predicted to have antagonistic mechanisms against the pathogens. *T. indica* and *W. anomalus* also showed an effect on the infection of fungal pathogens on rice seeds, such as *C. lunata* and *H. oryzae*, with *W. anomalus* completely inhibiting the infection by *H. oryzae* (Limtong et al. 2020). Kodamaea ohmeri demonstrated antimicrobial activity against the pathogen *F. moniliforme*, but not against *C. lunata* or *R. solani*. (Khunnamwong et al. 2020). Metabolite analysis of this yeast showed the production of 3-methyl-1-butyl acetate and enzymes like β-1,3 glucanase and chitinase.

In addition, *Lachancea kluyveri* SP132 (*Table 1*), isolated from paddy field soil in the Nakhom Pathom district of Thailand, demonstrated effectiveness against the *R. solani* and *C. lunata* (Matić et al. 2014). Exposure of the pathogens to the yeast metabolites specifically chitinase, cellulase and amylase resulted in effects on hyphal morphology and mycelial growth. *L. kluyveri* was found to produce indole-3-acetic acid (IAA) and ammonia which can promote growth and enhance the immune system of rice plants.

Bacteriophage

A bacteriophage or phage is a virus that can infects and propagates within bacterial hosts. Phages occur naturally in various environment. For instance, the *Xanthomonas* phage which infects *Xanthomonas* sp. can be in phyllosphere and rhizosphere of infected plants as well as in compost soil, sewage and aquatic environments (Nakayinga et al. 2021). Bacteriophages are classified into two types; lytic phages and lysogenic phage. Lytic phage inject their viral DNA into the host cell, synthesize new phages and ultimately kill the bacterial host (Nakayinga et al. 2021; Strathdee et al. 2023).

Lytic bacteriophages attach to bacterial hosts via receptors on the bacterial membrane and inject their viral genetic material into the host (Doss et al. 2017). The viral genome is replicated by commandeering the host's building materials and enzymes to synthesise new phage progeny. Simultaneously, phage-encoded proteins such as endolysin and holin are produced using the host's enzymes. Holin accumulates on the cell membrane surface enabling endolysin to degrade the host's peptidoglycan. This process allowed the progeny viruses to exit the cell.

On the other hand, lysogenic phages integrate their viral DNA into the bacterial host's genome. Lysogeny or temperate phages, can enter the lytic cycle under certain conditions (Doss et al. 2017; Nakayinga et al. 2021). The phage genome intergrade into the host's genome is called a prophage. The prophage remains dormant and replicates along with the bacterial genome. When the host is under stress, such as exposure to antibiotics, reactive oxygen species or DNA damage, the virus switches from lysogenic to lytic mode and produces new progeny (Doss et al. 2017). Lysogenic phages are less effective than lytic phages as biocontrol agents because they are not only less efficient in killing the host but some bacteriophages also carry genes that can make the bacteria more pathogenic when integrated into the host's genome (Kering et al. 2019). Furthermore, temperate phages facilitate the spread of pathogenic genes through transduction and can be transferred to daughter cells (Nakayinga et al., 2021). Additionally, temperate phages cause superinfection exclusion, which prevents infection by other phages in the host (Kering et al. 2019).

Some bacteriophages are highly specific, targeting only a few bacterial strains. This specificity can lead to bacterial mutations that confer resistance against the phages (Kering et al. 2019). For instance, bacteriophages NBP1-1, NBP4-7 and NBP4-8 infect *Burkholderia glumae* effectively targeting strain *B. glumae* BGLa14-8 but not strain *B. glumae* 336gr-1 (Jungkhun et al. 2021). To address this challenge, phage cocktails are created by mixing different phages with varying specificities (Kering et al. 2019). This approach enhances the host range of the phages and help prevent bacterial resistance, as bacteria find it difficult to develop resistance against all phages simultaneously.

A study on 19 phages effective against *Xanthomonas oryzae* pv. *oryzae* includes phages from the *Siphoviridae, Myoviridae*, and *Podoviridae* families (Jain et al. 2023). Among these, Phage vB_XooS_NR08 exhibits a broad spectrum of activity and does not infect non-pathogenic strain of *Xanthomonas campestris*. Phage NR08 is also resistant to temperature up to 40 °C, can survive in both acidic and alkaline conditions, and is resistant to UV radiation. Furthermore, another study demonstrated that phage φXOF4 has a broad infection range, affecting all eight tested Xoo strains. Treatment with this phage on rice seeds reduced symptoms of bacterial leaf blight (Ranjani et al. 2018).

Conclusion

Pathogen attacks have caused significant losses in rice cultivation, threatening food security and the economy, particularly in Malaysia. This situation presented a challenge as Malaysia must still meet its population's rice demand while relying on imports from other countries. Additionally, globalisation and climate change expose rice crops to new pathogens. The use of antibiotics and synthetic chemicals to control these infections can lead to side effects such as pollution and soil degradation. Furthermore, widespread exposure may result in resistance to these diseases. This review highlights the potential of microorganisms from bacteria, fungi and viruses as biological agents against rice diseases. Currently, biocontrol microorganisms cannot fully replace conventional chemicals due to their often lower effectiveness. However, advancements in genomic research and metabolite analysis provide opportunities for discovering new biocontrol microorganisms. Developments in proteomics and metabolomic technologies facilitate the identification of microorganism metabolites that play a role in pathogen control. Improving these technologies enhance our understanding of the interactions between biocontrol microorganisms and pathogens. Locally endemic biocontrol microorganisms found in their natural habitats have a minimal environmental impact.

The future for biocontrol microorganisms is promising especially as public awareness grow among farmers, agricultural investors and governments about the importance of alternatives to conventional chemicals. To achieve sustainability in agriculture, the biocontrol microorganisms that have been discovered must be optimised for commercial use. This optimisation includes developing formulation techniques that produce long-lasting biocontrol products and facilitate their delivery. Additionally, formulations need to enhance product stability under various climatic conditions, particularly in local environment. For instance, microorganisms should be able to survive changing water conditions in rice cultivation which allow biocontrol products to be exported to other countries. The formulations should also yield user-friendly products that do not require complicated procedures, enabling large scale use. Simple application methods should be encouraged such as watering the plants with a mixture containing the microorganisms or spraying them onto the leaves. Innovations in encapsulation and adjuvant use in formulations further enhance the effectiveness of biocontrol products against pathogens. In summary, biocontrol organisms have the potential to replace the conventional agrochemicals used today.

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Conflict of interest

No potential conflict of interest was reported by the author(s).

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