



Biocontrol properties of *Bacillus velezensis* SM1 enable field recovery from durian root rot

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ABSTRACT

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Biocontrol properties of *Bacillus* spp. are well known due to the antibiotics and bioactive compounds they produce, which promote plant growth and reduce the harm caused by plant pathogens. The role of *Bacillus velezensis* SM1 as a new biocontrol agent to address durian root rot caused by *Phytophthora* sp. infection was explored. Chemical, biochemical, and microbiological techniques, including Scanning Electron Microscopy (SEM), were employed to characterize the antagonistic mechanisms of SM1 on *Phytophthora palmivora* NKST002. Bacterial genomic DNA fragments encoding β -glucanase and cell wall hydrolase enzymes were cloned. The growth inhibitory effect of the expressed clones against the test fungus was evaluated. The bacterium was formulated as dry granules and used after reconstitution in water. The biocontrol property was assessed by applying the reconstituted SM1 on infected durian tissues four times per month and on the soil around the plant base twice per month in durian fields. Increased production of siderophore and superoxide dismutase enzyme was observed when SM1 was grown under stress conditions. Recombinant clones expressing β -glucanase and cell wall hydrolase enzymes were inhibitory to the test fungus. SEM observations showed the entry of SM1 into hyphae, vacuolation, and mycelial cracks in the coculture with NKST002. Diseased durians were cured after exposure to the reconstituted SM1 for one month. Long-term colonization of SM1 in the durian rhizosphere is expected. Overall, *Bacillus* SM1 demonstrated significant potential for treating durian root rot in crop fields.

Contribution/Originality: This study contributes to the existing literature by exploring the role of *Bacillus velezensis* SM1 as a biocontrol agent for treating durian root rot caused by *Phytophthora* infection. It demonstrates the feasibility of using SM1 to treat durian root rot in crop fields in the future.

1. INTRODUCTION

Thailand is the world's leading durian exporter, with China being the largest market [1]. In recent years, the nation's durian farming has faced significant threats from pathogens of the genus *Phytophthora* and *Pythium*, which cause root rot and have substantial economic impacts on durian production [2, 3]. Indeed, *Phytophthora palmivora* has been present in most orchards in Thailand [4]. Treatments using traditional fungicides are not fully effective and have raised concerns regarding environmental pollution and pathogen resistance. Grafting is another common method used in fruit tree production, where *Phytophthora*-resistant rootstocks are used to provide protection against the disease [5, 6]. But there are disadvantages recorded, such as the reduction of microbial diversity of rhizospheres by disrupting local ecosystems, and being costly and less sustainable techniques for long-term usage. Utilizing biological controls is one of the sustainable alternatives to chemical fungicides for plant disease management. For example, *Bacillus subtilis* has effectively controlled soilborne pathogens like *Fusarium* spp., *Rhizoctonia* spp.,

Phytophthora spp., and *Pythium* spp. in tomato, peppers, and cucumbers [7, 8]. Different achievements using *Bacillus amyloliquefaciens* to control *Colletotrichum dematium* in mulberry, *F. oxysporum* f. sp. cubense in banana, *Botrytis cinerea* in tomato, and *C. truncatum* in alfalfa have been reviewed [9, 10]. Indeed, *Bacillus velezensis* has been commercialized as a biological control against *B. cinerea* in tomato in Spain [11]. Previously, antagonisms between *B. velezensis* SM1 and two in-field *Phytophthora* isolates examined *in vitro* indicated that volatile organic compounds produced by the bacillus are inhibitory to mycelial growth. Thirteen gene clusters possibly associated with antimicrobial biosynthesis and production of hydrolase enzymes and siderophores have been identified using genomic DNA alignment tools [12]. Recent research emphasizes the importance of understanding the mechanisms and potential of this *Bacillus* species as a biological control to improve durian crop yields.

2. MATERIALS AND METHODS

2.1. Microorganisms and Growth Conditions

All of the used microorganisms were provided by Dr. Ashara Pengnoo, Soil Microbiology Laboratory, Faculty of Natural Resources, Prince of Songkla University, Thailand. The *Bacillus* SM1 (NCBI accession no. OR056195.1) was routinely maintained on potato dextrose medium (PDM) plates at 37°C in an incubator. The cells at the logarithmic growth phase were harvested by scraping and resuspended in sterile tubes using sterile water. This was followed by centrifugation at 5,000×g for 10 min and incubation at 80°C for 10 min to obtain a cell pellet. SM1 starter inoculum was prepared by resuspending the pellet in sterile water to a density of 3×10⁹ cells mL⁻¹. In some treatments, parts of the potato extract of PDM were replaced with tomato extract (98% w/w, Doi Kham, Thailand), resulting in a combined tomato extract medium (TEM). *Phytophthora palmivora* NKST002 was used as the test fungus. It was maintained on PDM plates at room temperature (RT).

2.2. Siderophore Producing Ability

The method of Ghazy and El-Nahrawy [13] and Hegde et al. [14] was used with some modifications to determine the siderophore produced by SM1. In brief, sterile EDTA solution was added to 20 mL PDM broth to a final concentration of 60 µM, into which the bacterial starter inoculum of 0.5 mL was inoculated. The cells were cultured by gently shaking at 37°C for 72 hours. The supernatant was obtained by centrifugation at 4,000×g for 15 minutes. It was then filtered through a 0.45 µm membrane filter and stored at 4–8°C until use. Saturated ferric chloride (FeCl₃) solution in water was freshly prepared and filtered through Whatman No. 42 filter paper before use as the titrant to titrate a 5 mL sample supernatant. The titrant volume was recorded upon reaching the titration endpoint, indicated by the appearance of a red color. All glassware was soaked in 6M hydrochloric acid overnight, thoroughly rinsed with distilled water, and autoclaved before use.

2.3. Effects of Ions and Ion Chelator on SM1 Viability and SOD Production

Sterile solutions of 25 mM magnesium chloride (MgCl₂), 1 M ferrous sulfate (FeSO₄), and 7 mM EDTA in water were prepared by filtering through 0.45 µm membrane filters. They were separately added to PDM broth to achieve final concentrations of 0–0.25 mM MgCl₂, 0–10 mM FeSO₄, and 0–70 µM EDTA. Into 20 mL of each solution, a 0.5 mL bacterial starter inoculum was added and cultured with gentle shaking at 37°C for 72 hours. The supernatant was collected by centrifugation at 4,000×g for 15 minutes, and the pH was measured. Bacterial cells were kept for analyzing viability and superoxide dismutase (SOD) activity using the Bacterial Viability (Abcam, USA) and SOD (Sigma-Aldrich, Germany) assay kits, respectively.

2.4. Bacterial Viability Test

The kit employs two highly specific, ultrasensitive fluorescent dyes to determine the percentage (%) of live and dead cells in a bacterial culture. Total bacterial count was calculated based on signals from the dye that permeates all

cells. The dead cell count was calculated based on signals from the dye that selectively stains only dead cells. The % dead cell was calculated directly from the measured signals, and the % live cell was acquired by subtracting the % dead cell from 100%. The bacterial cell pellet was gently resuspended in the wash buffer, centrifuged at $4,000\times g$ for 10 min at RT, and resuspended again in 1 mL of wash buffer. Subsequently, 1 μ L each of the total cell stain and the dead cell stain was added, followed by incubation at RT for 1 hour. Fluorescence was measured using a spectrofluorometer at Ex₄₉₀/EM₅₂₅ (Read 1) and Ex₅₃₆/EM₆₁₇ (Read 2). The % dead cell was calculated as (Read 2 / Read 1) x 100.

2.5. SOD Activity Assay

In principle, SOD catalyzes the conversion of superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Tetrazolium salt, 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, is simultaneously reduced to form a formazan product, which is quantitatively determined at 450 nm. Normally, SOD secreted in culture supernatants is several-fold diluted, and the accurate concentration cannot be measured. Recently, intracellular SOD activity was assessed instead. Briefly, the harvested cells were resuspended in wash buffer, and ultrasonication at 15°C for 30 min was applied. Cell lysates were obtained by centrifugation at $11,000\times g$ for 30 min at 10°C. Four reaction 0.5 mL tubes (“sample,” “bl1,” “bl2,” and “bl3”) were prepared. A 20 μ L aliquot of lysate was added to the “sample” and “bl2” tubes, while 20 μ L of ultrapure water was added to the “bl1” and “bl3” tubes. Dye solution of 200 μ L was added to all tubes, followed by the addition of 20 μ L dilution buffer to the “bl2” and “bl3” tubes and 20 μ L enzyme solution to the “sample” and “bl1” tubes. The mixtures were vortexed, incubated at 37°C for 20 min, transferred to a transparent 96-well plate, and measured at OD₄₅₀ using a microplate reader. SOD activity was calculated as % activation or % inhibition using the formulas provided below.

$$\begin{aligned}\% \text{ Activation} &= (A_{\text{sample}} - A_{\text{bl2}}) / (A_{\text{bl1}} - A_{\text{bl3}}) \times 100; \text{ if } (A_{\text{sample}} - A_{\text{bl2}}) > (A_{\text{bl1}} - A_{\text{bl3}}) \\ \% \text{ Inhibition} &= [(A_{\text{bl1}} - A_{\text{bl3}}) - (A_{\text{sample}} - A_{\text{bl2}})] / (A_{\text{bl1}} - A_{\text{bl3}}) \times 100; \text{ if } (A_{\text{sample}} - A_{\text{bl2}}) < (A_{\text{bl1}} - A_{\text{bl3}})\end{aligned}$$

2.6. Scanning Electron Microscopy (SEM)

A colony of SM1 was resuspended in 1 mL PDM broth. A 14-day-old *P. palmivora* NKST002 culture was dispersed in 10 mL PDM broth, into which 100 μ L bacterial suspension was added. The coculture was maintained statically at RT for 4 days. A 10 μ L aliquot was applied to a 1 cm² poly-L-lysine-coated coverslip and stored in a humid environment at RT for 1 h. The cells were dehydrated by sequential immersion in ethanol solutions (30-100%) for 10 min each. The cell sample was sputter-coated with an Au/Pd alloy and examined using SEM (HITACHI, SU3900).

2.7. Cloning of Genes Encoding β -Glucanase and Cell-Wall Hydrolase Enzymes

SM1 genomic DNA was extracted using the PureLink™ Microbiome DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Successful DNA purification was evaluated on an agarose gel using electrophoresis. DNA concentration was quantified using the Qubit dsDNA HS Assay Kit (Invitrogen). PCR primer pairs targeting a gene of interest, either β -glucanase or cell wall hydrolase, were designed based on SM1 genome sequences deposited in the NCBI database (accession no. PRJNA1205391), and the sequences are listed in Table 1. PCR reactions and thermal cycling conditions used were summarized in Tables 2 and 3. The RBC TA vector (RBC, Taiwan) was used for gene cloning, and the pQE-30Xa vector (Qiagen, Germany) was employed for protein expression. Recombinant plasmids were transformed into *Escherichia coli* competent cells (NEB C3037). Luria-Bertani (LB) medium supplemented with ampicillin (Amp) and IPTG was used for growing transformed *E. coli*. Recombinant clones were screened using the pour-plate technique against the test fungus NKST002. Colonies exhibiting clear inhibition zones were selected and propagated in LB-Amp broth. Plasmids were

extracted, and the insert DNA fragment length was assessed by restriction digestion and agarose gel electrophoresis. Gene identification was performed using PCR and DNA sequencing.

Table 1. Primer sequences for amplification of beta-glucanase and cell-wall hydrolase genes.

Gene	Coding DNA Sequence	F-primer (5'→3')	R-primer (5'→3')	Expected PCR product (bp)
Beta-glucanase	2273062.2273781	ATG AAA CGA GTG TTG CT	TAT TTT TTT GTA TAG CGC A	720
Cell wall hydrolase	Complement (1815248.1815676)	TTA AAA TGT ATT GTA CAC GTT	ATG GCA GTC GTA AAA G	430

Table 2. PCR reaction mixture for amplification of beta-glucanase and cell-wall hydrolase genes.

Component	Used concentration	Volume (μL)
Genomic DNA	n.d.	1
F-primer	100 μM	0.5
R-primer	100 μM	0.5
Tag polymerase (Bio-Helix)	5U/μL	0.25
dNTP	10 mM	1
MgCl ₂	50 mM	2
Tag buffer	10x	5
Ultra-purified water	-	44.75

Note: n.d., not determined.

Table 3. Thermal cycles of PCR for amplification of beta-glucanase and cell-wall hydrolase genes.

Stage	Temperature (°C)	Time
Denaturation	95	5 min
PCR cycling	30 cycles	
- Denaturation	95	30 sec
- Annealing	47	15 sec
- Extension	72	45 sec
Final extension	72	10 min
Storage	10	Until use

2.8. Field Experiment

SM1 was grown on PDM plates to the early stationary growth phase. The cells were harvested by scraping, washed using sterile water, and collected by centrifugation at 5,000×g for 10 min. The cells were mixed with pharmaceutical excipients, including lactose as a diluent and carboxymethyl cellulose as a binder. Water was gradually added and mixed until a homogeneous paste was obtained. The paste was extruded through a fine grid sieve and dried in an oven at 30°C for 6 hours to produce dry granules. Ten grams of the granules were reconstituted in 1 L of water immediately before use. Infected durian tissues were gently scratched. The reconstituted bacterium was applied to the rasped lesions by spraying once. This action was performed weekly, four times a month. Additionally, 5 g of granules were reconstituted in 10 L of water and applied once onto the land surrounding the tree base every two weeks, totaling twice a month. Recovery of the plants from the infection was recorded weekly by photography throughout the trial.

2.9. Statistical Analysis

Experiments were conducted in triplicate. Results were expressed as mean ± S.D. and subjected to normality tests before analysis. Significant differences were determined and evaluated using One-Way ANOVA followed by the post hoc Tukey test at a 95% confidence interval. All data were analyzed using SPSS Statistics software (Prince of Songkla University).

3. RESULTS

3.1. Siderophore Production

Titration of 5.0 mL culture supernatant of SM1 with saturated FeCl_3 required a mean titrant volume of 0.38 ± 0.04 mL to reach the endpoint.

3.2. Effects of Ions and Ion Chelator on Culture pH, Cell Viability, and SOD Production

The supernatant pH, cell viability, and SOD activity of SM1 cultured in PDM or TEM supplemented with ions (Mg^{2+} and Cl^- , Fe^{2+} and SO_4^{2-}) or EDTA for 72 hours were investigated. Results are summarized in Table 4, indicating no significant differences among treatments. PDM was intrinsically less acidic than TEM, with a greater decrease in pH observed after incubation. When 0-250 μM MgCl_2 or 0-70 μM EDTA were included, pH changes after culturing were insignificant. However, FeSO_4 supplementation caused a significant reduction in pH along with growth impairment. The OD_{670} (Table 5), indicating cell density, was highest for cultures using 60% PDM-supplemented TEM. Better cell propagation was suggested for the combined TEM culture compared to PDM alone. Although SOD production was activated in PDM culture, maximal SOD activity was observed in cultures with 40-50% PDM-supplemented TEM. Notably, SM1 viability was not proportionally correlated with its SOD-producing ability.

Table 4. Data of culture pH, viability (%), and SOD activation (%) of SM1 grown in PDM broth or TEM supplemented with MgCl_2 , FeSO_4 or EDTA at an indicated final concentration.

Supplemented compound	pH (supernatant)		% Viability		% SOD activation	
	PDB	TEM	PDB	TEM	PDB	TEM
MgCl_2 (μM)						
0	6.49	5.04	81.1	89.5	132.56	114.51
5	5.75	5.04	83.4	82.6	129.17	110.23
10	5.67	5.03	83.5	82.3	128.32	108.97
40	5.60	5.03	83.5	82.0	123.25	107.12
100	5.66	5.03	83.7	82.0	120.32	105.56
180	5.80	4.98	85.2	85.2	125.67	104.67
250	5.64	4.93	87.5	90.6	127.58	103.18
FeSO_4 (mM)						
0	6.49	5.04	81.1	89.5	132.56	114.51
3.5	3.46	4.52	ND	32.0	ND	ND
5.0	3.28	3.74	ND	ND	ND	ND
6.0	3.17	3.65	ND	ND	ND	ND
9.5	3.00	3.08	ND	ND	ND	ND
EDTA (μM)						
0	6.49	5.04	81.1	89.5	132.56	114.51
7.0	6.59	4.99	86.9	86.4	138.25	101.65
17.5	6.57	5.02	83.7	90.6	127.44	103.35
28.0	6.58	5.01	80.9	88.2	130.07	109.57
42.0	6.71	4.98	86.9	90.6	100.22	115.43
63.0	6.72	4.95	86.6	86.7	127.04	121.84

Note: ND, not detected; S.D. of the data was within $\pm 5\%$ of the corresponding mean.

Table 5. The OD_{670} , viability (%), and SOD production (relative activation) of SM1 grown in TEM containing PDB (0-100%).

% PDB	OD_{670} (cell density)	% Viability	Relative SOD activation
100	0.1413	87.06	1.29
60	0.4365	74.81 ^b	1.54 ^b
50	0.4253	63.27 ^a	1.86 ^a
40	0.4191	63.59 ^a	1.83 ^a
30	0.4073	67.34 ^b	1.74 ^b
20	0.4050	84.43	1.36
10	0.3001	82.77	1.38
0	0.4119	98.92	1.03

Note: ^a, $p < 0.01$; ^b, $p < 0.05$ significant difference compared to the control (100% PDB).

For SM1-NKST002 coculture, broader clear inhibition zones were apparent when 10-100 μM MgCl_2 or 28 μM EDTA were included (Figure 1a). The microorganisms' viability was clearly evaluated on 40% PDA-supplemented TEM plates, where the addition of EDTA reached 60 μM final concentration. This adjustment was based on information indicating that there are more abundant metal ions in tomatoes than in potatoes [15]. Under the modified conditions, SM1 proliferated significantly to encircle the fungus colony (Figure 1b).

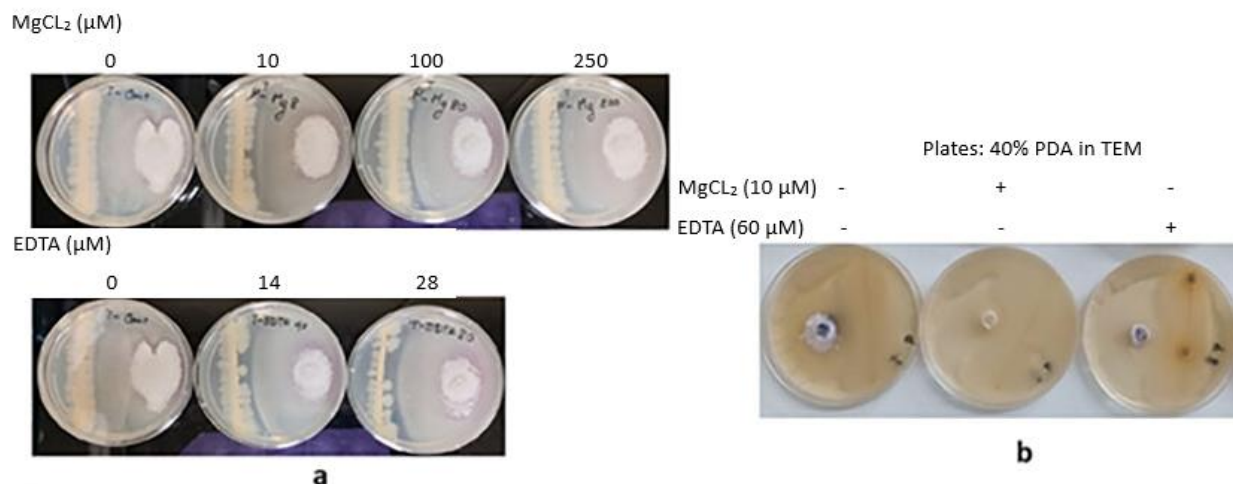


Figure 1. Coculture experiment between the bacillus SM1 and *P. palmivora* NKST002 on PDM plates (a) and TEM containing 40% PDM plates (b) supplemented with MgCl_2 or EDTA at the indicated concentration.

3.3. Scanning Electron Microscopy (SEM)

As shown in Figure 1, there was a particular antagonistic interaction between SM1 and NKST002. SEM was then used to observe structural changes in the hyphae when in direct contact with SM1. Results are shown in Figure 2, indicating the entry of SM1 into the hyphae, accompanied by infection peg-like structures (vacuolation) and mycelial cracking.

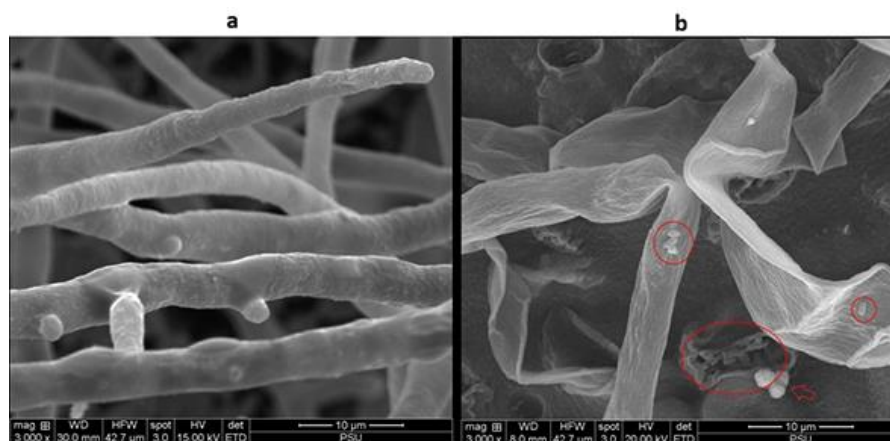


Figure 2. Representative SEM micrographs of *P. palmivora* NKST002 alone (a); the bacillus SM1 attached to and entered into the fungal hyphae (red circles) (b); Vacuolation (an arrow); Mycelia cracking (the largest red circle).

3.4. Cloning and Expression of β -Glucanase and Cell Wall Hydrolase Enzymes

Four recombinant clones of *E. coli* (namely #1, #2, #3, and #4) were selected due to exhibiting clear inhibition zones against *P. palmivora* NKST002. The antagonistic activities varied, ranked as #4 > #1 > #3 > #2 (Figure 3). The resident plasmids were extracted to determine the inserted DNA lengths and sequences. Results of double digestion using *Bam*HI and *Hind*III enzymes and agarose gel electrophoresis show that clones #3 and #2 harbored insert DNAs of approximately 860 base pairs, while clones #4 and #1 contained approximately 580 base pairs (Figure

4). An additional 140–150 base pairs relative to the expected gene length were indicated, likely due to vector-derived sequences included in the restriction sites. PCR amplification using primers specific to the gene (Table 1) identified clones #3 and #2 as harboring the β -glucanase gene, and clones #4 and #1 carrying the cell wall hydrolase gene. Sequences of the DNA inserts aligned approximately 95–98% with the corresponding gene sequences deposited in the NCBI database (unpublished data).

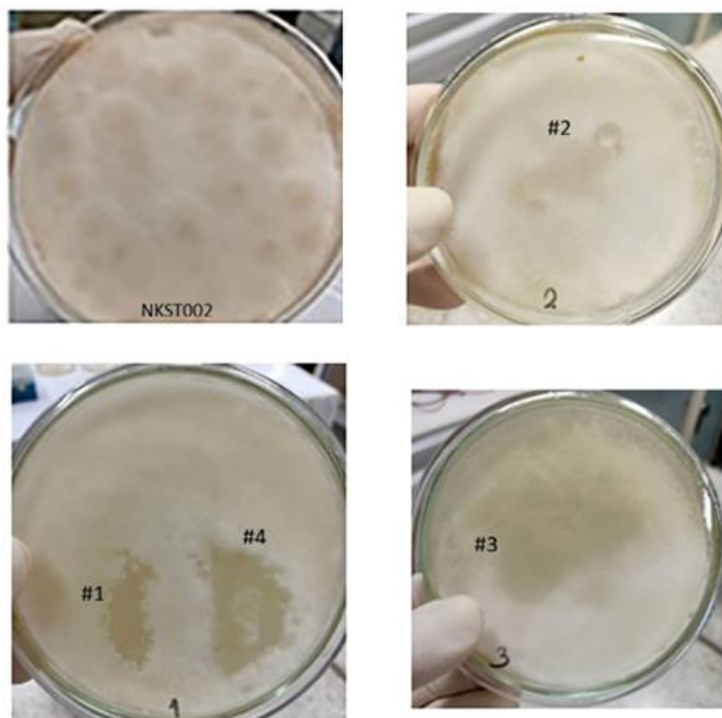


Figure 3. Four positive recombinant clones, #1, #2, #3, and #4, producing different clear inhibition zones against *P. palmivora* NKST002.

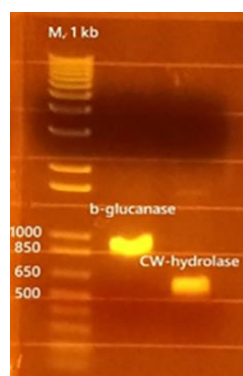


Figure 4. *Bam*HI-*Hind*III double-digested fragments of plasmids from the 4 selected clones, electrophoresed on 1% agarose gel; The 860 base pairs fragment for clones #2 and #3; The 580 base pairs fragment for clones #1 and #4.

3.5. Field Experiment

According to the described *in vitro* results, the potential of SM1 as a biocontrol agent against durian root rot pathogen, *P. palmivora*, was evaluated under field conditions. SM1 was formulated as dry granules. After reconstitution and application 4 times on the infected tissues and two times onto the land surrounding the plant base within a period of 1 month, the diseased plants recovered from the infection, and the infected tissues were cured and healed (Figure 5).

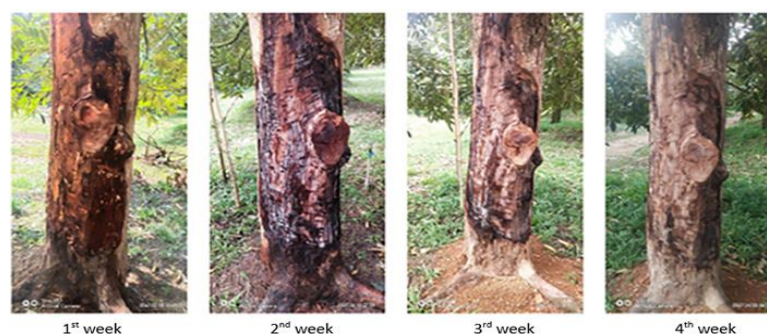


Figure 5. Photographs of a representative durian tree with root rot signs caused by *Phytophthora palmivora* infection; The diseased plant tissues were removed before the reconstituted SM1 granule was applied consecutively once a week for 4 weeks. The reconstituted SM1 product was additionally poured on the soil surrounding the plant base, every 2 weeks within a month. Photography was done every week in a month.

4. DISCUSSION

More than 90% of global durian exports are supplied by Thailand, as reported [1]. However, the national durian production still relies heavily on traditional practices passed down through generations, which have contributed to declining yields over time and reduced competitiveness in the global market. Extensive use of chemical agents for pest and disease control has led to substantial agricultural losses due to physiological drought and stunted growth, in addition to serious health and environmental concerns [16].

Fungi of the genus *Phytophthora* are among the most prevalent and destructive pathogens affecting durian trees in Southeast Asia, causing root rot and patch cankers on trunks and stems that are often lethal to the plant [17]. Indeed, symbiotic interactions among soil microorganisms are beneficial to plants by enhancing nutrient uptake, stimulating phytohormone production, improving soil structure for water retention and drainage, and protecting plants from pathogens through competitive exclusion and antibiotic production, thereby contributing to healthier and more resilient crops while reducing reliance on chemical inputs. Currently, biocontrol agents used for pest and disease management in agriculture are considered environmentally acceptable due to their targeted action against harmful organisms. In Thailand, BIOTEC-NSTDA released standardized guidelines for the application of biocontrol products in 2023. However, the success rate of this initiative has yet to be reported. Consequently, the combined use of plants and beneficial microorganisms has emerged as a major focus of current agricultural research.

Iron toxicity is a common problem affecting crops in Thailand, largely due to the excessive use of chemical fertilizers. Although durian trees appear to tolerate high iron levels, microorganisms of the rhizospheres contribute to iron detoxification by producing biosurfactants and siderophores [18, 19], and siderophore-mediated iron acquisition has been reported in *Bacillus subtilis* [20]. In the present study, siderophore production by SM1 was suggested to be inducible under low-metal conditions, including reduced availability of Fe^{2+} and Fe^{3+} . This response likely serves to maintain intracellular iron homeostasis necessary for normal bacterial growth [21, 22]. Iron toxicity in plant roots can trigger oxidative stress through the accumulation of reactive oxygen species (ROS), and superoxide dismutase (SOD) enzymes constitute a primary defense mechanism for catalyzing the dismutation of ROS [23, 24]. This study indicates increased SOD production of SM1 when grown under ionic, osmotic, and nutrient stress conditions (Tables 1 and 2). In accordance, the enzyme upregulation may be beneficial to durian trees by aiding in the dissipation of harmful ROS generated in the plant rhizospheres. The growth of *P. palmivora* NKST002 was inhibited when cocultured with SM1 (Figure 1). The possible fungicidal effect of SM1 included the lytic property of specific enzymes secreted by SM1 when in direct contact with the fungus, allowing the bacillus to penetrate the damaged cell wall. This resulted in vacuolation and cracking of the mycelia (Figure 2). These hypotheses were supported by gene cloning experiments, which indicated the inhibitory and lytic characteristics of the expressed β -glucanase and cell wall hydrolase enzymes of SM1 (Figures 3 and 4). These enzymes cleave glycosidic bonds of cell wall components, leading to pore formation and disruption of intracellular ion balance, ultimately causing cell lysis

and death [25]. Siderophore, SOD, and cell wall-associated lytic enzymes constitute the key antagonistic mechanisms employed by SM1 against *Phytophthora* spp. Field experiments demonstrated the recovery of durian trees from root rot disease within one month of SM1 application (Figure 5). The improvement of plant health by reducing disease incidence could be anticipated, correlating with the potential for persistent colonization of SM1 in durian rhizospheres [13]. These findings highlight the promising potential of SM1 as a novel biocontrol agent for durian agriculture.

5. CONCLUSION

For yield improvement and sustainability of durian cultivation, there is a strong push toward managing pests and pathogens using specific and effective biocontrol microorganisms. In this study, accumulating evidence demonstrated the potential of the *Bacillus* SM1 to treat root rot caused by *Phytophthora* sp. in durian fields. Siderophore production, SOD activation, and the expression of β -glucanase and cell wall hydrolase enzymes were identified as key biocontrol mechanisms of this bacterium. As infected durian trees can recover from the disease attack within 1 month of SM1 application, long-term colonization of SM1 in the rhizosphere is rational to propose. The use of SM1 as a biocontrol agent for durian root rot may represent an important strategy for sustaining durian agriculture in the future.

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Competing Interests: The author declares that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

- [1] FAO, *Durian global trade overview 2023*. Rome, Italy: Food and Agriculture Organization of the United Nations, 2023.
- [2] S. Somnuek, P. Kongtragoul, and T. Jaenaksorn, "Fungicide resistance of *Phytophthora palmivora* causing durian diseases in eastern and southern Thailand and the in vitro alternative control by cajeput leaf extracts," *International Journal of Agricultural Technology*, vol. 19, no. 2, pp. 703–720, 2023.
- [3] P. Kongtragoul, K. Ishikawa, and H. Ishii, "Metalaxyl resistance of *Phytophthora palmivora* causing durian diseases in Thailand," *Horticulturae*, vol. 7, no. 10, p. 375, 2021. <https://doi.org/10.3390/horticulturae7100375>
- [4] K. Sichai, P. Nianwichai, N. Taraput, V. Tongsri, and P. Songkumarn, "*Phytophthora palmivora* RPA1, a homolog of *Phytophthora infestans* RPA190, is irrelevant to metalaxyl resistance in *Phytophthora palmivora* causing root and stem rot of durian in Thailand," *The Korean Journal of Mycology*, vol. 52, no. 2, pp. 73–96, 2024. <https://doi.org/10.4489/kjm.520201>
- [5] A. Drenth and D. I. Guest, *Diversity and management of Phytophthora in Southeast Asia*. Canberra, Australia: Australian Centre for International Agricultural Research, 2004.
- [6] M. O. Adjei, R. Yu, X. Cao, and B. Fan, "The mechanisms of *Bacillus subtilis* as a plant-beneficial rhizobacterium in plant–microbe interactions," *Microorganisms*, vol. 13, no. 12, p. 2823, 2025. <https://doi.org/10.3390/microorganisms13122823>
- [7] I. Sarenkova, O. R. Razanakoto, S. I. Kreke-Kalnina, and K. Ulme, "The role of *Bacillus subtilis* in promoting plant growth under diverse agricultural and environmental conditions," *Journal of the Saudi Society of Agricultural Sciences*, vol. 25, no. 3, p. 41, 2026. <https://doi.org/10.1007/s44447-026-00128-w>
- [8] I. Zalila-Kolsi, A. Ben-Mahmoud, and R. Al-Barazie, "*Bacillus amyloliquefaciens*: Harnessing its potential for industrial, medical, and agricultural applications—a comprehensive review," *Microorganisms*, vol. 11, no. 9, p. 2215, 2023. <https://doi.org/10.3390/microorganisms11092215>

- [9] M. Stanislav, "Bacillus amyloliquefaciens: A versatile bacterium in food preservation, agriculture, and beyond," Retrieved: <https://www.indogulfbioag.com/post/bacillus-amyloliquefaciens-applications>. 2025.
- [10] M. F. Rabbee, B.-S. Hwang, and K.-H. Baek, "Bacillus velezensis: A beneficial biocontrol agent or facultative phytopathogen for sustainable agriculture," *Agronomy*, vol. 13, no. 3, p. 840, 2023. <https://doi.org/10.3390/agronomy13030840>
- [11] P. Jin *et al.*, "Bacillus velezensis, a new valuable source of bioactive molecules within plant microbiomes and natural weapons for the biocontrol of plant pathogens," *Tropical Plants*, vol. 4, no. 1, p. e001, 2025. <https://doi.org/10.48130/tp-0024-0044>
- [12] A. Pengnoo *et al.*, "Bacillus velezensis SM1: A promising biocontrol solution for Phytophthora durian root rot," *Applied Microbiology*, vol. 5, no. 1, p. 21, 2025. <https://doi.org/10.3390/applmicrobiol5010021>
- [13] N. Ghazy and S. El-Nahrawy, "Siderophore production by Bacillus subtilis MF497446 and Pseudomonas koreensis MG209738 and their efficacy in controlling Cephalosporium maydis in maize plant," *Archives of Microbiology*, vol. 203, no. 3, pp. 1195-1209, 2021. <https://doi.org/10.1007/s00203-020-02113-5>
- [14] G. M. Hegde, U. Premchand, and R. Mesta, "The potential of siderophores in biological control in plant diseases," Retrieved: <https://apbb.ftc.org.tw/article/658>. 2025
- [15] R. Sargsyan, A. Grigoryan, and A. Grigoryan, *Potato vs. tomato — Health impact and nutrition comparison*. Armenia: Food Struct, 2021.
- [16] P. Visutsak, "Ontology-based semantic retrieval for durian pests and diseases control system," *International Journal of Machine Learning and Computing*, vol. 11, no. 1, pp. 92-97, 2021. <https://doi.org/10.18178/ijmlc.2021.11.1.1019>
- [17] T. K. Lim and S. Sangchote, "Diseases of durian," *Diseases of Tropical Fruit Crops*, pp. 241-251, 2003. <https://doi.org/10.1079/9780851993904.0241>
- [18] L. Chen *et al.*, "Adaptation of rhizosphere and endosphere microbiome to heavy metal pollution in castor bean," *Rhizosphere*, vol. 24, p. 100618, 2022. <https://doi.org/10.1016/j.rhisph.2022.100618>
- [19] A. B. Caracciolo and V. Terenzi, "Rhizosphere microbial communities and heavy metals," *Microorganisms*, vol. 9, no. 7, p. 1462, 2021. <https://doi.org/10.3390/microorganisms9071462>
- [20] A. Rizzi, S. Roy, J.-P. Bellenger, and P. B. Beauregard, "Iron homeostasis in Bacillus subtilis requires siderophore production and biofilm formation," *Applied and Environmental Microbiology*, vol. 85, no. 3, pp. 1-10, 2019. <https://doi.org/10.1128/AEM.02439-18>
- [21] C. R. Deb and M. Tatung, "Siderophore producing bacteria as biocontrol agent against phytopathogens for a better environment: A review," *South African Journal of Botany*, vol. 165, pp. 153-162, 2024. <https://doi.org/10.1016/j.sajb.2023.12.031>
- [22] E. Ahmed and S. J. M. Holmström, "Siderophores in environmental research: Roles and applications," *Microbial Biotechnology*, vol. 7, no. 3, pp. 196-208, 2014. <https://doi.org/10.1111/1751-7915.12117>
- [23] G. Li, J. Wu, H. J. Kronzucker, B. Li, and W. Shi, "Physiological and molecular mechanisms of plant-root responses to iron toxicity," *Journal of Plant Physiology*, vol. 297, p. 154257, 2024. <https://doi.org/10.1016/j.jplph.2024.154257>
- [24] R. Batool, M. J. Umer, B. Hussain, M. Anees, and Z. Wang, *Molecular mechanisms of superoxide dismutase (SODs)-mediated defense in controlling oxidative stress in plants, Antioxidant Defense in Plants: Molecular Basis of Regulation*. Singapore: Springer, 2022, pp. 157-179.
- [25] A. Ansabayeva *et al.*, "Plant growth-promoting microbes for resilient farming systems: Mitigating environmental stressors and boosting crops productivity—a review," *Horticulturae*, vol. 11, no. 3, p. 260, 2025. <https://doi.org/10.3390/horticulturae11030260>

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