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RESEARCH ARTICLE

Efficiency of Antagonistic Bacteria and Thai Traditional Crude Extracts Against *Pyricularia Oryzae*-Cause Rice Blast Disease

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A B S T R A C T

Rice blast, caused by *Pyricularia oryzae*, is a major rice-yield-reducing disease. Although the disease can be controlled by fungicides, these are often harmful to agriculturists, and fungicide residues that remain in the environment can negatively affect other living organisms. To avoid such effects, antagonistic bacteria is an alternative solution for controlling plant pathogens. Therefore, this study aimed to isolate the plant pathogen *P. oryzae* and identify its antagonistic bacteria from paddy soil. Two antagonistic bacteria, *Bacillus inaquosorum* and *Streptomyces albus*, were found to inhibit *P. oryzae* with 54.44% and 94% inhibition, respectively. Subsequently, the cell free supernatant and volatile compounds (VOCs) of these two strains were screened. The result showed that VOCs did not affect mycelium growth, even though the culture filtrated solution distressed fungal mycelium expansion and spore formation, there was no secreted enzymes (amylase, chitinase, cellulase) inhibiting *P. oryzae* growth. However, for further study, some factors affecting fungal mycelium growth will be studied. Moreover, there was another technique to control the disease, plant crude extraction, three types of Thai traditional plants, Noni (*Morinda citrifolia L.*), Galangal (*Alpinia galanga* L.) and Siamese neem tree (*Azadirachta indica A. Juss. var. siamensis Valeton*.), and four solvents: water, ethanol, hexane and ethyl acetate, were done crude extraction. The result showed that galangal with hexane was effective against *P. oryzae* and the LD_{50} was 52% inhibition and the galangal ethyl acetate showed 33.3% inhibition at 300000 ppm while the other treatments failed to inhibit *P. oryzae* growth. Overall, these findings demonstrated that *Streptomyces* sp. possessed greater potential against *P. oryzae* as compared to *Bacillus* sp. *in vitro* and could be further explored in vivo. Furthermore, galangal contained effective compounds that inhibited *P. oryzae*, so the rice pathogen will be managed using both approaches.

Keywords: Antagonistic, *Bacillus* sp., *Actinomycetes* sp., rice blast disease, *Pyricularia oryzae*, crude extract.

INTRODUCTION

Rice (*Oryza sativa*) is a staple crop for more than half of the world's population in various parts of the world, and is mainly cultivated in Southeast Asia, East Asia, and South Asia (Plodpai *et al.,* 2013; Parinthawong and Poosin., 2020; Imran *et al.,* 2020; Khan *et al.,* 2022). Several rice cultivation areas have experienced problematic yield loss caused by many different insects and diseases. One particularly serious disease is rice blast caused by the ascomycete fungus *Pyricularia oryzae* (synonym: *Magnaporthe oryzae*), which previous studies have shown to reduce yield by about 90% (Singh *et al.,* 2015; Parinthawong and Poosin., 2020; Arooj *et al.,* 2023). *P. oryzae* can infect rice in various stages of cultivation, such as at the tillering, flowering, and milk stages, with infected rice leaves showing white-to-grey, diamond-shaped lesions (Kunyosying *et al.,* 2018; Shahbaz *et al.,* 2021).

Rice blast is commonly controlled by using chemical agents or synthetic fungicides harmful to agriculturists and the environment. A less environmentally harmful solution is the use of resistant cultivars; however, *P. oryzae* has a short life span and rapidly adapts to resistant cultivars and new environments, resulting in breeders having to continuously develop new resistant cultivars. An alternative solution to controlling rice blast is biological control. Antagonistic microorganisms are widely used, and are well known to control various plant pathogens. For example, *Trichoderma* sp. was effectively used to control many pathogens, such as *Rhizoctonia* spp., *Fusarium* spp., and *Phytophthora* spp. (Pfordt *et al.,* 2020; Zin and Badajuddin, 2020). However, subsequent studies reported that *Trichoderma* sp. also had harmful effects on mushroom cultivation because it infected crop mushrooms, resulting in 50% yield loss (Fletcher, 1997; Samuels *et al.,* 2002; Sobieralski *et al.,* 2012). Moreover, some *Trichoderma* sp. were shown to have negative effects on human health, especially through respiratory tract problems caused by a volatile compound produced by *Trichoderma* sp. (Larsen *et al.,* 1998; Alanio *et al.,* 2008).

Subsequently, other microorganisms were discovered to be effective biocontrol reagents. For example, *Bacillus* sp., known as Plant Growth Promoting Bacteria (PGPB), have been found to effectively antagonize plant pathogens, with an ability to manipulate a broad range of pathogens and resist various environmental conditions (Albayrak, 2019). *Bacillus* sp. produce volatile compounds, and extracellular enzymes such as pectinase, β-glucanase, chitinase, and so forth, to control pathogen growth. Volatile compounds of *B. subtilis* controlled the airborne fungal pathogen *Alternaria solani*, which causes potato early blight disease (Zhang *et al.,* 2020), while its hydrolytic enzymes and antimicrobial metabolite, HCN, were found to inhibit the mycelial growth of various fungal plant pathogens (Zhao *et al.,* 2014; Mardanova *et al.,* 2017; Zhao *et al.,* 2022). Furthermore, Actinomycetes are among the rhizospheric microorganisms with an ability to generate extracellular enzymes, including antibiotics, that can control fungal and bacterial pathogen growth, so are widely used as antagonistic bacteria (Hata *et al.,* 2015; Chauhan *et al.,* 2015; Djebaili *et al.,* 2020). These bacteria could inhibit *Fusarium oxysporum* and *Rhizoctonia solani*, *Pseudomonas syringae*, and others (Gangwar *et al.,* 2011; Djebaili *et al.,* 2020).

Moreover, plant crude extraction is another method widely used to manage plant pathogens because plants have various active compounds, secondary metabolites, such as flavonoids, tannins and phenols etc. to be antimicrobial activity that is also advantage for sustainable disease management. In Thailand, there are various plants; herbal plants and traditional plants having antimicrobial activity such as *Piper Betle* and *Terminalia bellirica* extracted with ethyl acetate were high efficiency controlling *Erwinia carotovara* that caused soft rot disease of Chinese cabbage (Barbin *et al.,* 2018). Crude extraction of galangal, lemon grass and garlic inhibited *Colletotrichum sp*., caused mango anthracnose disease (Sutthisa *et al.,* 2014). Therefore, this study aimed to isolate and identify antagonistic bacteria and screening Thai traditional plants for controlling *Pyricularia oryzae* causing rice blast disease in Thailand.

MATERIALS AND METHODS

***Pyricularia oryzae* Py-02 isolation:** Infected rice leaves were collected from several sampling areas in Lopburi province, central Thailand. The leaves were then cut into small pieces before being rinsed with sterilized distilled water. Dissected leaves were placed on potato dextrose agar (PDA) medium and incubated at 28-30°C for seven days. White to light-gray colonies were selected and moved to PDA for single-colony isolation. Subsequently, single conidia were observed and identified under a light field microscope. Positive colonies were transferred to PDA slant medium for maintenance before use.

***Bacillus* sp. Isolation:** Ten grams of soil sample was suspended in 90 mL sterile normal saline and heated at 80°C for 10 min to destroy vegetative cells. Subsequently, the soil suspensions were serially diluted in normal saline (10^{-1} to 10^{-4}) and spread onto nutrient agar (NA, Hi-Media, Mumbai, India) plate. After incubation at 37 °C for 48 h, single colonies were re-inoculated onto nutrient agar for purification. The isolated colonies were identified by gram staining, spore staining and catalase-production.

Actinomycetes isolation: Ten gram of soil sample was suspended in 90 mL sterile normal saline shaken at 180 rpm, 28-30°C for 10 min. Subsequently, the soil suspensions were serially diluted (10^{-1} to 10^{-6}) and the soil suspension each dilution was plated on starch casein agar medium (10 g soluble starch, 2 g K_2HPO_4, 2 g KNO_3, 0.3 g casein, 0.05 g $MgSO_4.7H_2O$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4.7H_2O$, 15 g agar, and 1 L distilled water). The plates were incubated at 28-30°C for 7-14 days. The colonies were sub-cultured on ISP-2 medium (10 g glucose, 4 g malt extract, 4 g yeast extract, 15 g agar, and 1 L distilled water) for purification and kept in 20% glycerol at −20°C as stock culture. This method followed Chaiharn *et al*. (2020).

In vitro* screening of antagonistic actinomycetes *and

Bacillus sp.: The actinomycetes and *Bacillus* isolates were screened for antagonistic activity against rice blast pathogen by using dual culture technique. For antagonistic activity of actinomycete was determined according to (Loqman *et al.,* 2009). The actinomycetes colonies on ISP2 agar was cut in 5 mm diameter discs with a cork borer and transferred to the PDA plates, placed 3 cm away from the center of the plate. After incubation at 28-30°C for 7 days, 5 mm in fungal disc was placed in the center of the plate. PDA plates with a fungal mycelia disc in the center of the plate as a control. The plates were then incubated at room temperature for 10 days. For *Bacillus*, the PDA plate was inoculated with 5 mm of fungal mycelia disc 1.5 cm away from the edge of the agar plate. Then, *Bacillus* colony on NA plate was streaked 3 cm at the opposite direction, 1.5 cm away from the edge of the agar plate. A control plate without streaking bacterial culture. Both the test and control plates were incubated at room temperature for 14 days. The percentage of inhibition was calculated using the formula:

Percentage of inhibition = $[(r1 - r2)/r1] \times 100$

where r1 represents the radial of fungal growth in the control and r2 is the radial of fungal growth in the test plate.

Determination of antimicrobial activity *in vitro* (Broth vs. VOC): Cell-free supernatant assay: Both antagonistic bacterial strains were cultured in nutrient broth for 5 days for secondary metabolic secretion, and then the supernatant was collected by centrifugation at 3000 rpm for 10 min before extracellular hydrolytic enzyme assay. A *P. oryzae* Py-02 plug was placed in the center of an PDA plate for 5 days' preincubation, and then the holes for the enzymatic assay were made with a 5-mm diameter cork borer. Subsequently, the supernatant was added to the hole in triplicate, then the plates were incubated for 3 days before measuring the diameter of *P. oryzae* Py-02 compared to the control plates, in which distilled water was used instead of bacterial supernatant.

Volatile organic compounds (VOCs) test: A *P. oryzae* Py-02 plug (5-mm diameter) was placed on the center of a PDA plate, and an antagonistic bacteria plug was placed on another PDA plate. All half-plates were positioned face to face and sealed with parafilm to prevent the loss of volatile compounds. After incubation at 28-30°C for 7 days and the experiment was done in triplicate, the diameter of *P. oryzae* Py-02 was measured (the distance between the bacterium and mycelium of *P. oryzae* Py-02) and compared with the control, which had no antagonistic bacteria plug.

Hydrolytic enzyme production: Cellulase: This was a manipulation of the technique of Bischoff *et al.* (2006). Carboxymethyl cellulose (CMC) medium was used as a substrate for the cellulase secreted from the antagonistic bacteria. The antagonistic bacteria were grown on CMC at 28-30°C for 24 hr (aerobic condition), the CMC plates were stained with 1% (w/v) Congo-red solution for 15 minutes and washing with 1 M NaCl to enhance the halo zone; then, the cellulase activity was calculated by the halo-zone formation visible around the positive colony. This experiment was done in triplicate.

Amylase: Starch agar was the substrate for the amylase test, containing 10 g starch, 10 g tryptone, 5 g meat extract, 15 g Agar, and 1000 mL distilled water, pH 7. The antagonistic bacteria were plated on starch agar and incubated at 28-30°C for 24 hr. These plates were then stained with 2% iodine solution for 15 min, after which the clear zone was measured (Chaiharn *et al.,* 2020). This experiment was done in triplicate.

Chitinase: Colloidal chitin (0.1% concentration) was the substrate for the chitinase test. Both antagonistic bacteria were grown on the medium and incubated at 28-30°C for 48 hr, after which the halo zones appeared around the positive colonies were measured (Chaiharn *et al.,* 2020).

Thai traditional plant crude extraction: Noni (*Morinda citrifolia L.*), Galangal (*Alpinia galanga* L.) and Siamese neem tree (*Azadirachta indica A. Juss.* var. *siamensis Valeton*.), Thai traditional plant having some medicinal properties, were studied to control *P. oryzae*. Noni leaves, Siamese neem leaves and Galangal rhizome were rinsed with tap water for cleaning before sliced into small and thin pieces then plant samples were dried at 50°C for 5 days before grinding into fine powder by electric grinder. Four solvents: distilled water, 99% ethanol, hexane and ethyl acetate were used in this study, then 10 grams of each dried sample was soaked in 100 ml of each solvent and shook at 120 rpm for 3 days for 3 times before filtration through No.1 Whatman filter paper. All filtrated solutions were done evaporation in water bath with temperatures below the solvent's boiling point (approximately 50-80°C) to get yield of crude extract. Subsequently, crude was dissolved in 0.2% DMSO. There were 12 treatments namely 3 traditional plant extracts and four solvents for this study.

In vitro* assay of Thai traditional crude extracts against *P. oryzae: Plant crude extraction activity was done by agar well diffusion. A plug of mature mycelium *P. oryzae* was placed at the center of PDA and then a sterilized cork borer punched on the PDA plate away from a fungal plug approximately 2.5 cm and done for four wells per plate. A hundred microliters of each of crude extracts (50000,

100000, 200000 and 300000 ppm) were filled into each well and done triplicate for each sample, moreover, 0.2% DMSO, solvent-only treatment, was negative control before incubation at 28°C for 3-5 days subsequently diameter of fungal colony growth was measured and calculated the percentage of growth inhibition by the formula (Tun *et al.,* 2018)

$$PIA = [(Rc- Rt)/Rc] \times 100$$

PIA = Percentage of inhibition activity

Rc = Radial of fungal mycelium control

Rt = Radial of fungal mycelium treatment

Molecular genetics identification: The mycelium of *P. oryzae* Py-02 and the antagonistic bacteria underwent DNA extraction. The ITS and 16S rRNA primers, used for *P. oryzae* Py-02 and the antagonistic bacteria, respectively (Table 1), were amplified by PCR. The PCR products were purified and sequenced, after which the ITS and 16Sr RNA nucleotide sequences were compared to those in the GenBank database (http://www.ncbi.nlm.nih.gov). Phylogenetic trees including reference sequences were constructed using the MEGA X program by the neighbor-joining model with 1000 bootstrap values.

The GenBank accession number of the sequences reported in this paper is PLK010801, CNT010110, AYA010801, SSK010110, SK010110 and NR_185623 for *P. oryzae* for phylogenetic tree construction whereas AB184522.1, MH_628441.1, NR_0434981, KP170481.1 and KP704806.1 used for Actinomycetes phylogenetic tree construction, moreover, GenBank accession numbers for Bacillus sp. phylogenetic tree construction were OK178285.1, EF532601.1, HM486497.1, ON242120.1 and NR036794.1.

Table 1. Nucleotide primers for PCR assay.

Target region	Primer name	Nucleotide sequences	Target size (bp)	Tm (°C)	Reference
ITS	ITS1	5′-TCCGTAGGTGAACCTGCGG-3′	649	55	White *et al*. 1990
	ITS4	5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3′			
16S	27F	5′-AGAGTTTGATCCTGGCTCAG-3′	1400	57	Heure *et al.,* 1997
	1492R	5′-TACGGYTACCTTGTTACGACTT-3′			

DATA ANALYSIS

All collected data were statistically analyzed based on SPSS software using an analysis of variance (One-way ANOVA test) and means were compared with at least 5% significant level using.

RESULTS

***Pyricularia oryzae* identification:** Infected leaves with lesions, which were white-to-brown and diamond shaped, were isolated on PDA after preliminary selection via spore morphological identification. Fungal colonies were stained with lactophenol blue, and spore morphology was observed under a microscope (Figure 1). Positive spore morphology was indicated by a pear shape, the presence of a hilum on the tip of the spore, and two septa dividing each conidiophore. Moreover, the positive fungal colonies had white mycelia, whereas the mycelia of negative colonies were green and black. Next, the colony underwent DNA extraction and PCR with ITS1 and ITS4, after which the extracted sequences were compared with GenBank sequences. The nucleotide sequences and phylogenetic tree revealed that the extracted isolate was *P. oryzae*, with 99% similarity. Furthermore, the phylogenetic tree showed that the isolate belonged in a group with *P. oryzae* from GenBank (Figure 2).

A B C

Figure 1. (A) Morphology of *Pyricularia oryzae* Py-02 -caused rice blast disease, (B) blast disease leaf lesion surrounded by a *P. oryzae* Py-02 colony, and (C) conidia and conidiophore of *P. oryzae* Py-02.

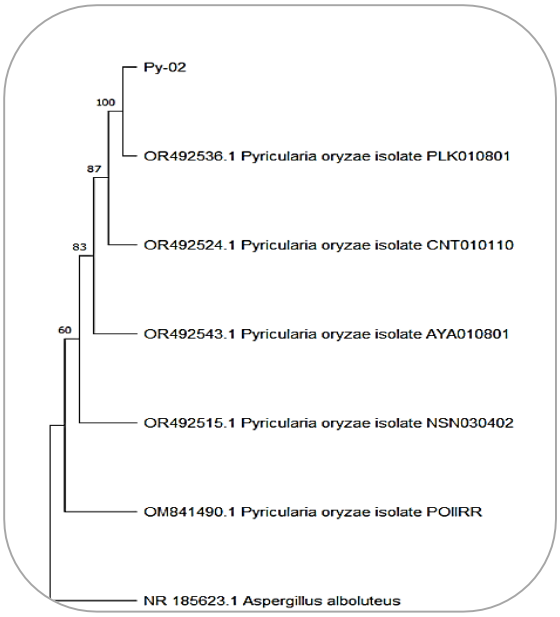

Figure 2. Phylogenetic tree based on the ITS gene of *P. oryzae* Py-02, constructed for comparison with related *P. oryzae* species using the neighbor-joining method in MEGA X.

Antagonistic bacteria identification: Paddy field soil was collected, and antagonistic bacteria were isolated on NA and GYM for *Bacillus* sp. and Actinomycetes sp., respectively. The *Bacillus* colony was creamy-white in color, gram-positive, and returned a positive catalase test, while the *Actinomycetes* sp. had white mycelium and was also a gram-positive colony. All antagonistic bacteria—four isolates of *Bacillus* sp. and eight isolates of Actinomycetes sp.—were tested for antagonistic activity. The results showed that four Actinomycetes and one *Bacillus* isolate could inhibit *P. oryzae* Py-02; however, only one Actinomycetes isolate, named Act-03, had strong antagonistic activity (approximately 94%), whereas the *Bacillus* Isolate S-04 yielded 54.44% inhibition (Table 2, Figure 3).

Table 2. Antifungal activity of Bacillus isolates and actinomycetes isolates

	Percent Inhibition (%)							
	Bacillus isolates							
P.oryzae Py-02	S-01		S-02		S-03		S-04	
	43±0.4^{b}		10.4±0.4^{c}		43.44±0.23^{b}		54.44±0.3^{a}	
	Actinomycete isolates							
	Act-01	Act-02	Act-03	Act-04	Act-05	Act-06	Act-07	Act-0 8
	88.78±0.25^{b}	87.5±0.2^{b}	94±0.3^{a}	72.74±0.5^{d}	83.81±0.1^{c}	62.4±0.1^{f}	72.33±0.4^{d}	67.78±0.5^{e}

a, b, c, d, e, f = the same letter superscript is not significantly different at 5% level of significance

Figure 3. Efficiency of antagonistic bacteria against *P. oryzae* Py-02 in a dual-culture test; (A) control, (B) *P. oryzae* Py-02 inhibition by *Bacillus* sp., and (C) *P. oryzae* Py-02 inhibition by Actinomycetes sp.

Then, two antagonistic bacteria, Act-03 and S-04, underwent spore morphological analysis, with the result showing that the Act-03 spore was of an open-spiral type, whereas the S-04 endospore was located at the center of the vegetative cell.

16S rDNA analysis: The positive antagonistic bacteria underwent DNA extraction and PCR with 16S rRNA primers. The resulting nucleotide sequences were then blasted, and the results revealed that Act-03 was a *Streptomyces* sp., whereas the S-04 antagonistic bacteria isolate was a *Bacillus* sp. A phylogenetic tree was constructed based on the antagonistic bacteria sequences as well as GenBank sequences. Figure 4 shows that Act-03 was grouped with *Streptomyces albus* with strong bootstrap support, while Figure 5 shows that S-04 was grouped with *Bacillus inaquosorum* with moderate bootstrap support.

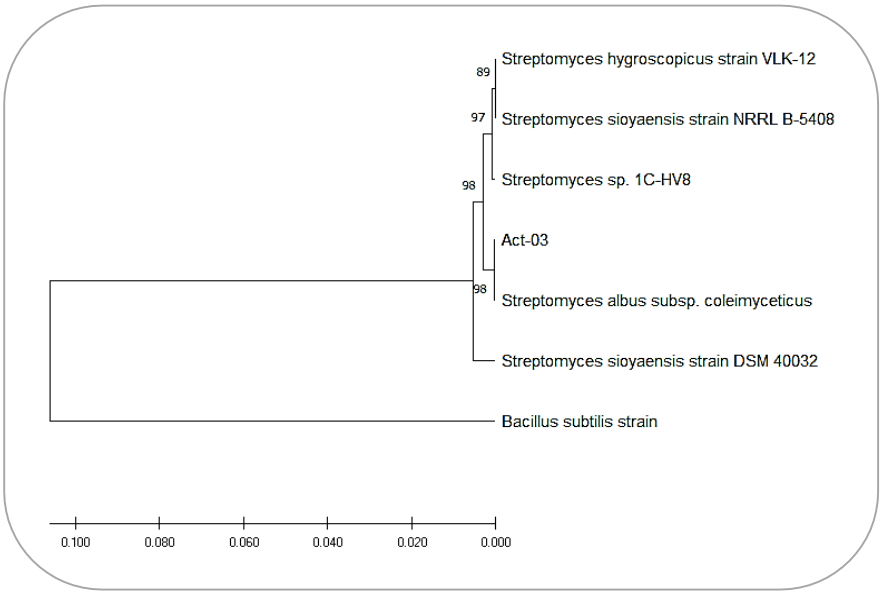

Figure 4. The phylogenetic tree based on the 16S rDNA gene of an Actinomycetes isolate, constructed using the neighbor-joining method in MEGA X, shows its relatedness with other *Streptomyces* species.

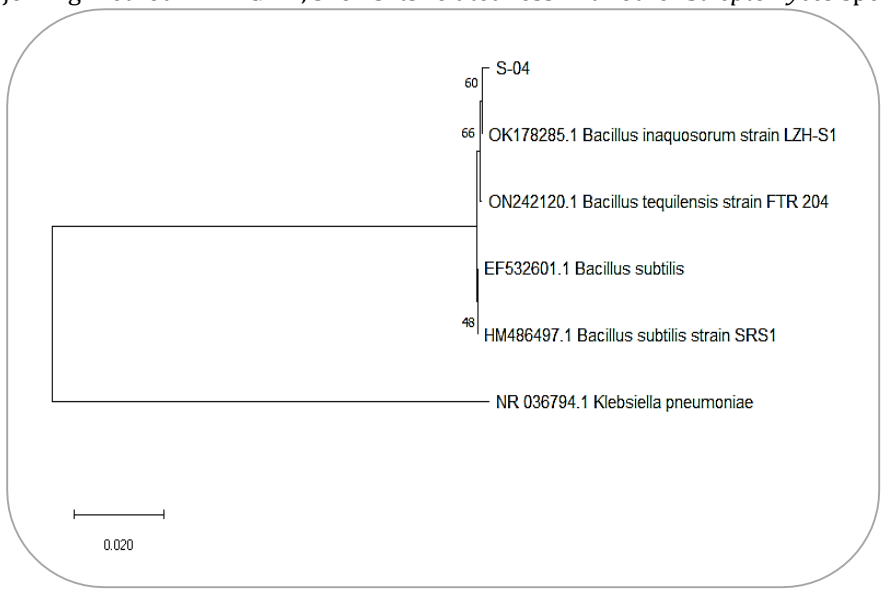

Figure 5. The phylogenetic tree based on the 16S rDNA gene of the *Bacillus* isolate, constructed using the neighbor-joining method in MEGA X, shows its relatedness with other *Bacillus* species.

Cell free supernatant screening: The antagonistic bacteria were screened for secondary metabolite secretion inhibiting *P. oryzae* mycelium growth. The results of the volatile organic compound (VOC) assay showed that these did not affect *P. oryzae* growth, whereas culture filtrated secretion could inhibit mycelium growth and spore formation, as shown in Figure 6.

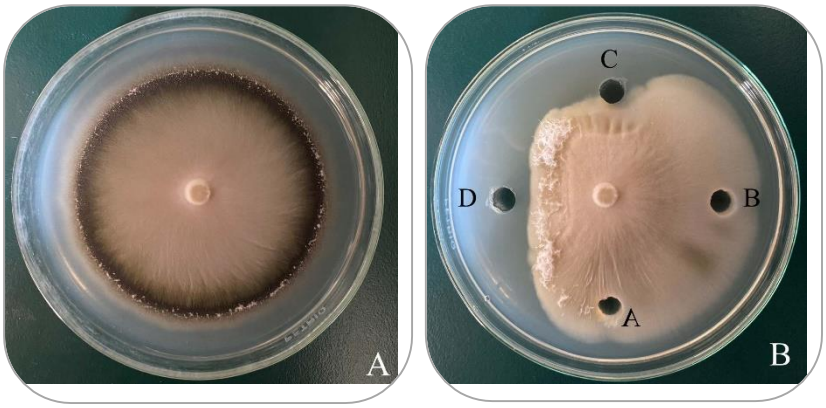

Figure 6. *In vitro* cell free supernatant test: A *P. oryzae* on PDA and B Inhibition of *P. oryzae* with cell free supernatant, (A) PDB broth, (B) distilled water, (C) cell free secretion from *Bacillus inaquosorum*, (D) enzyme secretion from *Streptomyces albus*.

Enzymatic assay: Both antagonistic bacteria were tested for enzyme secretion, namely of cellulase, amylase, and chitinase. The result showed there was even no enzyme to inhibit *P. oryzae* mycelium growth (Data not shown).

Thai traditional plant crude extracted assay: The table showed percent of inhibition from various treatments and the result showed that galangal hexane crude extract having the highest activity approximately 52% inhibition at 300000 ppm whereas the other concentrations of galangal hexane crude extract could be significant moderately inhibited *P. oryzae* that was found in galangal ethyl acetate crude extract at 300000 ppm. On the other hand, the other treatments could not be inhibited *P. oryzae* growth (Table 3).

Table 3 Effective inhibitory concentration of plant crude extractions against *P. oryzae*

plant	Type of solvent	Percentage of radial growth inhibition (%) Concentration of plant extraction (ppm)			
		50000	100000	200000	300000
galangal	Hexane	26.40±0.43^{d}	30.40±0.43^{c}	43.03±0.21^{b}	52±0.82^{a}
	Ethyl acetate	9.97±0.25^{g}	15.58±0.29^{f}	19.02±0.83^{e}	31.70±1.16^{c}
	Absolute ethanol	6.17±0.05^{h}	6.23±0.05^{h}	6.3±0.08^{h}	6.37±0.05^{h}
	water	6.23±0.09^{h}	6.50±0.16^{h}	6.4±0.08^{h}	6.23±0.05^{h}
Noni	Hexane	5.93±0.09^{h}	6.07±0.09^{h}	5.97±0.05^{h}	6.0±0.16^{h}
	Ethyl acetate	5.33±0.12^{h}	5.47±0.05^{h}	5.43±0.05^{h}	5.47±0.09^{h}
	Absolute ethanol	6.3±0.08^{h}	6.28±0.03^{h}	5.73±0.09^{h}	5.93±0.05^{h}
	water	6.10±0.08^{h}	6.27±0.05^{h}	6.47±0.17^{h}	6.4±0.05^{h}
Siamese neem tree	Hexane	6.19±0.01^{h}	6.23±0.02^{h}	6.07±0.09^{h}	6.03±0.12^{h}
	Ethyl acetate	6.19±0.07^{h}	6.22±0.05^{h}	6.32±0.02^{h}	6.59±0.01^{h}
	Absolute ethanol	6.0±0.16^{h}	6.33±0.09^{h}	6.31±0.02^{h}	6.06±0.19^{h}
	water	5.70 ±0.08^{h}	5.86±0.10^{h}	5.73±0.09^{h}	5.87±0.12^{h}

a, b, c, d, e, f, g, h = the same letter superscript is not significantly different at 5% level of significance

DISCUSSION

Rice is a major crop in Thailand for both domestic consumption and export; however, it faces many diseases caused by various pathogens. One of the major pathogens is *P. oryzae*, which causes rice blast disease and drives yield loss. Agriculturists commonly control the disease by using chemical or synthetic fungicides; however, the fungicide residues remain in soil, air, and water, and may be encountered by agriculturists. To protect the environment and agriculturists, biocontrol agents have been developed against fungal pathogens, with previous studies reporting that *Bacillus* sp. and Actinomycetes both functions to control fungal pathogens (Gangwar *et al.,* 2011; Rai *et al.,* 2018; Chen *et al.,* 2019; Gao *et al.,* 2020). Though biological control may be slow to control pathogens, it is not harmful to living organisms in the soil, thus demonstrating a board spectrum of antifungal activity while supporting the natural balance of ecosystems (Ramanathan *et al.,* 2002; Gao *et al.,* 2020). Many studies have used *Bacillus* sp. to control crop pathogens through indirect mechanisms, such as the production of hydrolytic enzymes to damage pathogen cell walls (Shakeel *et al.,* 2015; Majeed *et al.,* 2016). Moreover, *Bacillus* sp. can trigger induced systemic

resistance (ISR), which causes plant physiological change by increasing the content of antioxidant enzymes such as superoxide dismutase, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase (Gajbhiye *et al.,* 2010; El-Sayed *et al.,* 2014; Hassan *et al.,* 2015; Fatima and Anjum, 2017; Rai *et al.,* 2018). Additionally, previous studies have reported that *Bacillus* sp. is able to increase plant antioxidant enzymes such as superoxide dismutase, polyphenol oxidase, and peroxidase to increase plants' *P. oryzae* resistance (Ng *et al.,* 2016; Rais *et al.,* 2017).

This study shows that the most highly antagonistic *Bacillus inaquosorum* and *Streptomyces albus* isolates from paddy soil secreted elicitors into cell free solution to inhibit fungal mycelium. Natural fibrous materials such as cellulose, chitin, proteins, etc. produced at the hyphal tip of the fungal cell wall can be destroyed by these extracellular enzymes—which may be proteolytic, cellulolytic, and amylolytic—produced by antagonistic bacteria. Furthermore, the results revealed that the isolates secreted cell free supernatant to inhibit the fungal mycelium, with 54.44% and 94% inhibition for *B. inaquosorum* and *S. albus*, respectively. The inhibition efficiency of *B. inaquosorum* was similar to that of the *Bacillus* strains KFP-5, KFP-7, and KFP-17, which were reported to have 40–52% inhibition against blast disease (Rais *et al.,* 2018). Although, enzymatic assay showed that there was even no secreted enzyme inhibiting *P. oryzae*, there were other biological agents against pathogens such as antibiotics, soluble compounds (lipopeptides, polyketides, siderophores) or bacteriocins (pediocin, thuricin or lantibiotics) (Clavo *et al.,* 2020). Previous studies showed that the defense mechanism of *Bacillus* sp. against *P. oryzae* was demonstrated in the *Bacillus* strains KFP-5, KFP-7, and KFP-17, while *B. subtilis* strain GB519 induced the ISR mechanism, secreted protease and glucanase, and produced siderophores (Rais *et al.,* 2017, Rai *et al.,* 2018; Zhu *et al.,* 2022). *Bacillus velezensis* was shown to antagonize *P. oryzae* by secreting hydrolytic enzyme, and increasing the accumulation of salicylic acid and hydrogen peroxide (Chen *et al.,* 2021). Additionally, the present study examined whether VOCs, which are produced by organisms such as fungi, yeast, and bacteria and are beneficial for interactions between host plants and microorganisms, inhibited *P. oryzae* growth. Previous studies have found that VOCs can control plant–pathogen interaction and promote plant growth (Tahir *et al.,* 2017; Zhao *et al.,* 2023). However, the present study found that the VOCs of *B. inaquosorum* and *S. albus* could not inhibit *P. oryzae* growth. This contrasts with the results of a previous study in which *B. subtilis* produced VOC to control *Alternaria solani* (Zhang *et al.,* 2020).

Unlike *Bacillus* sp. antagonistic bacteria, there are very few reports of Actinomycetes antagonistic to pathogens, even though this phylum includes a powerful antagonistic genus— *Streptomyces*—that can control pathogens such as *Colletotrichum gloeosporioides*, *Cephalosporium maydis*, *Rhizoctonia solani*, and *P. oryzae* (El-Mehalawy *et al.,* 2005; Prapagdee *et al.,* 2008; Pan *et al.,* 2015; Awla *et al.,* 2017). Moreover, it is a Plant Growth Promoting Rhizobacteria (PGPR), thus improving plant growth and yield. *Streptomyces* is one of the free-living genera of Actinomycetes, and has been easy to study as it consists of more than 500 species in natural environments (Seipke *et al.,* 2012). These bacteria are efficient antagonists as they have colonized numerous root crops and inhabit the rhizosphere; consequently, they have evolved abilities to promote plant growth and produce secondary lytic enzymes to inhibit plant pathogens (Palaniyandi *et al.,* 2013; Bonaldi *et al.,* 2015; Kunova *et al.,* 2016). In the present study, *S. albus* had higher efficiency than *Streptomyces* strain UPMRS4 (67.9%), *S. albidoflavus* OsiLf-2 (74.1%), and rhizobacterial isolate IS4 (81.7%) to inhibit *P. oryzae* (Awla *et al.,* 2017; Gao *et al.,* 2020; Nabila, 2021). *Streptomyces* sp. reportedly combine various mechanisms against plant pathogens; for instance, *S. mutabilis*, *Streptomyces* strain DBT204, and *S. endus* OsiSh-2 secreted chitinase and antibiotics and produced siderophores to inhibit *R. solani*, *F. proliferatum*, and *P. oryzae*, respectively (Goudjal *et al.,* 2014; Passari *et al.,* 2015; Xu *et al.,* 2017), while *S. albus* secreted cellulase to inhibit *P. oryzae* in the present study. According to crude extraction, several studies showed that crude extraction was another management for instance *Alpinia galanga* strongly against *Ceratocystis* sp. moreover *Fusarium oxysporum* (Suprapta and Khalimi, 2009) and *Tectona grandis L.f.* was board range to inhibit various fungi namely *Arthrinium phaeospermum, Aspergillus flavus, Acremonium butyri, Nigrospora sp., and Penicillium citrinum* that were wood-pathogen to *Albizia falcataria* (Suprapta, 2012) due to the efficiency of active compounds to control phytopathogens such as phenolic compounds, tannin, flavonoids etc. Various researches showed that *P. oryzae* was inhibited by plant crude extractions such as *Epicoccum* sp, *chromoluena odorata*, *Prosophis juliflora* and *Ziziphus jujuba* (Sena *et al.,* 2013,

Suriani *et al.,* 2015) and this result showed that *A. galangal* hexane was potential crude extraction to inhibit *P. oryzae* similar to previous studies that used *C. longa* hexane to inhibit *P. oryzae* including *Botrytis cinerea, Chaetomium olivaceum*, *Fusarium graminearum,* and *Magnaporthe grisea* (Nabila *et al.,* 2021) while *Z. officinale* hexane was slightly activity to inhibit *P. oryzae* (Supratra and Khalimi, 2009). Additionally, this result showed that *A. galangal* ethyl acetated at 300000 ppm was slightly inhibited *P. oryzae* consequent to previous study stated *A. galangal, C. longa and Z. officinale* were wide-ranged ability to against several bacterial and fungi depending on kind and the number of active components in crude extraction (Nabila *et al.,* 2021).

CONCLUSION

In conclusion, biological control and plant crude extraction are an important solution for inhibiting plant pathogens, and is harmless to agriculturists and the environment. Moreover, some antagonistic bacteria are also PGPRs for plant growth. *S. albus* demonstrated greater potential as a candidate for a biocontrol agent to manage rice blast disease. Moreover, *A. galangal* with hexane was high potential to against *P. oryzae* as well, so further study will examine its PGPR properties such as IAA production, potassium and phosphorus solubility etc. and *A. galangal* hexane crude extraction will be used as spray on rice in pot experiment.

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REFERENCES

Alanio, A., B. Brethon, M. Feuihade de Chauvin, E. Kerviler, T. Leblanc, C. Lacroix, A. Baruchel, and J. Menotti. 2008. Invasive pulmonary infection due to *Trichoderma longibrachiatum* mimicking invasive Aspergillosis in a neutropenic patient successfully treated with voriconazole combined with caspofungin. Clinical Infectious Diseases, 46(10): 116–118.

Albayrak, C. 2019. Bacillus Species as Biocontrol Agents for Fungal Plant Pathogens. Bacilli and Agrobiotechnology: Phytostimulation and Biocontrol, 2: 239-265

Arooj, S., S. Ahmad, M. Akhter, M. Atiq, A. Rashid, M.E.U. Haq, M.A. Rehman, M. Kamran, A. Intisar and M. Asim, 2023. Epidemiology of Rice Blast (*Pyricularia oryzae*) Disease in Central Punjab, Pakistan. International Journal of Phytopathology, 12(2): 129-139.

Awla, H., J. Kadir, R. Othman, T. S. Rashid, H. Sathyapriya, and W. Mui-Yun. 2017. Plant growth-promoting abilities and biocontrol efficacy of *Streptomyces* sp. UPMRS4 against *Pyricularia oryzae*. Bio Control, 112: 55-63.

Barbin, K.B., S. Leizel, B. E.Ruth, L. Angelyn and J.H. Ekman. 2018. Efficacy of guava and mangosteen extracts in reducing soft rot (*Pectobacterium carotovorum*) in harvested Chinese cabbage. Acta Horticulturae, 393-400.

Bischoff, K. M., A. P. Rooney, X. Li, S. Liu, and S. R. Hughes. 2006. Purification and characterization of a family 5 endoglucanase from a moderately thermophilic strain of *Bacillus licheniformis*. Biotechnololy Letter, 28: 1761–1765.

Bonaldi, M., X. Chen, A. Kunova, C. Pizzatti, M. Saracchi and P. Cortesi. 2015. Colonization of lettuce rhizosphere and roots by tagged *Streptomyces*. Frontiers in Microbiology, 5(25): 10 pages.

Calvo, H., I. Mendiara, E. Arias, A. P. Gracia, D. Blanco and M. E. Venturini. 2020. Antifungal activity of the volatile organic compounds produced by Bacillus velezensis strains against postharvest fungal pathogens. Postharvest Biology and Technology, 166: 111208.

Chaiharn, M., T. Theantana and W. Pathom-aree. 2020. Evaluation of Biocontrol Activities of *Streptomyces* spp. against Rice Blast Disease Fungi. Pathogens, 9(2): 126. https://doi.org/10.3390/pathogens9020126

Chauhan, H., D. Bagyaraj, G. Selvakumar and S. P. Sundaram. 2015. Novel plant growth promoting rhizobacteria—Prospects and potential. Applied Soil Ecology, 95: 38-53.

Chen, P., C. Zhang, X. Ju, Y. Xiong K. Xing and S. Qin. 2019. Community Composition and Metabolic Potential of Endophytic Actinobacteria From Coastal Salt Marsh Plants in Jiangsu, China. Frontiers in Microbiology, 10: 1063.

Chen, Z., L. Zhao, Y. Dong, W. Chen, C. Li, X. Gao, R. Chen, L. Li and Z. Xu. 2021. The antagonistic mechanism of *Bacillus velezensis* ZW10 against rice blast disease: Evaluation of ZW10 as a potential biopesticide. PLoS One, 16(8): e0256807.

Chen, Z., L. Zhao, W. Chen, Y. Dong, C. Yang, C. Li, H. Xu, X.

Gao, R. Chen, L. Li and Z. Xu. 2020. Isolation and evaluation of *Bacillus velezensis* ZW-10 as a potential biological control agent against Magnaporthe oryzae. Biotechnology & Biotechnological Equipment, 34(1): 714–724.

Djebaili, R., M. Pellegrini, M.Bernardi, M. Smati, K. Mahmoud and M. Del Gallo. 2020. Biocontrol Activity of *Actinomycetes* Strains against Fungal and Bacterial Pathogens of *Solanum lycopersicum L*. and *Daucus carota L*.: In Vitro and in Planta Antagonistic Activity. Biology and Life Sciences Forum, 4: 1305.

El-Mehalawy, A., N. Abd-Allah, R. Mohamed. and M. Abu-Shady. 2005. *Actinomycetes* Antagonizing Plant and Human Pathogenic Fungi. II. Factors Affecting Antifungal Production and Chemical Characterization of the Active Components. International Journal of Agricultural and Biological Engineering, 7(2): 188-196.

El-Sayed, W. S., A. Akhkha, M. Y. El-Naggar and M. Elbadry. 2014. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Frontiers in Microbiology, 5: 118165.

Fatima, S. and T. Anjum. 2017. Identification of a potential ISR determinant from *Pseudomonas aeruginosa* PM12 against Fusarium wilt in tomato. Frontiers in Plant Science, 8: 255436.

Fletcher, J.T. 1997. Mushroom spawn and the development of *Trichoderma* compost mould. Mushroom News, 45: 6-11.

Gajbhiye, A., A. R. Rai, S. U. Meshram and A. B. Dongre. 2010. Isolation, evaluation and characterization of *Bacillus subtilis* from cotton rhizospheric soil with biocontrol activity against *Fusarium oxysporum*. World Journal of Microbiology and Biotechnology, 26(7): 1187–1194.

Gangwar, M., S. Dogra and N. Sharma. 2011. Antagonistic bioactivity of endophytic actinomycetes isolated from medicinal plants. Journal of Advanced Laboratory Research in Biology, 2(4): 154–157.

Gao, H., G. Taylor, S. K. Evans, P. C. M. Fogg and M. C. M. Smith. 2020. Application of serine integrases for secondary metabolite pathway assembly in *Streptomyces*. Synthetic and Systems Biotechnology, 5: 111–119.

Goudjal, Y., O. Toumatia, A. Yekkour, N. Sabaou, F. Mathieu and A. Zitouni. 2014. Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. Microbiological Research, 169(1): 59-65.

Hassan, M. N., S. Zia-Ul-Husnain Shah, S. Afghan and F. Y. Hafeez. 2015. Suppression of red rot disease by *Bacillus sp*. based biopesticide formulated in non-sterilized sugarcane filter cake. BioControl, 60: 691-702.

Hata, M. E., K. Sijam, Z. Ahmad, M. T. Yusof and N. Azman. 2015. In vitro Antimicrobial Assay of Actinomycetes in Rice Against *Xanthomonas oryzae pv. oryzicola* and as Potential Plant Growth Promoter. The Brazilian Archives of Biology and Technology, 58: 821-832.

Imran, M., S.T. Sahi, M. Atiq and A. Rasul. 2020. Disparity in mineral contents of rice genotypes infected with brown leaf spot. Pakistan Journal of Agricultural Sciences, 57(6).

Khan, M.F., A. Riaz, I. Ahmad, S. Ullah, M.U. Raja, F.A. Shaheen, R. Ahsan and M. Zakria. 2022. Current status of brown leaf spot in rice growing areas of Punjab, Pakistan. Pakistan Journal of Phytopathology, 34(2).

Kunova, A., M. Bonaldi, M. Saracchi, C. Pizzatti, X. Chen and P. Cortesi. 2016. Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. BMC Microbiology, 16(1): 272.

Kunyosying. D., C. To-anun and R. Cheewangkoon. 2018. Control of rice blast disease using antagonistic yeasts. International Journal of Agricultural Technology, 14: 83-98.

Larsen, F. O., P. Clementsen, M. Hansen, N. Maltbaek, T. Ostenfeldt-Larsen, K. F. Nielsen, S. Gravesen, P.S. Skov and S. Non. 1998. Volatile compounds from the indoor mould *Trichoderma viride* cause histamine release from human bronchoalveolar cells. Inflammation Research, 47(1): 55–56.

Loqman, S., E.E.A. Barka, C.E. Clement and E.Y. Ouhdouch. 2009. Antagonistic actinomycetes from Moroccan soil to control the grapevine gray mold. World Journal Microbiology and Biotechnology,25: 81–91.

Majeed, M., S. Majeed, K. Nagabhushanam, S. Natarajan, A. Sivakumar and F. Ali. 2016. Evaluation of the stability of *Bacillus coagulans* MTCC 5856 during processing and storage of functional foods.

International Journal of Food Science & Technology, 51: 894–901.

Mardanova, A.M., G.F. Hadieva, M.T. Lutfullin, I. V. Khilyas, L. F. Minnullina, A. G. Gilyazeva, L. M. Bogomolnaya and M. R. Sharipova. 2017. *Bacillus subtilis* Strains with Antifungal Activity against the Phytopathogenic Fungi. The Agricultural Science Journal, 8: 1-20.

Nabila, K. R. S. 2021. Antagonistic activity of siderophore-producing bacteria from black rice rhizosphere against rice blast fungus *Pyricularia Oryzae*. Microbiology and Biotechnology Letters, 49(2): 217–224.

Ng, L. C, M. Sariah, O. Sariam, O. Radziah, A. M. A. Zainal. 2016. PGPM-induced defense-related enzymes in aerobic rice against rice leaf blast caused by *Pyricularia oryzae*. European Journal of Plant Pathology, 145: 167-175.

Palaniyandi, S. A., S. H. Yang, L. Zhang and J. W. Suh. 2013. Effects of actinobacteria on plant disease suppression and growth promotion. Applied Microbiology and Biotechnology, 97: 9621–9636.

Pan, H. Q., S. Y. Yu, C. F. Song, N. Wang, H. M. Hua, J. C. Hu and S. J. Wang. 2015. Identification and characterization of the antifungal substances of a novel *Streptomyces cavourensis* NA4. Journal of Microbiology and Biotechnology, 25(3): 353-7.

Palaniyandi, S.A., S.H. Yang, L.Zhang, and J.W. Suh, 2013. Effects of Actinobacteria on Plant Disease Suppression and Growth Promotion. Applied Microbiology and Biotechnology, 97: 9621-9636.

Parinthawong, N. and P. Tanasian. 2020. Pathogenicity of *Pyricularia oryzae* on elite rice cultivars and geographical distribution of avirulence genes causing blast disease in Thailand. International Journal of Agricultural Technology, 16(4): 897-906.

Passari, A. K., V. K. Mishra, R. Saikia, V. K. Gupta and B. P. Singh. 2015. Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. Frontiers in Microbiology, 6: 273.

Pfordt, A., L. Ramos Romero, S. Schiwek, P. Karlovsky and A. Tiedemann. 2020. Impact of environmental conditions and agronomic practices on the prevalence of *Fusarium* species associated with ear-and stalk rot in maize. Pathogens, 9(3): 236.

Plodpai, P., S. Chuenchitt, V. Petcharat, S. Chakthong and S. P.Voravuthikunchai. 2013. Anti- *Rhizoctonia solani* activity by Desmos chinensis extracts and its mechanism of action. Crop Protection, 43: 65–71.

Prapagdee, B., C. Kuekulvong and S. Mongkolsuk. 2008. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. International Journal of Biological Sciences, 4(5): 330–337.

Rai, K., S. Khadka and B. Shrestha. 2018. Actinomycetes: Isolation, Characterization and Screening for Antimicrobial Activity from Different Sites of Chitwan. International Journal of Medical Biochemistry. 3(1): 25-30.

Rais, A., Z. Jabeen, F. Shair, F. Y. Hafeez and M. N. Hassan. 2017. *Bacillus spp*., a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae*. PLoS One, 12(11): e0187412.

Ramanathan, A., V. Shanmugam, R. Thiruvengadam and S. Ramasamy. 2002. Induction of systemic resistance in ragi against blast disease by *Pseudomonas flourescens*. Annals of Plant Sciences, 10: 313-318.

Samuels, G.J., S. Dodd, W. Gams, L.A. Castlebury and O. Petrini. 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown Agaricus bisporus. Mycologia, 94: 146–168.

Seipke, R. F., Kaltenpoth, M., Hutchings, M. I. 2012. *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiology Reviews, 36(4): 862-76.

Sena, A., A. C. Amanda, D. C. B. Côrtes, M. Vinicius, S. Gisele, L. Valacia, P. Anne, F. Marta and A. Leila. 2013. Increased enzymatic activity in rice leaf blast suppression by crude extract of *Epicoccum* sp. Tropical Plant Pathology, 38: 387-397

Shahbaz, M., U. Shakir, A. Palwasha, F. Ahmad, M. Riaz, Z. Ali, G. Nabi, A. Hussain, S. Muhammad, A.A. Khan and M.S. Kashif. 2021. Evaluation of different fungicides against blast, brown leaf spot and sheath blight of rice under field conditions in Sheikhupura, Pakistan. Pakistan Journal of Phytopathology, 33(1).

Shakeel, M., A. Rais, M. N. Hassan and F. Y. Hafeez. 2015. Root Associated *Bacillus Sp.* Improves Growth, Yield and Zinc Translocation for Basmati Rice (*Oryza sativa*) Varieties. Frontiers in Microbiology, 6: 1286.

Singh, W., R. Kapila, T. Sharma and R. Rathou. 2015.

Genetic and physical mapping of a new allele of Pik locus from japonica rice 'Lijiangxintuanheigu'. Euphytica, 205: 889-901.

Sobieralski, K., M. Siwulski, M. Kommon-Żelazowska, L. Błaszczyk, I. Sas-Golak and D. Frużyńska-Jóźwiak. 2012. Impact of *Trichoderma pleurotum* and *T. pleuroticola* isolates on yielding of *Pleurotus ostreatus* (Fr.) Kumm. Journal of Pharmacy Practice and Research, 52: 165–8.

Suprapta, D.N. and K. Khalimi. 2009. Efficacy of plant extract formulation to suppress stem rot disease on vanilla seedlings. ISSA Journal, 15, 34-41.

Suprapta, D.N. 2012. Potential of Microbial Antagonists as Biocontrol Agents against Plant Fungal Pathogens. Journal of International Society for Southeast Asian Agricultural Sciences, 18, 1-8.

Suputtra J., C. Rattanakreetakul and C. Leksomboon. 2015. Zingiberaceous plant crude extracts unfavourable towards growth and spore gemination of postharvest disease fungi. Postharvest Technology Innovation Center (Proceeding), 142.

Suriani L. N., D. N. Suprapta, N. Nazir, D. K. Agung, A. P. Susun, S. W. Ni. and Y. M. Bohari. 2020. Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants. Indian Journal of Agricultural Research, 54(4): 411-419.

Tahir, H. A. S., Q. Gu, H. Wu, W. Raza, A., Safdar, Z. Huang, F. Uddin Rajer and X. Gao. 2017. Effect of volatile compounds produced by *Ralstonia solanacearum* on plant growth promoting and systemic resistance inducing potential of *Bacillus* volatiles. BMC Plant Biology, 17: 133.

Tun Z. M., M. Zaw, S. S. Aye, S.P. Oo and T. A. A. Naing. 2018. Evaluation of plant extracts against rice blast disease caused by *Pyricularia grisea.* Journal of Agricultural Research, 5(1):37-43.

Xu, T., Y. Li, X. Zeng, X. Yang, Y. Yang, S. Yuan, X. Hu, J. Zeng, Z. Wang, Q. Liu, Y.,Liu, H. Liao, C. Tong, X. Liu and Y. Zhu. 2017. Isolation and evaluation of endophytic *Streptomyces endus* OsiSh-2 with potential application for biocontrol of rice blast disease. Journal of the Science of Food and Agriculture, 97(4): 1149-1157.

Zhang, D., S. Yu, Y. Yang, J. Zhang, D. Zhao, Y. Pan, S. Fan, Z. Yang and J. Zhu. 2020. Antifungal Effects of Volatiles Produced by *Bacillus subtilis* Against *Alternaria solani* in Potato. Frontiers in Microbiology, 11: 1196.

Zhao, P., C. Quan, Y. Wang, J. Wang and S. Fan. 2014. *Bacillus amyloliquefaciens* Q-426 as a potential biocontrol agent against *Fusarium oxysporum*. f. sp. *spinaciae*. Journal of Basic Microbiology, 54: 448-456.

Zhao, S., Z. Ning, L. Bingxia, H. Ying, L. Jiaxing, Z. Yingning, X. Xinting, Q. Chenyu, D. Qunpeng, L. Bin, J. Dongfu, Q. Yibin, L. Jingzhuan, C. Zhongwei and Z. Wu. 2022. Effect of probiotics on growth performance, immune function, serum indices and intestinal flora of broilers. Frontiers in veterinary science, 9: 846649.

Zhao, Q., J. Cao, X. Cai, J. Wang, F. Kong, D. Wang, J. Wang, F. Kong, D.Wang and J. Wang. 2023. Antagonistic Activity of Volatile Organic Compounds Produced by Acid-Tolerant *Pseudomonas protegens* CLP-6 as Biological Fumigants to Control Tobacco Bacterial Wilt Caused by *Ralstonia solanacearum*. Applied and Environmental Microbiology Journal, 89(7): e0083923.

Zhu, H., H. Zhou, Z. Ren and L. Erming. 2022. Control of *Magnaporthe oryzae* and Rice Growth Promotion by *Bacillus subtilis* JN005. Journal of Plant Growth Regulation, 41: 2319–2327.

Zin, N. and N. A.Badaluddin. 2020. Biological functions of *Trichoderma spp*. for agriculture applications. Annals of Agricultural Sciences, 65: 168-178.

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