Research Article

Biocontrol Potential of *Paenibacillus* sp. and *Bacillus thuringiensis* Against *Fusarium proliferatum* in Melon Manis Terengganu

Sarina Mat Rosid[1], Siti Nurul Aini Abdul Rahman[1], Noor Afiza Badaluddin[1,*], Mariana Mohammad[1], Mohammad Moneruzzaman khandaker[1] and Mohd Eeyad Arief Mohd Nor Asri[2]

[1]School of Agricultural Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia; [2]Tembila Agrotech Resources, Agropreneur Incubator Park UniSZA, Besut Campus, 22200 Besut, Terengganu, Malaysia.

Abstract | *Cucumis melo* var. Inodorus cv. Manis Terengganu 1 or Melon Manis Terengganu (MMT), is an iconic fruit widely cultivated in Terengganu, Malaysia. However, over-reliance on chemical pesticides and synthetic fertilizers has degraded soil fertility, posed health risks, and increased environmental pollution. As a new variety, research on its common diseases is limited. Due to that, effective microbes (EM), such as *Paenibacillus* sp. and *Bacillus thuringiensis*, offer environmentally friendly alternatives for disease control. The objectives of this study were to identify the cause of MMT disease and to evaluate the potential of EMs as biological control agents. *Paenibacillus* sp. and *B. thuringiensis* were successfully identified from soil culture collection from Universiti Sultan Zainal Abidin (UniSZA). From Koch's postulates test, it showed that severe infections occurred on MMT leaves and stems as early as day 3 with 100% incidence. Dual culture assays revealed that *Paenibacillus* sp. achieved the highest inhibition with 61.1% followed by *B. thuringiensis* (51.8%) and consortium (47.5%). This is similar with EM testing that demonstrated strong antagonistic activity against *Fusarium proliferatum*, with single strains being more effective than consortium. Furthermore, EMs also exhibit plant growth promoted properties with *Paenibacillus* sp. significantly improved root growth (p= 0.004). These results highlight the potential of EM as biological control agents and plant growth promoters, contributing to sustainable MMT cultivation.

Received | July 29 2025; **Accepted** | Sep 5, 2025; Published | December 08, 2025
***Correspondence** | Noor Afiza Badaluddin, University Sultan Zainal Abidin, Malaysia. **Email:** noorafiza@unisza.edu.my
Citation | Rosid, S.M., S.N.A.A. Rahman, N.A. Badaluddin, M. Mohammad, M.M. Khandaker and M.E.A.M.N. Asri. 2025. Biocontrol potential of *Paenibacillus* sp. and *Bacillus thuringiensis* against *Fusarium proliferatum* in melon manis terengganu. Sarhad Jurnal of Agriculture, 41(5): 24-33. DOI | https://dx.doi.org/10.17582/journal.sja/2025/41.5.24.33
Keywords | Biological control agent, Disease, Effective microbes, Melon manis terengganu, Pathogens.

Introduction

Cucumis melo var. Inodorus cv. Manis Terengganu 1, commonly known as Melon Manis Terengganu (MMT), is a new variety of *Cucumis melo L.* cultivated mainly in Terengganu, Malaysia. Since 2014, melon production in Malaysia has been relatively high, yet it remains insufficient to meet increasing demand, particularly in Selangor and Perak. Globally, melons are widely cultivated, with China, Iran, and Turkey leading production. In Malaysia, watermelon, rockmelon, and honeydew are the most planted melon types, with

a total production of 220,226 metric tons in 2014. However, factors like adverse weather conditions and pest and disease infestations significantly challenge melon producers, causing substantial losses. In response, chemical pesticides are often heavily relied upon, contributing to environmental degradation. To address these issues, alternative approaches to disease control are essential. Exploring environment friendly solutions, such as biological control agents and sustainable agricultural practices, can help reduce reliance on chemical pesticides and support the growing demand for melons in Malaysia.

Effective microbes (EM), or beneficial microbes, are introduced to crops for biological disease control and play multiple roles in the soil-plant ecosystem. EMs enhance plant resistance by priming defense mechanisms without directly interacting with pathogens. They suppress plant diseases, balance soil microbes, solubilize soil minerals, preserve resources, and boost photosynthesis and nitrogen fixation (Olle and Williams, 2013). Common biological control agents include rhizobacteria and mycorrhizal fungi, which help plants resist pathogens by influencing their chemical and metabolic reactions. This reduces the risk of crop loss, providing a sustainable alternative to chemical pesticides. By improving soil health and plant resilience, EMs contribute to environmentally friendly agricultural practices and promote long-term productivity for farmers.

Fusarium proliferatum is a destructive phytopathogen that poses a serious threat to agricultural productivity by causing growth retardation and considerable yield reduction in many economically important crops (Ekwomada and Mwanza, 2023; Hof and Schrecker, 2024). This fungus is capable of infecting plants systemically, disrupting water and nutrient transport, and ultimately leading to chlorosis, slow growth, and premature wilting. Its high pathogenicity is mainly attributed to aggressive tissue colonization and the secretion of mycotoxins, which compromise host defense mechanisms and accelerate disease progression (Ismaiel and Papenbrock, 2015). In melons, *F. proliferatum* is especially harmful, as it induces severe rotting of leaves, stems, and flowers. These infections manifest as necrotic lesions, stem tissue collapse, and impaired photosynthetic activity, all of which compromise plant vigor and fruit development (Seo and Kim, 2017; Yu *et al*., 2022). Beyond reducing fruit quality, the pathogen also causes substantial yield loss, making it a critical barrier to sustainable melon cultivation. Addressing *F. proliferatum* infections therefore requires innovative and eco-friendly management strategies to safeguard both crop productivity and quality.

EMs suppress plant pathogens through antagonistic reactions. Efficient antagonists produce antimicrobial metabolites in micro-niches at concentrations high enough to outcompete rivals or low enough to perform other functions, such as signaling or nutrient metabolism (Köhl *et al*., 2019). Common mechanisms for microbial antagonism include metabolite synthesis, competition, and direct parasitism. Other factors, such as induced resistance, often reduce pathogen enzyme activity (Alsohiby *et al*., 2016). This study evaluates the potential of selected EMs (*Paenibacillus* sp. and *Bacillus thuringiensis*) as biological controls for pathogenic fungi on Melon Manis Terengganu (MMT). The findings aim to contribute to sustainable disease management in Malaysia's melon industry, offering an eco-friendly alternative to chemical pesticides and supporting long-term agricultural productivity.

Materials and Methods

This study comprises three phases, where the first phase involves characterization of selected Ems using morphological and molecular approaches. For the second phase, the causal agents of MMT diseases were identified and the third phase involves the evaluation of potential EMs as biological control agents against pathogenic fungi.

Materials

This study utilized bacterial isolations from the soil bacteria stock culture collection of the Faculty of Bioresources and Food Industry (FBIM), UniSZA. The selected bacteria were *B. thuringiensis* (T2) and *Paenibacillus* sp. (T1). Both isolates were streaked on Nutrient Agar (NA) plates and incubated at 28 °C for 24 hours (Kim *et al*., 2016). The fungal pathogen used in the experiment was *Fusarium proliferatum*, obtained from the fungal stock culture of Mekar Greentech Solutions, where it was previously identified as a suspected pathogenic fungus. The fungus was subcultured on Potato Dextrose Agar (PDA) and incubated at 25 °C for seven days (Mohammad *et al*., 2013). These experiments have been done at FBIM, UniSZA campus Besut for 4 months.

The plant material used in this study was leaves part of Melon Manis Terengganu (MMT). The MMT leaves were obtained from Mekar Greentech Solutions. For planting preparation, MMT seeds were soaked in water overnight before being sown in seedling trays filled with potting soil. Germination took place in a greenhouse, and after 10 days, the seedlings were transplanted into polybags containing a 1:1 mixture of topsoil and cocopeat. After 60 days, the plants were ready for harvest. A total of 200 leaves were picked randomly and utilised to assess the potential of *B. thuringiensis* and *Paenibacillus* sp. as biological control agents against *F. proliferatum* on MMT.

Characterization of selected effective microorganism (EM)

Gram staining

A thin bacterial colony layer was stained on a slide, which was then heat-fixed by briefly passing it over a flame. Following Coico (2005), the staining process began with the smear being covered with crystal violet for 30 seconds, then rinsed gently with water. Iodine was applied for one minute to form a crystal violet-iodine complex (VIC) and washed off. Next, a decolorizer was slowly added to remove the purple color until the slide appeared clear. Safranin was applied as a counterstain for one minute. The stained bacteria's morphology and color were then observed under a 100× oil immersion microscope, providing detailed structural insights.

Biochemical tests

Five tests were conducted in this study to characterize the selected bacteria. The oxidase test determined the presence of cytochrome C by placing a bacterial colony on filter paper and adding a drop of 1% oxidase reagent. For the catalase test, bacterial colonies were placed on a clean glass slide, followed by two drops of 3% hydrogen peroxide, with immediate bubble formation indicating catalase activity (Hemraj *et al.*, 2013). Starch hydrolysis was assessed using the amylase test, while the Triple Sugar Iron (TSI) test examined carbohydrate fermentation, H_2S production, and gas emission. In the TSI test, bacterial colonies were inoculated into TSI agar using a wire loop and incubated at 37 °C for 24 hours. Carbohydrate fermentation was detected by a color change of phenol red (pH indicator) from red to yellow, and H_2S production was indicated by blackening at the agar's base. Lastly, the SIM (sulfur, indole, motility) test evaluated bacterial motility and indole production. An isolated colony was inoculated into SIM medium, and turbidity around the inoculation site indicated motility. For the indole assay, three to five drops of Kovacs reagent were added to the agar slant after incubation, confirming indole production by a red color at the medium's surface (Badaluddin *et al.*, 2020).

Environmental impact analysis

The environmental impact analysis assessed the optimum bacterial growth under favorable conditions. Table 1 presents the parameters evaluated during the analysis.

Table 1: *Environmental effect test*

Test	Temperature (°C)	pH	Salinity (%) (NaCl)
Condition (temperature, pH, and salinity levels)	10, 25, 30, 37, 45, 50, and 60	Control, 4, 5, 6, 7, 8, and 9	Control, 2.5, 5.0, 7.5, and 12.5

Identification of selected EMs using a molecular approach

DNA extraction

DNA extraction from *Paenibacillus* sp. and *B. thuringiensis* was performed using the Wizard® Genomic DNA Purification Kit with lysozyme modification. Bacterial colonies were cultured overnight in nutrient broth at 30 °C. A 1 mL aliquot was transferred to a 1.5 mL microcentrifuge tube, centrifuged at 13,400 rpm for 2 minutes, and the supernatant discarded. The pellet was resuspended in 480 µL of 50 mM EDTA, followed by 60 µL of lysozyme and incubated at 37 °C for 30 minutes. After centrifugation, the supernatant was discarded, and 600 µL of Nuclei Lysis solution was added, resuspending the cells and incubating at 80 °C for 5 minutes. After cooling, 3 µL of RNase solution was added, and the sample incubated at 37 °C for 30 minutes. Next, 200 µL of protein precipitation solution was added, mixed, and cooled on ice for 5 minutes. After centrifugation, the DNA-containing lysate was mixed with 600 µL isopropanol, inverted to form DNA strands, and centrifuged. The pellet was washed with 600 µL of 70% ethanol, centrifuged, air-dried, and rehydrated with 20 µL DNA rehydration solution at 65 °C for 1 hour. The DNA was stored at 4 °C (Sajili *et al.*, 2018).

Polymerase chain reaction (PCR)

PCR amplification was conducted using 16S primer pairs: 16SF2 (5' - GAG TTT GAT CCT GGC

TCA - 3') as the forward primer and 16SR2 (5' - ACG GCT AAC TTG TTA CGA - 3') as the reverse primer. The reaction was performed in a 50 µL volume containing 25 µL ExTEN master mix, 1 µL DNA template, 20 µL deionized distilled water, and 2 µL of each primer. The PCR protocol included initial denaturation at 95 °C for 2 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 1 minute, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 10 minutes. The amplified products were separated using 1.5% agarose gel electrophoresis, with a 1 kb DNA ladder as a marker (Sajili *et al*., 2018).

DNA sequencing and BLAST analysis

Purified PCR products (EMs samples) were sent to 1st BASE DNA Sequencing Services for sequencing. Sequences were aligned using BLAST and compared with bacterial sequences from the NCBI database (Jamali *et al*., 2020).

Koch's postulates

Fungi sample was harvested by adding 15 mL of sterilized distilled water (dH_2O) to agar plates with matured fungi. The spores and water were mixed using a cell distribution stick and transferred to a sprayer. Koch's postulates were conducted to evaluate the effect of pathogenic fungi on MMT leaves. Fungal isolates and a water-only control were prepared as suspensions. Fresh MMT plants (three replicates) were placed in water containers and sprayed with the fungal suspensions on leaves, stems, and buds. Plants were maintained at 30 °C for seven days, and disease incidence (%) was calculated using Equation 1.

$$\frac{\text{Number of infected plants}}{\text{Total number of plant}} \times 100\%$$

Evaluation of EMs as biological control agents against Fusarium proliferatum on MMT

Dual-culture assay

A 0.5 cm disc of *F. proliferatum* culture was placed 2.0 cm from the right side of PDA agar plates. Bacterial strains were then spread 2.5 cm away on the left side. Four treatment types were tested: control (C), *F. proliferatum* with *Paenibacillus* sp. (T1), *F. proliferatum* with *B. thuringiensis* (T2), and *F. proliferatum* with the bacterial consortium (T1+T2), each with three replicates. *F. proliferatum* radial growth was measured and compared to the control after 7 days at 25 °C (Mohammad *et al*., 2013). The growth of fungi versus bacteria was assessed daily, and the highest inhibition zone was used to determine the most effective biological control agent using Equation 2.

$$\%\ \text{inhibition zone} = \frac{RC-RD}{RC} \times 100\%$$

where;
RC= radius growth of control fungi
RD= radius growth of fungi in dual-culture assay

EMs tests on MMT leaves

The antagonistic activity of *Paenibacillus* sp. (T1) and *B. thuringiensis* (T2) was tested against *F. proliferatum* on MMT leaves. Five treatments were applied: control, *F. proliferatum* only, *F. proliferatum* + T1, *F. proliferatum* + T2, and *F. proliferatum* + T1T2 (consortium), with five replicates per treatment. MMT leaves and stems were sprayed with respective fungi to initiate inoculation. Subsequently, EMs suspension was sprayed to assess antagonistic activity. Disease symptoms and root length were monitored over two weeks.

Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) at a 0.05 significance level. Relationships were determined using the latest SPSS software version 17 for data analysis.

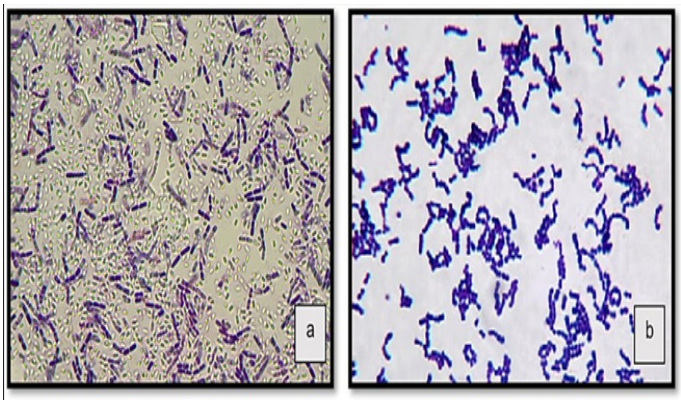

Figure 1: *Gram-staining morphology of (a) Paenibacillus sp., T1 and (b) B. thuringiensis, T2 bacteria*

Results and Discussion

Gram staining

From Figure 1, both *Paenibacillus* sp. (T1) and *B. thuringiensis* (T2) were identified as Gram-positive bacteria due to their purple-stained, rod-shaped colonies, unaffected by safranin pink color. Gram-

positive bacteria retain crystal violet, whereas Gram-negative bacteria turn pink. This test helps classify bacteria based on their structural characteristics (Smith and Hussey, 2005).

Table 2: *The summary of Gram-staining and biochemical tests*

Bacteria	*Paenibacillus* sp.	*B. thuringiensis*
Shape	Rod-shaped	Rod-shaped
Gram-stain	+	+
Oxidase	+	+
Catalase	+	+
Amylase	+	+
Fermentation of glucose	-	-
Motility	+	+
Indole production	-	-
H_2S production	-	-

+ indicates positive, - indicates negative

Biochemical tests

As shown in Table 2, in the oxidase test, both T1 and T2 were oxidase-positive, as their bacterial colonies turned purple when treated with a 1% oxidase reagent. The test detects the presence of cytochrome oxidase, an enzyme that catalyzes cytochrome c in the electron transport chain (Wanger *et al.*, 2017). Cytochrome is a catalytic enzyme tightly bound to the plasma membrane in prokaryotic cells. The presence of this enzyme indicates that the bacteria are aerobic, as the electron transport chain is a key step in aerobic respiration. Meanwhile, in the catalase test, both T1 and T2 were positive for catalase activity. Catalase is a heme-containing enzyme found in most aerobic organisms, responsible for decomposing hydrogen peroxide (H_2O_2) into water and oxygen (Karakus, 2020). When the bacterial colonies were exposed to 3% H_2O_2, bubbles formed immediately, indicating decomposition. In aerobic bacteria, oxygen oxidizes redox enzymes, generating H_2O_2, a reactive oxygen species (ROS) that can potentially damage cells.

Furthermore, amylase production was detected in the amylase test, where α-amylase hydrolyzes starch into glucose. Starch, a complex carbohydrate, cannot pass the cell membrane, whereas glucose, a simple carbohydrate, can enter the cell cytoplasm (Hemraj *et al.*, 2013). Both T1 and T2 were positive for amylase production, indicating their ability to hydrolyze starch. Based on the results (Table 2), both T1 and T2 were unable to ferment sugar, as indicated by the red color remaining in the slant and butt after overnight incubation. No H_2S production was observed, as there was no blackening in the medium, and no gas production was noted, as there was no cracking in the medium. Therefore, T1 and T2 do not ferment sugar, produce H_2S, or produce gas. The SIM test was conducted to assess sulfur production, motility, and indole formation (Warren *et al.*, 2005). If sulfur is released as H_2S, it blackens the medium. In this study, both bacteria did not produce H_2S. For motility, bacteria extending from the streaked line indicates movement. Indole production was tested by detecting tryptophan breakdown in the SIM medium, but no positive result for indole formation was observed (Cooper, 2019).

Optimum growth condition

Both bacteria were cultured in different salinity, pH, and temperature conditions to determine their optimal growth environments. As shown in Table 3, both T1 (*Paenibacillus* sp.) and T2 (*B. thuringiensis*) grew well in a 2.5% NaCl solution. In a 5.0% NaCl culture, T1 grew well, while only a few colonies of T2 were present. At 7.5% and 12.5% NaCl, no bacterial colonies were observed. This suggests that T1 is more salt-tolerant, as supported by Sukweenadhi *et al.* (2018). Regarding pH, both bacteria grew at pH 6, 7, 8, and 9, but not at pH 4 and 5. T1's optimal pH is between 7 and 8 (Patowary and Deka, 2020), while T2 grows best at pH 8 (Sidorova *et al.*, 2020). For temperature, both bacteria grew well at 25°C, 30°C, and 37°C. At 45°C, only T1 showed minimal growth, while T2 failed to grow. Neither bacteria grew at 50°C and 60°C. *Paenibacillus* sp. thrives best at temperatures

Table 3: *The environmental impact analysis of both bacteria*

Bacteria	Environmental impact analysis																
	Salinity (% NaCl)				pH						Temperature (°C)						
	2.5	5.0	7.5	12.5	4	5	6	7	8	9	10	25	30	37	45	50	60
T1	+	+	-	-	-	-	±	+	+	±	-	+	+	+	±	-	-
T2	+	±	-	-	-	-	+	+	+	+	-	+	+	+	-	-	-

+ indicates positive, - indicates negative, ± indicates small colony

between 25°C and 45°C (Patowary and Deka, 2020), while *B. thuringiensis* prefers temperatures between 25°C and 39°C (Cahya *et al.*, 2019).

Molecular identification of EMs

DNA extraction and BLAST alignment identified T1 as *Paenibacillus* sp. (97% similarity) and T2 as *B. thuringiensis* (98% similarity), confirmed by comparison with bacterial sequences in the NCBI database. This process validated the identities of both bacterial strains.

Figure 2: *Disease symptoms appeared on MMT leaves and stem parts after seven days. (A) treatment with Fusarium proliferatum (stem) and (B) treatment with Fusarium proliferatum (leaves),*

Table 4: *The day of symptom appearance and disease incidence caused by Fusarium proliferatum*

Suspected pathogenic fungus	Symptom appearance (Day)	Disease incidence (%)
Control	-	0
Fusarium proliferatum	3	100

Koch's postulates

Koch's Postulates were applied to MMT leaves to evaluate the pathogenicity of fungal infections. Disease symptoms were observed within seven days of fungal inoculation, as shown in Figure 2. Disease severity was measured by examining the affected host tissue or organ. Table 4 reveals that severe infections occurred on MMT leaves as early as day 3. Fungal infections produce proteinaceous effector genes and non-proteinaceous effectors (NPE), such as short RNA, to target and suppress the host defense system, facilitating colonization (Jaswal *et al.*, 2020).

Dual-culture assay

The dual culture assay assessed the impact of effective microorganisms (EMs) on the mycelial radial growth of pathogenic fungi (Pellan *et al.*, 2020). The treatments were tested as follows: 1) *Paenibacillus* sp., 2) *B. thuringiensis*, and 3) a consortium (*Paenibacillus* sp. + *B. thuringiensis*). Table 5 shows *Paenibacillus* sp. achieved the highest inhibition of *F. proliferatum* mycelial growth after seven days (61.1%) followed by the *B. thuringiensis* and consortium. This inhibition highlights the antagonistic potential of *Paenibacillus* sp., attributed to hydrolytic enzymes like chitinase, β-1,3-glucanase, siderophores, and fusaricidins, which degrade fungal cell walls, causing cytoplasmic leakage and growth inhibition (Ali *et al.*, 2021). These results establish *Paenibacillus* sp. as a promising EM for controlling fungal infections in MMT by leveraging its broad-spectrum antifungal activity (Hasim and Coleman, 2019).

Table 5: *Percentage inhibition by EMs treatments against Fusarium proliferatum after seven days*

Type of EMs treatment	Percentage inhibition (%)
Paenibacillus sp	61.1
B. thuringiensis	51.8
consortium (*Paenibacillus* sp. + *B. thuringiensis*)	47.5

On the other hand, T2 (*B. thuringiensis*) inhibited *F. proliferatum* mycelial growth by 51.8%, attributed to its production of volatile organic compounds (VOCs) like 2,3,6-trimethylphenol, nonan-2-one, decan-2-one, and 2-methyl pyrazine, which enhance plant resistance to fungal pathogens (He *et al.*, 2020). Meanwhile, the T1+T2 consortium only inhibited mycelial growth of *F. proliferatum* by 47.5%.

EMs test on MMT leaves

F. proliferatum was subjected to three treatments: *Paenibacillus* sp., *B. thuringiensis*, and a consortium of both. As illustrated in Figure 3, single strains of *Paenibacillus* sp. and *B. thuringiensis* demonstrated stronger antagonistic effects against *F. proliferatum* than the consortium. The consortium treatment in EMs tests proved less effective, leading to severe leaf infection symptoms such as lesions and anthracnose. This reduced efficacy may be attributed to nutrient limitations in the EMs test, which likely triggered competition between the bacteria. Antagonistic interactions often involve competition for nutrients and adhesion sites, the production of toxic metabolites, and antimicrobial substances like bacteriocins (Júnior *et al.*, 2011). These findings

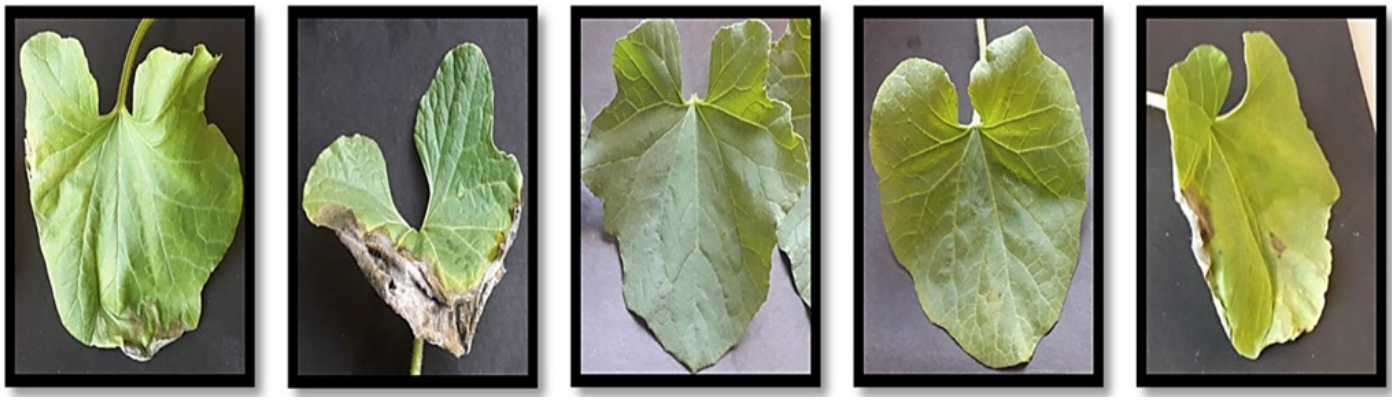

Figure 3: *EMs test on MMT leaves against fusarium proliferatum. The sample indicates (A) control, (B) Fusarium proliferatum, (C) treatment of Paenibacillus sp., (D) treatment of B. thuringiensis and (E) treatment of consortium against fusarium proliferatum*

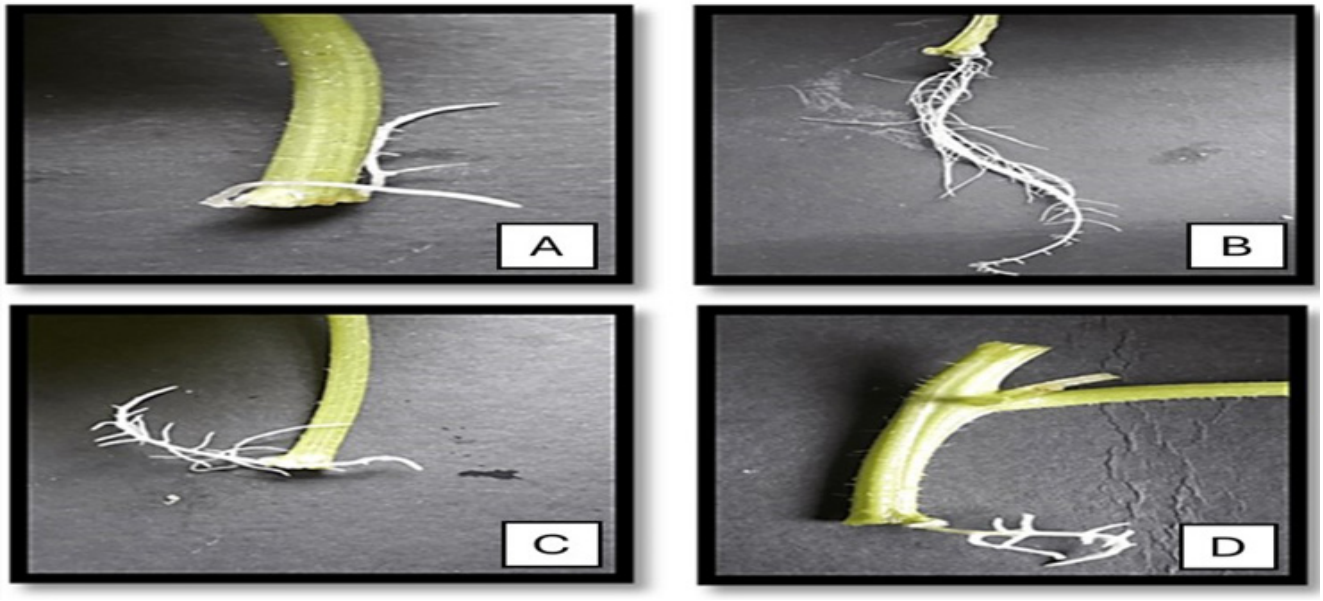

Figure 4: *Root length of MMT by EMs treatment against fusarium proliferatum after two weeks. The sample indicates (A) control, (B) Paenibacillus sp. (C) consortium against fusarium proliferatum and (D) B. thuringiensis*

highlight the superior efficacy of individual bacterial strains as biological control agents, underscoring their potential in managing *F. proliferatum* infections in MMT cultivation.

Potential of EMs as a plant growth promoter

Paenibacillus sp. and *B. thuringiensis* also exhibit plant growth-promoting properties. Figure 4 shows the root growth of MMT treated with EMs against *F. proliferatum* after two weeks. The treatments with *Paenibacillus* sp., *B. thuringiensis* and consortium resulted in significant root elongation compared to the control, highlighting their role in enhancing root development.

The root length of MMT was measured and compared after two weeks. One-way ANOVA analysis revealed significant differences in root length among five treatments against *F. proliferatum* ($F(4,20) = 6.145$, $p = 0.000$). The post hoc Tukey HSD test showed that *Paenibacillus* sp. treatment significantly improved root growth against *F. proliferatum* ($p = 0.004$). *Paenibacillus* sp. promotes root growth by producing indole acetic acid (IAA), a phytohormone of the auxin class, which is involved in nitrogen fixation and phosphate solubilization (Patowary and Deka, 2020). Auxin enhances root elongation, aiding MMT in water and mineral absorption. Roots play a vital role in plant systems, not only absorbing water through root hairs and the epidermis but also supporting mineral uptake and structural stability. This highlights the importance of *Paenibacillus* sp. in fostering healthier root systems and overall plant resilience.

Conclusions and Recommendations

In conclusion, the potential of *Paenibacillus* sp. and *B. thuringiensis* as biological control agents against *F. proliferatum* on Melon Manis Terengganu (MMT) were evaluated. The bacteria were identified and characterized through morphological and molecular approaches. Environmental tests revealed their optimal conditions: salinity at 2.5%, pH levels of 7 and 8, and temperatures of 25, 30, and 37 °C. The antagonistic activities of *Paenibacillus* sp. and *B. thuringiensis* were assessed through dual culture assays and EM tests on MMT leaves. While both strains were effective individually, their consortium showed reduced efficacy. Despite this, *Paenibacillus* sp. and *B. thuringiensis* remain promising biological control agents against pathogenic fungi on MMT, offering potential for sustainable disease management in agricultural practices.

Acknowledgements

The author would like to thank the laboratory staff of Faculty of Bioresources and Food Industry UniSZA for their technical help and assistance throughout this study. This work was supported by Dana Penyelidikan Universiti (DPU) 2.0 by Universiti Sultan Zainal Abidin (UniSZA/2022/DPU2.0/07) (R0406).

Novelty Statement

The novelty of this manuscript is the evaluation of effective microbes, specifically *Paenibacillus* sp. and *Bacillus thuringiensis*, as biological control agents against *Fusarium proliferatum*, a pathogenic fungus affecting *Cucumis melo* var. *Inodorus cv.* Manis Terengganu (Melon Manis Terengganu). As a newly developed melon variety in Malaysia, research on its associated diseases and eco-friendly management strategies remains limited.

Author's Contribution

Sarina Mat Rosid: Wrote and editing the paper.
Siti Nurul Aini Abdul Rahman: Wrote the first draft of the manuscript, performed the experiment and analysed the data.
Noor Afiza Badaluddin: Designed the experimentation and supervised the experiment.
Mariana Mohammad: Analysed the data.
Mohammad Moneruzzaman Khandaker: Coordinated the experiment and analysed the data.
Mohd Eeyad Arief Mohd Nor Asri: Contributed analytic tools. All authors read and approved the final manuscript.

Generative AI or AI assisted technology statement
The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in this manuscript.

Conflicts of Interest
The authors declare that there is no conflict of interests regarding the publication of this article".

References

Alsohiby, F.A.A., S. Yahya and A.A. Humaid. 2016. Screening of soil isolates of bacteria for antagonistic activity against plant pathogenic fungi. PSM Microbiol., 1(1): 5–9.

Ali, M.A., Y. Lou, R. Hafeez, X. Li, A. Hossain, T. Xie, L. Lin, B. Li, Y. Yin, J. Yan and Q. An. 2021. Functional analysis and genome mining reveal high potential of biocontrol and plant growth promotion in nodule-inhabiting bacteria within Paenibacillus polymyxa complex. Front. Microbiol., 11: 1–16. https://doi.org/10.3389/fmicb.2020.618601

Badaluddin, N.A., S.Y. Yong, R. Umar, N.H. Sabri, N.S. Hasshim, M.K. Ali, A.R. Mohamed. 2020. Isolation and characterization of high embient electromagnetic radiation (EMR) bacteria. Malays. Appl. Biol., 49(4): 165-172. https://doi.org/10.55230/mabjournal.v49i4.1608

Cahya, V.A., C. Hanim, L.M. Yusiati, Z. Bachruddin and Y. Erwanto. 2019. Growth optimization of *Bacillus subtilis* 11A isolated from Indonesian native chicken (*Gallus domesticus*) for bacteriocin production. IOP Conf. Ser.: Earth Environ. Sci., 387(1): 9–14. https://doi.org/10.1088/1755-1315/387/1/012013

Coico, R. 2005. Gram staining basic protocol commonly used techniques. Curr. Protoc. Microbio., 3–4. https://doi.org/10.1002/9780471729259.mca03cs00

Cooper, C.R. 2019. Sulfide-Indole-Motility (SIM) Test. Microbiol. Laborat. (BIOL 3702L): 1–5. https://doi.org/10.3390/agriculture13091810

Ekwomadu, T.I. and M. Mwanza. 2023. *Fusarium*

fungi pathogens, identification, adverse effects, disease management and global food security: A review of the latest research. Agric., 13(1810): 1-20. https://doi.org/10.4155/fmc-2018-0465

Hasim, S. and J.J. Coleman. 2019. Targeting the fungal cell wall: Current therapies and implications for development of alternative antifungal agents. Future Med. Chem., 11(8): 869–883. https://doi.org/10.3390/molecules25153360

He, C.N., W.Q. Ye, Y.Y. Zhu and W.W. Zhou. 2020. Antifungal activity of volatile organic compounds produced by *Bacillus methylotrophicus* and *Bacillus thuringiensis* against five common spoilage fungi on loquats. Molecul., 25(15): 3360.

Hemraj, V., S. Diksha and G. Avneet. 2013. A review on commonly used biochemical test for bacteria. Innov. J. Life Sci., 1(1): 1–7.

Hof, H. and J. Schrecker. 2024. *Fusarium* spp.: infections and intoxications. GMS Infec. Dis., 12: 1-8.

Ismaiel, A.A. and J. Papenbrock. 2015. Mycotoxins: Producing fungi and mechanism of phytotoxicity. Agric., 5: 492-537. https://doi.org/10.3390/agriculture5030492

Jamali, S.A.M., N.A. Badaluddin, S.N. Baharum, J.M. Salim, A. Ahmad, M. Taib. 2020. *Trichoderma atroviride* isolated from mangroves of the east coast of peninsular Malaysia exhibited high tolerance against heavy metal cadmium. Malays. Appl. Biol., 49(4): 113-120. https://doi.org/10.55230/mabjournal.v49i4.1600

Jaswal, R., K. Kiran, S. Rajarammohan, H. Dubey, P.K. Singh, Y. Sharma, R. Deshmukh, H. Sonah, N. Gupta and T.R. Sharma. 2020. Effector biology of biotrophic plant fungal pathogens: Current advances and future prospects. Microbiol. Res., 241: 126567. https://doi.org/10.1016/j.micres.2020.126567

Júnior, G.M., A.P.R. Sobrinho, B.H.S. Bambirra, F.H.S. Bambirra, M.A.R. Carvalho, L.M. Farias, J.R. Nicoli and E.S. Moreira. 2011. Synergistic growth effect among bacteria recovered from root canal infections. Brazilian J. Microbiol., 42(3): 973–979. https://doi.org/10.1590/S1517-83822011000300017

Karakus, Y.Y. 2020. Typical catalases: Function and structure. Glutathion. Sys. Oxidat. Stress Health Diseas., reaction 3.

Kim, Y.S., K. Balaraju and Y. Jeon. 2016. Biological control of apple anthracnose by *Paenibacillus polymyxa* APEC128, an antagonistic rhizobacterium. Plant Pathol. J., 32(3): 251–259. https://doi.org/10.5423/PPJ.OA.01.2016.0015

Köhl, J., R. Kolnaar and W.J. Ravensberg. 2019. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. Front. Plant Sci., 10: 1–19. https://doi.org/10.3389/fpls.2019.00845

Mohammad, A.M., M.M. El-Fatih and K.G. Helmy. 2013. Antifungal activity of *Bacillus thuringiensis* strains and their efficacy against the cotton leaf worm *Spodoptera littoralis*. *Arch.* Phytopathol. Plant Prot., 46(20): 2420–2427. https://doi.org/10.1080/03235408.2013.796695

Olle, M. and I.H. Williams. 2013. Effective microorganisms and their influence on vegetable production - A review. *J. Hortic. Sci. Biotechnol.* 88(4): 380–386. https://doi.org/10.1080/14620316.2013.11512979

Patowary, R. and H. Deka. 2020. *Paenibacillus*. Benefi. Microb. Agro-Ecol. Elsev. Inc. https://doi.org/10.1016/B978-0-12-823414-3.00017-4

Pellan, L., N. Durand, V. Martinez, A. Fontana, S. Schorr-Galindo and C. Strub. 2020. Commercial biocontrol agents reveal contrasting comportments against two mycotoxigenic fungi in cereals: *Fusarium graminearum* and *Fusarium verticillioides*. Toxin., 12(3): 152. https://doi.org/10.3390/toxins12030152

Sajili, M.H., A.A. Azman, N.A. Badaluddin, A.S.M. Ghazali, S. Mohamed, N. Ngah. 2018. Potential of *Pseudomonas* sp. & *Bacillus* sp. for controlling Fusarium oxysporum, a causal agent for rockmelon *Fusarium* wilt disease. J. Agrobiotech., 9(1s): 269-282.

Seo, Y. and Y.H. Kim. 2017. Potential reasons for prevalence of *Fusarium* wilt in oriental melon in Korea. Plant Pathol. J., 33(3): 249-263. https://doi.org/10.5423/PPJ.OA.02.2017.0026

Sidorova, T.M., A.M. Asaturova, A.I. Homyak, N.A. Zhevnova, M.V. Shternshis and N.S. Tomashevich. 2020. Optimization of laboratory cultivation conditions for the synthesis of antifungal metabolites by *Bacillus subtilis* strains. Saudi J. Biol. Sci., 27(7): 1879–1885.

https://doi.org/10.1016/j.sjbs.2020.05.002

Smith, A.C. and M.A. Hussey. 2005. Gram stain protocols. ASM., 1(September 2005): 14.

Sukweenadhi, J., S.R. Balusamy, Y.J. Kim, C.H. Lee, Y.J. Kim, S.C. Koh and D.C. Yang. 2018. A growth-promoting bacteria, *Paenibacillus yonginensis* DCY84T enhanced salt stress tolerance by activating defense-related systems in *Panax ginseng*. Front. Plant Sci., 9: 1–17. https://doi.org/10.3389/fpls.2018.00813

Wanger, A., V. Chavez, R.S.P. Huang, A. Wahed, J.K. Actor and A. Dasgupta. 2017. Biochemical tests and staining techniques for microbial identification. Microbiol. Mol. Diagn. Pathol., 61–73. https://doi.org/10.1016/B978-0-12-805351-5.00005-3

Warren, Y.A., D.M. Citron, C.V. Merriam and E.J.C. Goldstein. 2005. Biochemical differentiation and comparison of *Desulfovibrio* species and other phenotypically similar genera. J. Clinic. Microbiol., 43(8): 4041–4045. https://doi.org/10.1128/JCM.43.8.4041-4045.2005

Yu, X., J. Zhang, L. Guo, A. Yu, X. Wang, W. Xiang, J. Zhao. 2022. First report of *Fusarium proliferatum* causing fruit rot on muskmelon (*Cucumis Melo L.*) in China. Plant Dis., 106(4): 1085-1313. https://doi.org/10.1094/PDIS-09-21-2015-PDN