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Research

Three Isolate of Actinomycetes As Biological Control Against *Magnaporthe orzyae* and *Fusarium solani*

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ABSTRACT

Phytopathogens are causing global food security concerns, resulting in approximately 12.5% crop loss. These fungi significantly impact plant physiology, growth, and development. Traditional fungicides used for control are known to be harmful to both humans and the environment. Therefore, this study advocates an eco-friendly approach using biological control agents to curb phytopathogenic fungi growth. This research focuses on identifying potential antagonistic microorganisms capable of inhibiting two common phytopathogenic fungi: *Magnaporthe oryzae*, responsible for rice blast disease, and *Fusarium solani*, causing *Fusarium* wilt disease. The inhibitory strength of the microorganisms isolated from six different locations in Peninsula Malaysia was tested *in vitro* via dual culture assays. Our findings revealed three actinomycete species isolated from Bangi Forest Reserve, UKM, namely *Streptomyces morookaense* UKM1, *Streptomyces rubrisoli* UKM1, and *Streptomyces gelaticus* UKM1 exhibit a remarkable ability to inhibit the growth of both *M. oryzae* and *F. solani*, with a percentage inhibition radial growth (PIRG) exceeding 70%. Additionally, distinct differences in pathogens mycelia were observed after being grown together with the antagonistic microorganisms. In summary, our research identifies promising microorganisms with potent inhibition capabilities against multiple plant pathogens, offering potential solutions for sustainable agriculture and improved food security.

Key words: Actinomycete, biocontrol agent, fungal pathogens, *Streptomyces*

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INTRODUCTION

It was reported that more than 12.5% of global crops were lost due to various diseases by phytopathogens. The phytopathogens regularly disrupt plant development and physiological processes, including photosynthesis, cell division, and the translocation of water (Shahid *et al*., 2021). Among the numbers, 70–80% of the phytopathogens are fungi, comprising over 20,000 fungal species that can cause diseases in agriculture (Isleib, 2012; Peng *et al*., 2021). This is a crucial issue that must be considered because it can cause global financial losses of over USD220 billion every year and potentially lead to a food crisis and inflation (FAO, 2021).

Magnaporthe oryzae and *Fusarium solani* are Ascomycetes, which are often reported as notorious filamentous pathogenic fungi towards plants and animals (Arie, 2019; Chai *et al*., 2023). that commonly cause blast disease to rice or grain and *Fusarium* wilt, which mainly leads to wilting, weakening, and blocking of vascular vessels in most plants, respectively. Several factors induce their

pathogenicity towards plant hosts, such as cell wall-degrading enzymes, toxins, growth regulators, effector proteins, and fungal viruses (Peng *et al*., 2021; Bharudin *et al*., 2022). For instance, *M. oryzae* was reported can lower the plant's defenses by inducing plant growth regulators, such as abscisic acid and jasmonic acid (Ding *et al*., 2016; Lievens *et al*., 2017). Other examples are the activation of an effector protein such as monooxygenase that converts jasmonic acid to the hydroxyl compound 12OH-JA, which leads to a reduction in plant disease resistance (Patkar *et al*., 2015). Meanwhile, the expression cell wall-degrading enzymes like pectinesterase, pectinase, and cellulase were found to increase the efficiency of plant lignin degradation during spore germination by *F. solani* (Koller *et al*., 1982; Lozovaya *et al*., 2007). Moreover, *F. solani* also produces certain toxins such as deoxynivalenol, ovoid, ellipsoid, or reniform, and 1-2-celled micronidia that increase its pathogenicity towards the plant roots (Kurt *et al*., 2020).

Fungicide is regularly used in the agricultural industry due to its widely varied purposes such as being persistent and effective against fungal pathogens, being versatile applications and easy to access, and being cost-effective (Hassal, 1965; Jepson, 2001). It was reported that among Asian countries, China is the highest region in using fungicides with more than one million tonnes, followed by India and Malaysia (Parajuli *et al*., 2021). Nevertheless, the improved awareness in recent years towards environmental value has encouraged farmers to switch to organic fungicide, which is safer and more ecologically friendly yet is found to be more expensive, too selective for certain fungal species, and needs to be applied frequently, leaving most farmers in a dilemma (Prakash & Sikdar, 2019). Other approaches, such as plant breeding, genetically engineered crops, agrochemicals, and physical methods (i.e heat treatments, UV irradiation, cold storage, and an elicitors application), have been applied in agriculture, but the approaches are expensive and may cause antibiotic resistance (Azadi *et al*., 2015; Thambugala *et al*., 2020). The microorganisms could be the best candidate for improving plant health; acting as biological control agents (BCA) (Yusoff *et al*., 2021) and plant growth promoting (PGP) (Rafedzi *et al*., 2024) which has been proven to be environmentally safe and cost-effective.

To date, there are many potential biological control microorganisms been studied, either bacteria or fungi such as *Bacillus*, *Burkholderia*, *Agrobacterium*, *Enterobacter*, *Pseudomonas*, *Streptomyces*, *Arthrobacter*, *Trichoderma*, *Alternaria*, and *Penicillium* (Thambugala *et al*., 2020; Wahab *et al*. 2022; Bonaterra *et al*., 2022). However, many studies only focus on single-plant pathogens. Our study tries to identify a potential biological control microorganism that can be used to overcome the growth of two main plant pathogens, *M. oryzae,* and *F. solani*. In this study, we have successfully isolated three actinomycetes with high potential to be used as biological control agents against two of the main fungal pathogens in the agriculture sector.

MATERIALS AND METHODS

Maintaining the plant pathogens culture

The plant pathogenic fungi that were used in this study are *M. oryzae* and *F. solani*, which are obtained from the fungal culture collection of Laboratory 3166, Department of Biological Sciences and Biotechnology, Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia. These phytopathogenic fungi were cultured on potato dextrose agar (PDA) (Oxoid, UK) with a maximum incubation period of 14 days for growth optimization of two pathogens until they reach an optimum radius on the plate. The optimum temperature that is being used for the fungi is 28±2°C.

Isolation of soil microbes

Soil samples were obtained from six different places in Peninsular Malaysia (Table 1). The soil samples were stored in sterile bottles (250 mL) before being transferred to the laboratory for microbiological isolation for no more than 24 hr. Serial dilution was done by taking into account the dilution factor from 10^{-3} up to 10^{-5}. The media used were potato dextrose agar (PDA) (Oxoid, UK) and Rose-Bengal agar with chloramphenicol (RBC) (Friendeman Schmidt, Australia) for the isolation of fungi or yeast, and nutrient agar (NA) (Friendeman Schmidt, Australia) for the isolation of bacteria. The incubation for the media was at a temperature of 28±2°C for 24 to 72 hr. All single colonies with different sizes, colors, and morphologies were transferred to a new agar plate for further analysis.

Dual culture assay activity

All pure culture isolates were tested for their ability to be inhibitory (antagonistic) against *M. oryzae* and *F. solani* through the dual culture assay technique. The dual culture technique by culturing each isolate together with each pathogen simultaneously on the same PDA plate (Yusoff *et al*. 2021). Dual

culture slope assay positions of isolates and pure fungal cultures are attached in Figure 1A. The growth development of mycelia of pathogens upon isolates was observed daily until the positive controls of each pathogen reached optimum growth in the plate. The level of inhibition strength of each isolate against each pathogen was determined and evaluated based on the percentage inhibition of radial growth (PIRG) formula as shown in Figure 1B (Shariffah-Muzaimah *et al*., 2015). A PIRG value of 70% to 100% indicates the strongest level of inhibition produced by isolates, followed by moderate inhibition (PIRG value (%) = 50-69) and no inhibition (PIRG value (%) = 0-49). The PIRG value of more than 70% is chosen as an antagonistic candidate and will be used for further species identification. Three replicates were prepared for each sample with positive and negative controls.

Table 1. The sampling sites across Peninsular Malaysia

Soil source	Sample collection	Location
Reserved forest	6	Bangi Forest Reserve, UKM, Bangi, Selangor, 2°55'11.8452”N, 101°46'02.4132”E
Rubber plantation	6	Rubber Plantation, Durian Tunggal, Melaka, 2°20'46.965”N, 102°14'35.458”E
Oil palm plantation	3	Oil palm plantation, Jasin, Melaka, 2°13’13.184”N, 102°27’39.74”E
Oil palm plantation	10	Oil palm plantation, Jenderam Hilir, Selangor, 2°52’48.7”N, 101°42’29.5”E
Paddy plantation	3	Paddy Plantation, Pekan, Pahang, 3°35'14.5"N, 103°21'18.8"E
Paddy plantation	3	Paddy Plantation, Kuala Pilah, Negeri Sembilan, 2°44'47.2"N, 102°9'11.2"E

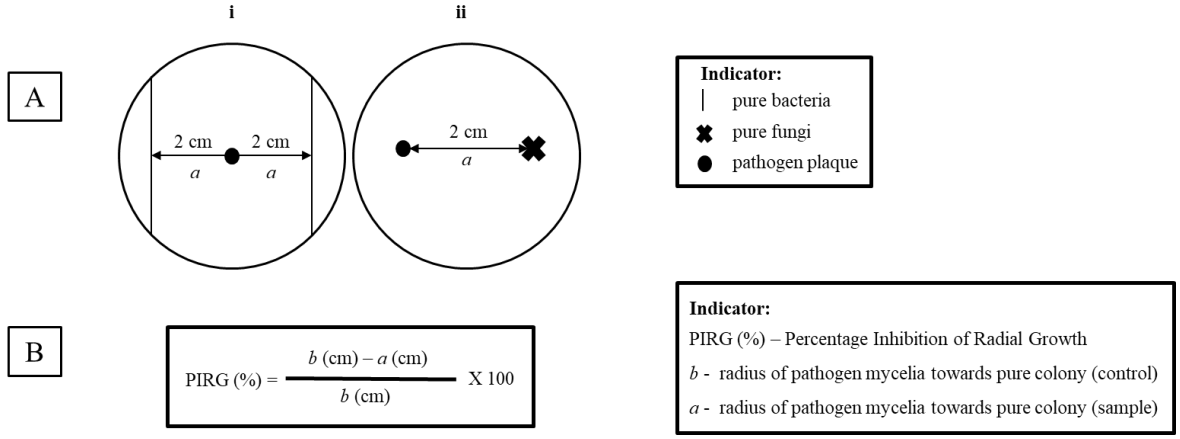

Fig. 1. The PIRG value is used in a dual culture assay to determine the inhibition effect of a pure colony against pathogens. (A) shows the figure of how to arrange the location of (i) pure bacteria or (ii) pure fungi with the fungal pathogen on PDA media, with a 2 cm distance between the isolate and the pathogen. (B) The PIRG formula in percentage, indicates the inhibition strength of pure isolates against pathogens.

DNA sequencing and species identification

The candidates that were selected as potential antagonistic microorganisms against two pathogens were used for species identification. Each candidate was cultured in 100 mL of flask containing potato dextrose broth (PDB) (Difco, USA) and incubated at 30±2°C with 250 rpm shaking for at least three to five days. The suspended cells were filtered using sterile cotton cloth and one spoon of suspended cells was crushed in cell powder by using liquid nitrogen. DNA extraction of each candidate was carried out using cetyl-trimethyl-ammonium bromide (CTAB) extraction buffer (100 mM Tris-HCl pH 8.4, 1.4 M NaCl, 25 mM EDTA, 2% CTAB), according to Yusoff *et al*. (2021). The polymerase chain reaction (PCR) was carried out for each candidate with a total product of 25 µL (16S rRNA forward primer 27F 0.5 µL, 16S rRNA reverse primer 1492R 0.5 µL, 10 mM dNTPs 0.5 µL, Dream*Taq*® DNA Polymerase 0.2 µL (ThermoScientific, USA), 5X Dream*Taq* buffer 2.5 µL, DNA template 2 µL, dH_2O 18.8 µL). The PCR was performed using a Tpersonal Thermocycler (Biometra, Germany) with amplification of one cycle of

denaturation at a temperature of 95°C for 3 min, followed by 30 cycles of denaturation (94°C, 30 sec), annealing (50°C, 30 sec), elongation (68°C, 1 min) and final elongation (68°C, 5 min). The PCR product obtained was observed through agarose gel electrophoresis and was further sequenced by Apical Scientific Sdn. Bhd. The DNA sequence obtained from each potential antagonistic microorganism was analyzed using the Basic Local Alignment Search Tool (BLAST) through Nucleotide BLAST analysis from the National Centre for Biotechnology Information (NCBI). Then, the relationship among all the candidate species was estimated using an estimated algorithm of Maximum Likelihood with a 1000 bootstrap value by including different species with distinct accession numbers from the databases (Table 2). The phylogenetic tree analysis was constructed via MEGA11 Software (Tamura *et al*., 2021).

Table 2. Accession number of each species based on 16S rRNA species identification from blast ncbi databases

Strains	Accession Number of GenBank
Kitasatospora kepongensis SUK113	KY908509.1
Kitasatospora setae S9b	KF591082.1
Kitasatospora phosalacinea	LC010672.1
Streptomyces microflavus NBRC3717	NR_041210.1
Streptomyces fulvissimus DSM40593T	NR_103987.1
Streptomyces typhae p1417	NR_180976.1
Streptomyces alboflavus NBRC3438	AB184775.1
Streptomyces morookaense Da08006	EU595362.1
Streptomyces morookaense UKM1	OQ694622.1
Streptomyces morookaense LMG20074	NR_042300.1
Streptomyces rubrisoli UKM1	OQ694767.1
Streptomyces rubrisoli FXJ1.725	NR_137264.1
Streptomyces rubrisoli FXJ1.526	KC137299.1
Streptomyces gelaticus UKM1	MT249312.1
Streptomyces gelaticus SR6-39	MN421537.1
Streptomyces gelaticus SK4-17	MN421123.1
Streptomyces griseus KACC20084	NR_042791.1
Streptomyces griseus MATT2019	MK799836.1
Bacillus subtilis SSN02	LN831326.1
Bacillus thuringiensis DNB-Bt4	AM293342.1
Bacillus mycoides S2-163	LT604365.1

Micrograph analysis using Scanning Electron Microscopy (SEM)

The interaction between candidates against each pathogen that has a high PIRG value (>70%) was examined under scanning electron microscopy (SEM) protocols, which were carried out at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Malaysia, utilizing the JSM-IT100 InTouchScope. The hyphal interactions of each pathogen in the treated samples (dual culture) were evaluated and compared with healthy mycelium (control) through SEM. The procedures were referred to Graham and Orenstein's (2007) work, in which the plug agar at the inhibition zone was divided into 1 cm^3 pieces, fixed for 4-6 hr at 4°C in 2.5% glutaraldehyde fixative, and centrifuged to separate the supernatant for each sample. The sample was then washed three times in 0.1 M sodium cacodylate buffer for 10 min between changes. Each washed sample was post-fixed with 1% osmium tetroxide for 2 hr at 4°C, and then the washing procedure was repeated. Finally, each sample was serially dehydrated using acetone in the following concentrations: 35%, 50%, 75%, and 90% for 10 min, and 100% for 15 min (three changes). The samples are put into a specimen basket for critical point drying and placed on aluminum foil (10 cm) coated with albumin. The specimen was dried in the critical drier for 1.5 hr, mounted using colloidal silver on the stub, and then covered in gold and examined under the SEM.

Statistical analysis

The t-test and two-way ANOVA analysis were conducted through GraphPad Prism ver. 9.5.1 to determine the quantitative PIRG value for all potential candidates against two pathogens *in vitro*.

RESULTS

Isolation of pure cultures from soil

There are 102 pure cultures consisting of 70 bacteria, 16 actinomycetes, and 16 fungi that have been successfully isolated with different morphology (Supplementary File A). Three of the isolates showed inhibition signs towards other organisms on PDA during the screening process, which were expected to be potential antagonistic microorganisms toward plant pathogens. Two of them are from actinomycete species (A23 and A24), which have a specific soil smell that is believed to contain particular antimicrobial secretions (Gerber, 1967). At the same time, isolate TX3, originally isolated by Yusoff *et al*. (2021) at Bangi Forest Reserve has also demonstrated potential signs as an antagonistic candidate to the other microbes. The soil depth isolated was referred from Hao *et al*. (2021) which is 10-30 cm and compared with less than 10 cm to determine the potential beneficial microorganisms and rhizospheres.

Screening of antagonistic effect by all pure cultures against *Magnaporthe oryzae* and *Fusarium solani*

All of the pure cultures were screened for the inhibitory effect against *M. oryzae* and *F. solani* corresponding to the optimum incubation period of each pathogen (Figure 2). The strains used exhibited optimum mycelial growth at 10 days for *F. solani* and 14 days for *M. oryzae* at a temperature of 28±2°C. From the formation of an inhibition zone between the 102 pure cultures against the pathogens, three actinomycete isolates, including isolate TX3, show a significant effect of inhibition (PIRG > 70%) towards *M. oryzae* and *F. solani*, while another three actinomycete and bacterial isolates display moderate inhibition with a PIRG value greater than 50% (Table 3).

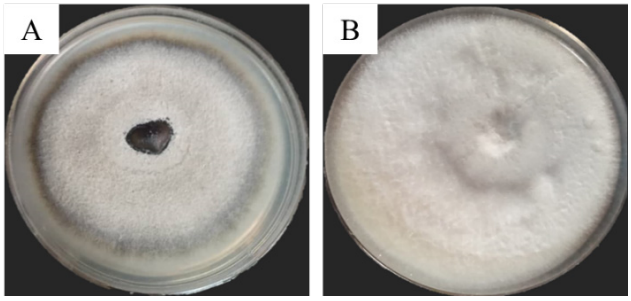

Fig. 2. The controls that represent plant pathogens of (A) *Magnaporthe oryzae* and (B) *Fusarium solani.*

Table 3. The PIRG value of isolates consists of microorganisms that are potentially inhibiting *M. oryzae* and *F. solani*

Isolate	Organism	Percentage Inhibition of Radial Growth (%)	
		Magnaporthe oryzae	*Fusarium solani*
A23	Actinobacteria	90.00	85.63
A24	Actinobacteria	78.49	87.40
TX3	Actinobacteria	88.45	72.78
A36	Bacteria	81.76	54.50
A38	Bacteria	69.59	56.80
A48	Actinobacteria	62.84	64.86

From the dual culture outcomes, it seems like the isolates from Bangi Forest Reserve, UKM (isolate A23 and A24) show the ability to inhibit the growth of two different pathogenic fungi, particularly *M. oryzae* and *F. solani*, compared to other places. The result was also supported by the inhibition effect shown by TX3, which was also obtained from the same location. Meanwhile, the isolates from oil palm plantations (isolates A36, A38 & A48) may reduce the diseases caused by these two pathogens, yet the isolates are resulting in only a moderate antagonistic effect with a PIRG value range of 50% to 69%. The isolates from the rubber plantation were not able to inhibit either one of the pathogens, according to a PIRG value of less than 50% during the screening test. Contradictory, the isolates from paddy plantations showed that only five isolates were able to inhibit either one of the pathogens, with PIRG values reaching 70% (Supplementary file). Figure 3 below displays the significant difference between the strong inhibition effect produced by A23, A24, and TX3. Figure 4 indicates the comparison between moderate and weak inhibition effects caused by other isolates against the pathogens.

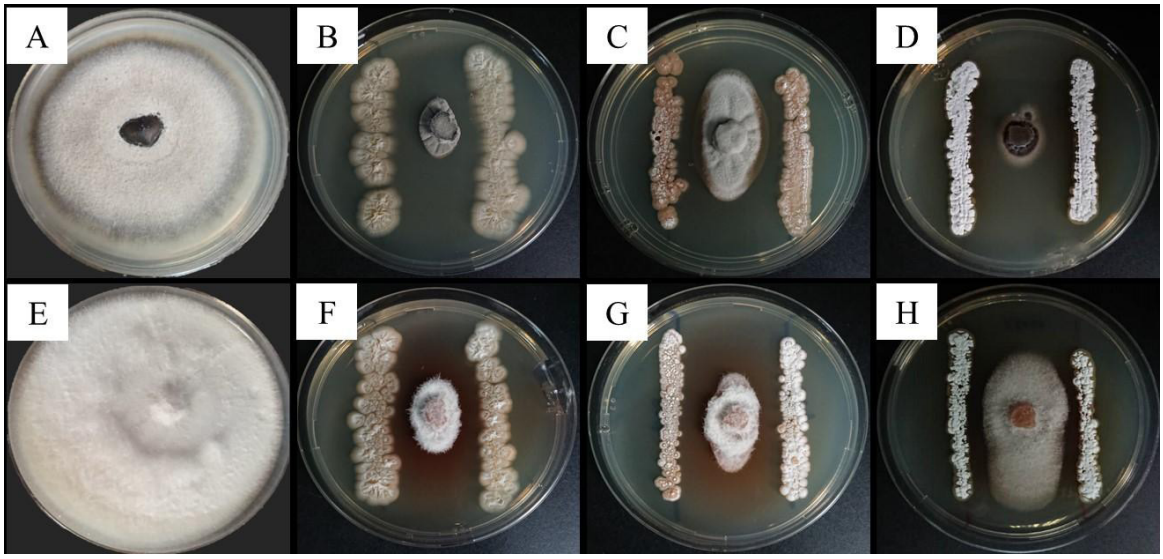

Fig. 3. Dual culture assay on PDA agar with strong candidates, where (A) is a healthy *M. oryzae* (control), and (B-D) refers to treated samples with A23, A24, and TX3, respectively. Similarly, (E) is a healthy *F. solani* (control), and (F-H) are treated samples with mentioned candidates.

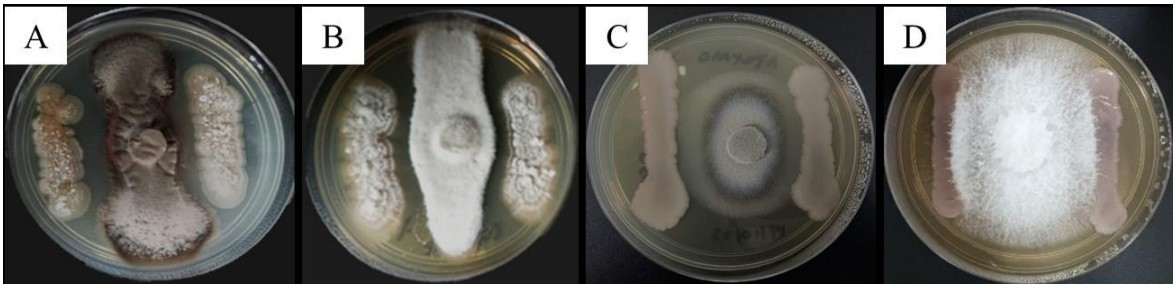

Fig. 4. Dual culture assay with moderate candidate by A48 against (A) *M. oryzae* and (B) *F. solani*, with PIRG value over 60%, and weak candidate by A57 against (C) *M. oryzae* and (D) *F. solani*, with PIRG value less than 50%.

Species identification of potential antagonists and phylogenetic analysis

Species identification of potential antagonistic microorganisms, A23 and A24, were identified as a genus of *Streptomyces*. The DNA sequencing of 16S rRNA through BLASTN (ncbi.nlm.nih.gov) showed pure culture A23 (*Streptomyces morookaense* UKM1) has significant similarity to 16S rRNA sequencing of *Streptomyces morookaense* strain Da08006 with a percentage identity of 99.07% and an e value of 0.0, while DNA sequencing of 16S rRNA pure culture A24 (*Streptomyces rubrisoli* UKM1) has significant similarity to 16S rRNA sequencing of *Streptomyces rubrisoli* strain FXJ1.725 with a percentage identity of 98.97% and an e value of 0.0. *Streptomyces gelaticus* UKM1 (TX3) has been identified as having similarity with 16S rRNA *Streptomyces gelaticus* strain SK4-17, with a percentage identity of 97.34% and an e value of 0.0. All of the identified 16S rRNAs from DNA sequencing that are from the same genus but different strains, taken from NCBI databases, were analyzed through phylogenetic analysis using a Maximum Likelihood tree with distinct strains. All of the DNA 16S rRNA genes are chosen for phylogenetic analysis based on their high percentage identity, which is more than 95%, and query coverage of more than 90%. To differentiate their lineages, two clades were divided into the phyla Actinomycete and Firmicute (Figure 5). The analysis has proved that most of the 16S rRNA of the chosen actinomycetes are closely related and may differ from *Bacillus* sp., which is phylum Firmicute.

Analysis of SEM of the antagonistic activity by strongest isolates against *M. oryzae* and *F. solani*

Analyses of scanning electron microscopy (SEM) were performed to determine the effect of antagonistic activity by three chosen candidates against the pathogens. Without being induced by any isolate(s), the hyphae of both healthy pathogens are regular in shape, long, linear, and thick (Figure 6A & 6D). However, noticeable morphological abnormalities were observed after the pathogens were cultured with the three candidates within the incubation period. The smooth hyphae were turning into

wrinkled and shortened hyphae, and some of them were lysed, as seen on the hyphae of *M. oryzae* in Figure 6B and Figure 6C. Similarly, the hyphae of infected *F. solani* in Figure 6E and Figure 6F were shown as coiled and broken hyphae. Most of the infected hyphae become dehydrated, shrink, and twitch, unlike the control.

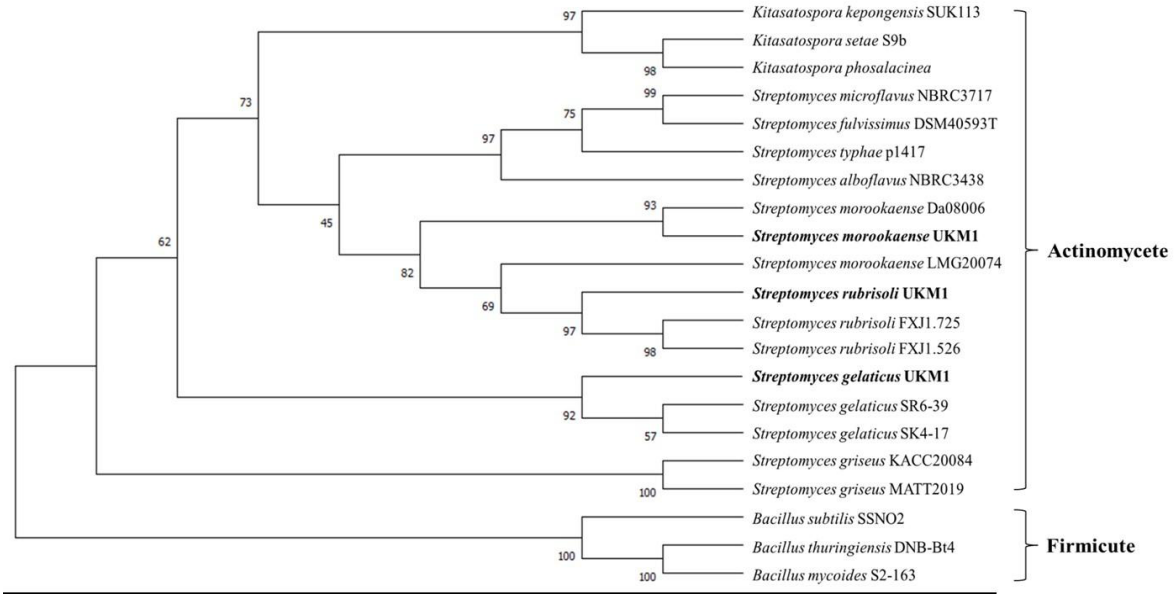

Fig. 5. Phylogenetic relationships among 16 species of the phyla Actinomycetes, which includes *Streptomyces* and *Kitasatospora,* are based on 16S rRNA gene sequencing. Three species of *Bacillus* are classified under the phylum Firmicute. The tree is analyzed using Maximum Likelihood with 1000 bootstraps.

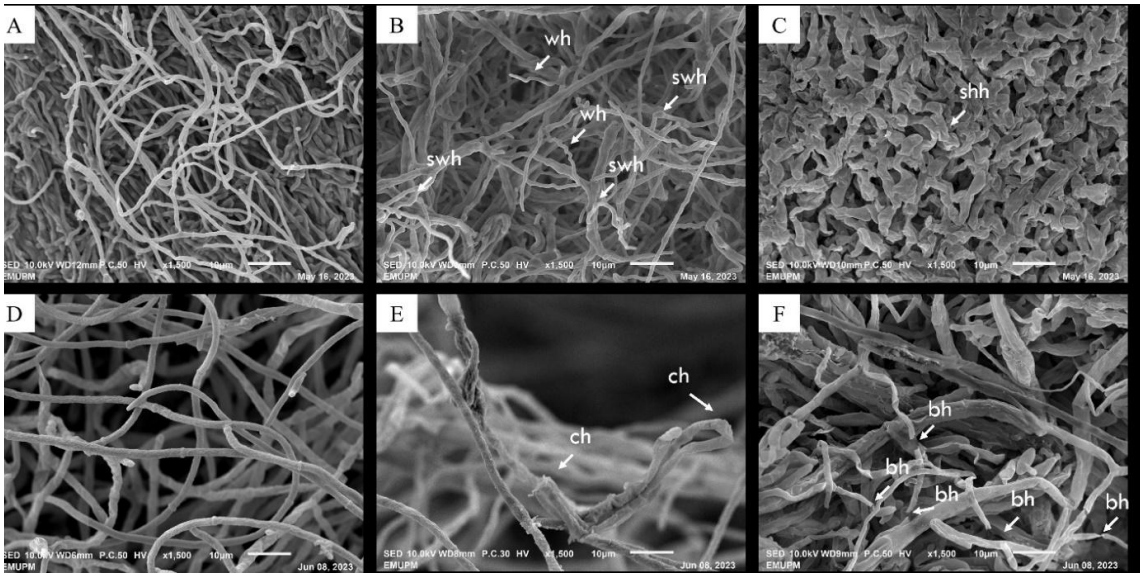

Fig. 6. The morphological mycelia structure of *M. oryzae* and *F. solani* after being grown together with *Streptomyces* sp. (A) Healthy mycelium of *M. oryzae* (control), while (B) and (C) show disrupted hyphae of *M. oryzae* such as wrinkle hyphae (wh), swollen hyphae (swh), and shorten hyphae (shh) after being infected with *S. morookaense* UKM1 and *S. gelaticus* UKM1, respectively. (D) Healthy mycelium of *F. solani* (control), while (E) and (F) display the coiled hyphae (ch) and broken hyphae (bh) of *F. solani* after being induced by *S. rubrisoli* UKM1. The magnification used in these SEM observations is 1,500X with a scale of 10 µm.

Statistical analysis of the treatment samples

The PIRG value (%) of each sample in triplicate was compared to the controls *via* t-test and the result showed there was a significant difference within the samples of each candidate (P<0.05). Two-way ANOVA with replication was conducted between the candidates as the p-value obtained was less than 0.0001. As the result showed a p-value less than 0.05, there is a significant difference between the PIRG values produced by candidates against both pathogens compared to the controls. The data

has also been supported by an F-value greater than the F_{crit} value, which is F=340.22 and F_{crit}=3.89, respectively.

DISCUSSION

Diversity of beneficial microorganisms within different locations

From the study, six out of 102 isolates are potentially inhibiting both *M. oryzae* and *F. solani*, with PIRG values greater than 50%. However, only three isolates coming from *Streptomyces* sp. can suppress pathogens by more than 70%. The result demonstrates that the most potent antagonistic microorganisms against *M. oryzae* and *F. solani* are from the actinomycete phyla, compared to bacteria or fungi. Six locations chosen for soil sampling in this study show the existence of actinomycete based on the morphologies illustrated by Li *et al*. (2016), yet the strongest isolates were found in Bangi Forest Reserve (BFR) rather than in other places. BFR is known as a green area of Universiti Kebangsaan Malaysia, which was gazetted as a UKM Permanent Reserved Forest in 1993 (Mat Salleh, 1999). It is approximately 81 ha of land reserved for ecological research purposes and has more than 600 species of flora and fauna. The samples were obtained from six different locations in BFR, including the exposed surface, nearby dead plants, and nearby the roots of healthy trees. In general, a variety of good microorganisms are prone to grow near plants to have a symbiotic interaction with each other, such as nitrogen fixation by the bacteria to plants and carbon sources as a nutrient from plants to bacteria. Their relationship plays a vital role in biogeochemical cycles and ecosystem stability, especially in disturbance conditions (Aqeel *et al*., 2023). Not only that, forests rich with decomposers or saprophytes are responsible for degrading dead plants or dead animals into small fragments before being reused by other plants as nutrient sources (Lopez-Mondejar *et al*., 2018). They tend to be found in fragmented forests like BFR that consider naturality and low toxin content (Tee *et al*., 2018; Yaakop & Aman, 2013). Unlike farmland areas such as rubber plantations in Durian Tunggal, Melaka, oil palm plantations in Jenderam Hilir, Selangor, or even paddy plantations in Pahang and Negeri Sembilan, the sterility of soil conditions might be lower compared to primary forests due to soil degradation and pollution (Panday *et al*., 2019; Aditya *et al*., 2021). The overuse of chemical pesticides or fertilizers was reported to have a bad impact on the population of good microorganisms in the soil while increasing the plant pathogen's resistance, which contributes to ecosystem instability and weakens plant immunity over a long period (Peng *et al*., 2021; Singh *et al*., 2023).

Antagonistic effects on fungal hyphae

Streptomyces morookaense UKM1 (A23), *S. rubrisoli* UKM1 (A24), and *S. gelaticus* UKM1 (TX3) show a significant inhibition effect based on the PIRG value towards *M. oryzae* and *F. solani*, where *S. morookaense* UKM1 is the strongest. *S. morookaense* UKM1 seems to have a heavy and hard feature, as it is strongly pasted on the surface of potato dextrose agar (PDA). It produces soil odor as well as *S. gelaticus* UKM1, which might be their kind of secondary metabolite. Previous research has found that the strong smell of soil mostly comes from *Streptomyces*, as they contain a certain compound called geosmin, which acts as protection for *Streptomyces* from predators like fruit flies as well as their catalyst to spread the spores through spore-coated animals such as ants and mosquitoes (Gerber, 1967; Becher *et al*., 2020). *S. morookaense* UKM1, *S. rubrisoli* UKM1, and *S. gelaticus* UKM1 produced certain bioactive compounds during the interaction with *M. oryzae* and *F. solani*, since they showed some inhibition effects through the morphological abnormalities in the mycelium formation of the pathogens. The morphology micrographs in the experiment have revealed significant changes in the pathogens hyphae cultured with either three *Streptomyces*, including wrinkle, twitch, coil, lysis, and broken, compared to the controls (healthy pathogen). Before the microscopic observations, the gross morphology on the dual culture plate also gave indicators of inhibition effects, such as the empty zone between pathogens and bacteria, lifted pathogenic mycelium, and color-changing on the agar, which support the presence of potential compounds either from *Streptomyces* or pathogens. In general, all the microorganisms in the population secrete their secondary metabolites to survive; they release the toxins for food or surface competition, adaptability, stress response, and symbiotic or parasitic interaction (Kohl *et al*., 2019). To date, the study by Pacios-Michelena *et al*. (2021) has discovered that the secondary metabolite secreted by certain microorganisms may alter and induce changes in the gene expression of nearby microorganisms.

CONCLUSION

In conclusion, three potential actinomycetes that have significant antagonistic activity against the causative agent of rice blast disease, *M. oryzae,* and the causative agent of *Fusarium* wilt, *F. solani,*

were successfully isolated from soil samples in the Bangi Forest Reserve. *In vitro* analyses have shown that *S. morookaense* UKM1, *S. rubrisoli* UKM1, and *S. gelaticus* UKM1 showed antagonistic activity through an inhibition zone in a dual culture assay with a PIRG value greater than 70%. Therefore, we proposed that these three actinomycetes are good candidates to be used as biological control agents against *M. oryzae* and *F. solani*.

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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