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Isolation and optimization of *Bacillus* sp. from corn planting soil to inhibit *Fusarium moniliforme* causing stalk rot disease in corn

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ABSTRACT

Corn is an economic crop. Nowadays, plant diseases such as Fusarium stalk rot have been found in corn cultivation. Chemical fungicides are widely used to inhibit this fungus. However, using biological controls is one of the approaches for preventing and solving the problem of plant diseases. Soil samples were collected from corn planting soils using random sampling techniques. The morphological characteristics of the 17 isolated bacteria were studied under a microscope. Isolate SCFPSU17 was evaluated for its ability to inhibit *Fusarium moniliforme* that cause stalk rot disease in corn. After identification, the results revealed that DNA sequencing of SCFPSU17 was similar to *Bacillus* sp. with percentage similarity of 99.86%. The culture filtrate of *Bacillus* sp. showed the best inhibition of *F. moniliforme* at 38.63%. Then, the optimal conditions of *Bacillus* sp. isolated SCFPSU17 were tested, including the optimal medium, optimal pH of medium, and optimal temperature of culture and cultivation time. The result found that *Bacillus* sp. isolated SCFPSU17 in nutrient broth pH 8 at 30°C for 36 hours demonstrated the most effective inhibition of *F. moniliforme*, up to 70%. From this result, bioactive agents from *Bacillus* sp. for inhibiting plant fungal pathogens may be an important discovery to develop bioactive compounds production for sustainable agriculture in the future.

1. INTRODUCTION

Maize (*Zea mays* L.) is considered an economically significant crop due to its substantial export rate, which generates considerable income for a large number of farmers. Botanical details of maize: The family name (Poaceae), type of inflorescence (a compound panicle), and type of fruit (caryopsis) [1]. Maize is cultivated annually on approximately 197 million hectares worldwide, making it the second most widely grown crop globally, after wheat. Global maize production amounts to over 1,137 million metric tons [2]. Thailand, the country ranks as the 7th largest maize exporter globally, with its export value in 2021 increasing by 87 million baht compared to 2020. Thailand's largest maize export market is Japan [3]. Additionally, maize is popularly consumed both as corn and in various processed forms, such as maize flour and cornflakes. Furthermore, maize is frequently used in the animal feed industry, thereby adding value to agricultural produce and promoting optimal utilization of natural resources. Presently, economic crops are experiencing a reduction in yield due to various factors, including natural disasters, pests, and diseases. Notably, plant diseases, particularly stalk rot caused by *Fusarium moniliforme*, significantly contribute to crop damage. *Fusarium moniliforme*, specifying its order (Hypocreales) and class (Sordariomycetes) [4], the causal agent of maize stalk rot, spreads through contaminated seeds, soil, and infected plant debris. The pathogen can spread throughout in stalk of maize and proliferate under suitable conditions. *Fusarium* sp. is deteriorated by long-term monoculture in the same plots. The pathogen can survive for up to 10 years as chlamydospores in soil [5]. Global maize yield losses from *Fusarium* sp. infections in some areas with severe outbreaks can result in reductions of up to 30%–50% [6]. In Thailand, outbreaks of ear rot caused by *Fusarium* sp. fungi have been reported in several areas [7]. Although *Fusarium* sp. infections in maize have occurred in the country, specific data on the extent of yield losses remain limited. However, *Fusarium* sp. infections can lead to reduced yields and a decline in maize quality. Additionally, *F. moniliforme* is capable of producing fumonisins, a group of polyketide derivatives known for their high toxicity to both humans and animals [8,9]. In modern agriculture, integrating biological controls with innovative technological approaches can provide sustainable solutions for crop protection. Studies such as Enhancing Agriculture Crop Classification with Deep Learning have demonstrated the use of deep

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learning to improve agricultural practices by accurately classifying crops and identifying potential risks early [10]. Such advancements not only enhance yield management but also complement biological strategies, such as using *Bacillus* species for disease control, to address challenges in agricultural systems comprehensively.

The primary approach chosen by the farmers to mitigate the issue is the use of chemical agents for controlling plant diseases, particularly within the phenylamide, quinone, and carboxylic acid amide groups [11]. This preference stems from the rapid efficacy of these chemical agents in disease management. However, these chemical substances pose significant environmental and consumer health risks. Furthermore, prolonged chemical usage for fungal eradication has been observed to induce resistance in fungi against these agents [12].

Therefore, it is necessary to use biologically active substances that are effective in addressing the aforementioned issues. The use of such biologically active substances constitutes a safe and efficacious method for inhibiting fungi, which are the causative agents of plant diseases. Relevant research has demonstrated that employing biologically active substances derived from bacteria within the *Bacillus* group exhibits efficacy in suppressing fungal pathogens. For example, *Bacillus subtilis* N3 can produce flagellin A, which possesses inhibitory properties against *Curvularia lunata*, the fungus causing disease in orchids [13], or *Bacillus* sp. isolated from mangrove soil has shown inhibition against *Fusarium* spp., *Phytophthora palmivora*, and *Colletotrichum gloeosporioides*, which are fungal pathogens in plants [7].

Bacillus sp. is a gram-positive bacterium characterized by rod-shaped morphology and motility via flagella. It is an aerobic bacterium requiring oxygen for growth, although some species are facultative anaerobes. *Bacillus* sp. can produce various biologically inhibitory substances, such as lactic acid, chitinase, chitosanase, lipase, laminalinase, and protease, which can inhibit the formation of fungal mycelium [14]. *Bacillus* sp. is a spore-forming bacterium, enabling it to tolerate high temperatures, dry conditions, chemicals, and various adverse environmental conditions effectively [15]. However, the production of biologically active substances derived from *Bacillus* sp. requires suitable growth conditions to maximize efficacy. Therefore, this research aims to identify optimal conditions for *Bacillus* sp. to produce biologically active substances for inhibiting fungal pathogens, potentially providing a new alternative for Thai farmers in plant disease management, replacing chemical agents.

2. MATERIALS AND METHODS

2.1. Fungal Strains and Culture Conditions

Plant pathogenic fungi, isolated P2T2R1/1, and P2T2R4/2 were isolated previously from durian leaf disease in Prachuap Khiri Khan Province, Thailand, and shown as *Fusarium* sp. under a microscope [16], and *F. moniliforme* was kindly provided by Assoc. Prof. Panan Rerngsamran, Department of Microbiology, Faculty of Science, Chulalongkorn University, Thailand. *Fusarium moniliforme,* P2T2R1/1, and P2T2R4/2 were grown on Potato Dextrose Agar (PDA) at 30°C for 5 days.

2.2. Isolation of Bacteria from Soil Samples

Soil samples were collected from maize fields at the agricultural farm of Prince of Songkla University, Surat Thani Campus. The sampling was conducted randomly at 5 points, including the 4 corners and the center of the maize plot, with each sample weighing 5 g. Subsequently, the samples were diluted with 45 ml of 0.85% NaCl. The diluted samples were then spread onto nutrient agar (NA) medium and incubated at 37°C for 24 hours [17]. Colonies of bacteria were observed, and the bacterial count was calculated as colony-forming units per gram (CFU/g). The single colony of bacteria was Gram-stained to identify gram-positive bacteria and characterize their morphology as bacilli. Subsequently, biochemical tests were performed, including catalase test, oxidase test, motility test, and starch hydrolysis [18].

2.3. Identification of Bacteria

The isolated SCFPSU17 was cultured in NA medium at 37°C for 24 hours [17]. The identification of pure culture was done by using the 16S rDNA sequencing method [19] at the Thailand Bioresource Research Center, National Center for Genetic Engineering and Biotechnology (Khlong Luang, Pathum Thani).

2.4. Screening of Antagonistic Bacteria by Plate Confrontation Assay

A cork borer No.3 was used to punch holes in fungal plates containing *F. moniliforme,* isolated P2T2R1/1 and P2T2R4/2 after 7 days of culture. The fungal plug was inoculated in the center of a PDA plate, and the isolated SCFPSU17 was inoculated in parallel lines 2 cm away from the fungal plug. The plates were subsequently incubated at room temperature. When the fungal colonies in the control group overgrew the bottom of the dish, the width of the inhibition zone in the treatment group was measured. The experiment was repeated 4 times [20].

2.5. Bioassay for Antifungal Activity

Bacteria isolated SCFPSU17 were cultured in 20 ml of nutrient broth (NB) and incubated with agitation at 150 rpm and 37°C for 24 hours. The turbidity of the culture was measured at OD_{600} and adjusted to 0.5 OD_{600}. Subsequently, the cell-free culture obtained from a 1% inoculum in NB was incubated at 150 rpm and 37°C for 24 hours and then centrifuged at 8,000 rpm for 15 minutes. A Millipore filter (0.2 μm) was used to filter-sterilize the culture supernatant. This cell-free culture filtrate was mixed with PDA and tested against *F. moniliforme*. The plates were then incubated at room temperature, and the percentage inhibition was calculated accordingly [21].

$$\% \text{ inhibition} = \{1-(\text{Fungal growth}/\text{Control growth})\} \times 100$$

2.6 Optimal Conditions for Bacterial Antifungal Production

2.6.1. Optimization of culture medium

Bacillus sp. isolated SCFPSU17 was cultured in various liquid media including NB, luria-bertani broth (LB) and tryptic soy broth (TSB) at 37°C with shaking at 200 rpm for 24 hours. The 10 ml of cell-free culture filtrate was prepared and mixed with 10 ml of molten PDA medium before pouring into Petri dishes for testing against *F. moniliforme*. The plates were then incubated at room temperature, and the percentage inhibition was calculated [21].

2.6.2. Optimization of culture medium pH

Bacillus sp. isolated SCFPSU17 was cultured in suitable liquid media with pH adjusted to 6, 7, 8, and 9, followed by testing similar to the optimization of culture medium conditions.

2.6.3. Optimization of incubation temperatures

Bacillus sp. isolated SCFPSU17 was cultured in suitable liquid media with adjusted pH, incubated at various temperatures including 30°C,

37°C, and 40°C, followed by testing similar to the optimization of culture medium conditions.

2.6.4. Optimization of incubation times

Bacillus sp. isolated SCFPSU17 was cultured in suitable liquid media with adjusted pH and temperature. Growth rates were measured, and antifungal activity was tested over a period of 36 hours, with data collected every 3 hours, following a procedure similar to that used for optimizing culture medium conditions.

2.7. Statistical analysis

All experimental data were collected in 4 replicates. The mean value for each experimental group was calculated. Differences among the experimental groups were analyzed using one-way ANOVA, followed by Duncan's multiple range test for mean comparisons at a 95% confidence level ($p < 0.05$). The analysis was performed using SPSS software version 11.0 (IBM Corp., Armonk, NY, USA) [22].

3. RESULTS

3.1. Basic Microbiological and Biochemical Characterization of Bacteria from Soil Samples

A total of 17 isolates from soil samples were obtained and quantified on NA agar, yielding a colony count of 6.65×10^5 CFU/g. These isolates were then Gram-stained and subjected to microscopic examination, revealing 11 isolates that stained positive and exhibited rod-shaped or bacilli morphology resembling *Bacillus* sp. These isolates were designated as SCFPSU01, SCFPSU03, SCFPSU05, SCFPSU07, SCFPSU09, SCFPSU10, SCFPSU13, SCFPSU14, SCFPSU15, SCFPSU16, and SCFPSU17 (Table 1). The biochemical characterization of the 11 isolates included tests for catalase, oxidase, starch hydrolysis, and motility. The results indicated positive reactions for catalase, starch hydrolysis, and motility tests, while the oxidase test yielded negative results. Importantly, isolate SCFPSU17 exhibited biochemical characteristics similar to *Bacillus* sp. (Table 1). Furthermore, the molecular classification of isolate SCFPSU17 based on 16S rDNA sequencing showed a nucleotide sequence similarity of 99.86% with *Bacillus* sp.

3.2. Screening of Antagonistic Bacteria by Plate Confrontation Assay

Through dual-culture inhibition assays of the pathogenic fungi using *Bacillus* sp. isolated SCFPSU17, it was observed that this bacterium could effectively inhibit the growth of pathogenic fungi, demonstrating an inhibition zone against *F. moniliforme* (Fig. 1a) and the isolate P2T2R4/2 (Fig. 1b). However, *Bacillus* sp. isolated SCFPSU17 was unable to inhibit the growth of the fungal strain P2T2R1/1 (Fig. 1c). Therefore, both fungal species, *F. moniliforme* and P2T2R4/2, were subjected to further antifungal testing using the culture filtrate method.

3.3. Bioassay for Antifungal Activity of Active Substance on *F. moniliforme*

In the antifungal inhibition test using the cell-free supernatant from *Bacillus* sp. isolated SCFPSU17, the supernatant demonstrated the highest inhibition of the pathogenic fungus *F. moniliforme*, with an inhibition percentage of 38.63% ± 1.64%, and also exhibited a 14.54% ± 2.09% inhibition of P2T2R4/2 ($p < 0.05$), as shown in Table 2. Based on these results, *F. moniliforme* was selected for further experiments to determine the optimal conditions for the bacterial production of biological control agents aimed at inhibiting plant pathogens.

3.4. Optimization of Culture Medium for Antifungal Activity

The evaluation of optimal growth conditions for three different culture media NB, LB, and TSB for cultivating *Bacillus* sp. isolated SCFPSU17 revealed that all media effectively inhibited the growth of *F. moniliforme* compared to the control (Figs. 2 and 5). Specifically, when *Bacillus* sp. isolated SCFPSU17 was cultured in NB, LB, and TSB media, the inhibition percentages against *F. moniliforme* were 41.33% ± 8.98%, 32.00% ± 8.84%, and 18.67% ± 4.62%, respectively, as shown in Table 3. Based on these results, NB medium was selected for the cultivation of *Bacillus* sp. isolated SCFPSU17 due to its highest inhibition percentage of *F. moniliforme* ($p < 0.05$). This medium was then used to investigate optimal pH conditions for the subsequent experiments.

Table 1. Characteristic and biochemical test of isolated bacteria from corn planting soil.

Isolate	Gram strain	Shape	Biochemical test			
			Catalase	Oxidase	Starch hydrolysis	Motility
SCFPSU01	+	Rod	+	+	+	+
SCFPSU03	+	Bacilli	+	+	+	+
SCFPSU05	+	Bacilli	−	+	+	+
SCFPSU07	+	Bacilli	+	+	+	+
SCFPSU09	+	Rod	+	−	−	+
SCFPSU10	+	Bacilli	+	+	+	+
SCFPSU13	+	Bacilli	−	+	+	+
SCFPSU14	+	Rod	+	+	+	+
SCFPSU15	+	Bacilli	+	+	−	+
SCFPSU16	+	Rod	+	+	+	+
SCFPSU17	+	Bacilli	+	−	+	+

(+) in column rod shapes is gram-positive bacteria and positive of biochemical test.

(−) in column rod shapes is gram-negative bacteria and negative of biochemical test.

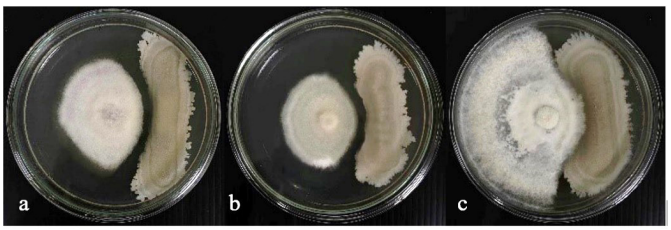

Figure 1. Inhibition zone of *Bacillus* sp. isolated SCFPSU17 with fungal pathogens: (a) *F. moniliforme*, (b) P2T2R4/2, and (c) P2T2R1/1.

Table 2. Percentage of inhibition by culture filtrate of *Bacillus* sp. isolated SCFPSU17.

Fungal pathogen	% Inhibition
F. moniliforme	38.63 ± 1.64[a]
P2T2R4/2	14.54 ± 2.09[b]

Letter a and b are statistically different in the percentage inhibition of each fungus at the 95% confidence level.

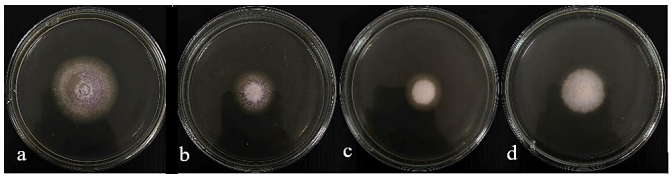

Figure 2. The growth of *F. moniliforme* that was inhibited by cell-free supernatant of *Bacillus* sp. isolated SCFPSU17 in three types of culture media compare to (a) Control, (b) NB, (c) LB, and (d) TSB.

Table 3. Percentage of inhibition by cell-free supernatant in *F. moniliforme* at different variable media.

Media	% Inhibition
NB	41.33 ± 8.98[a]
LB	32.00 ± 8.84[a]
TSB	18.67 ± 4.62[b]

Letter a and b are statistically different in the percentage inhibition of each fungus at the 95% confidence level.

Table 4. Percentage of inhibition by cell-free supernatant in *F. moniliforme* at different pH of medium.

pH of medium	% Inhibition
pH 6	22.22 ± 3.27[b]
pH 7	30.76 ± 9.41[b]
pH 8	41.88 ± 2.79[a]
pH 9	-

Letter a and b are statistically different in the percentage inhibition of each fungus at the 95% confidence level.

3.5. Optimization of Culture Medium pH for Bacterial Antifungal Production

The assessment of pH conditions for the culture medium of *Bacillus* sp. isolated SCFPSU17 revealed that growth was not possible in the NB medium at pH 9, thus excluding it from antifungal activity testing against *F. moniliforme*. However, NB medium at pH 8 showed the highest inhibition percentage of *F. moniliforme* at 41.88% ± 2.79% ($p < 0.05$), while at pH 7 and 6, the inhibition percentages were 22.22% ± 3.27% and 30.76% ± 9.41%, respectively ($p < 0.05$), as shown in Table 4 and Figures 3 and 5. Consequently, *Bacillus* sp. isolated SCFPSU17 was cultured in the NB medium at pH 8 for further experiments to determine the optimal temperature conditions for bacterial incubation.

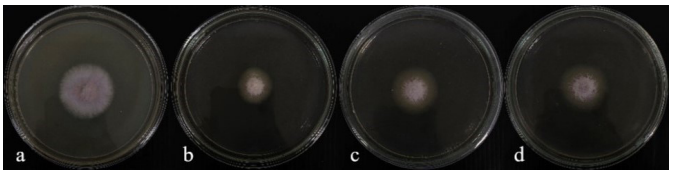

Figure 3. The growth of *F. moniliforme* that was inhibited by cell-free supernatant of *Bacillus* sp. isolate SCFPSU17 in three different pH level of NB compare to (a) Control, (b) pH 8, (c) pH 7, and (d) pH 6.

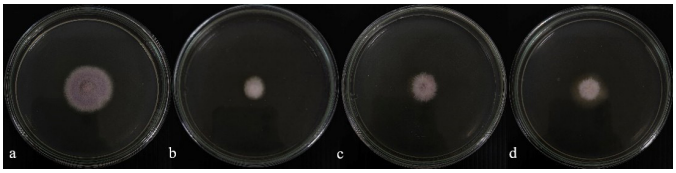

Figure 4. The growth of *F. moniliforme* that was inhibited by cell-free supernatant of *Bacillus* sp. isolated SCFPSU17 in three different temperatures of NB with pH 8 compare to (a) Control, (b) 30°C, (c) 40°C, and (d) 37°C.

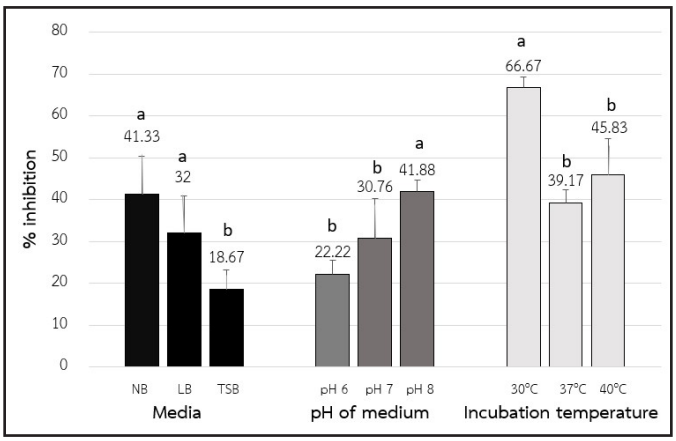

Figure 5. Percentage of inhibition by cell-free supernatant in *F. moniliforme* at different media, pH of medium, and incubation temperature.

3.6. Optimization of Incubation Temperatures for Antagonist Bacteria

The study to determine the optimal incubation temperatures for *Bacillus* sp. isolated SCFPSU17 cultured in the NB medium at pH 8; three different temperatures—30°C, 37°C, and 40°C—were tested. The results showed that *Bacillus* sp. isolated SCFPSU17 cultured at 30°C in the NB medium at pH 8 exhibited the highest inhibition of *F. moniliforme* with an inhibition percentage of 66.67% ± 2.72%. At 37°C and 40°C, the inhibition percentages were 39.17% ± 3.19% and 45.83% ± 8.77%, respectively ($p < 0.05$) (Table 5; Figs. 4 and

Table 5. Percentage of inhibition by cell-free supernatant in *F. moniliforme* at different incubation temperature.

Incubation temperature	% Inhibition
30°C	66.67 ± 2.72[a]
37°C	39.17 ± 3.19[b]
40°C	45.83 ± 8.77[b]

Letter a and b are statistically different in the percentage inhibition of each fungi. at the 95% confidence level

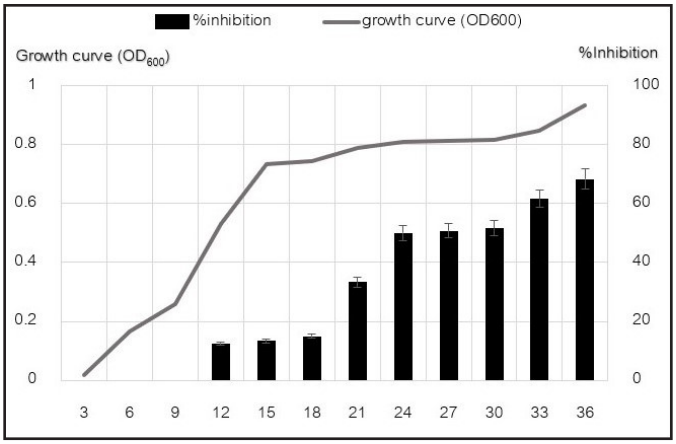

Figure 6. The growth curves and percentage of inhibition of *Bacillus* sp. isolate SCFPSU17 to inhibit *F. moniliforme*.

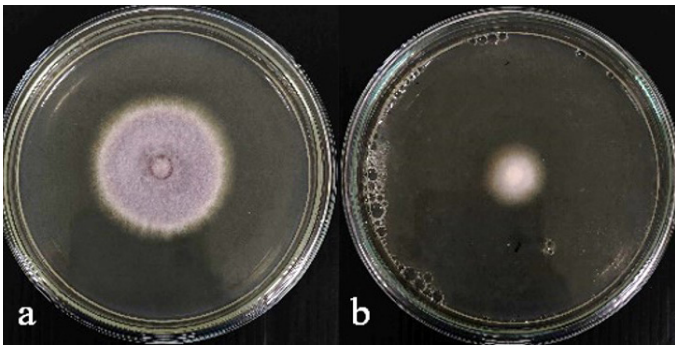

Figure 7. The growth of *F. moniliforme* that was inhibited by cell-free supernatant of *Bacillus* sp. isolate SCFPSU17 in NB with pH 6 incubated at 30°C for 36 hours: (a) Control and (b) 36 hours.

5). Therefore, *Bacillus* sp. isolated SCFPSU17 was cultured in the NB medium at pH 8 and 30°C for further experiments to determine the optimal incubation period for the production of antifungal compounds.

3.7. Optimization of Incubation Times for Bioassay

The investigation into the optimal incubation time for *Bacillus* sp., grown in the NB medium at pH 8 and incubated at 30°C, involved testing the cell-free supernatants for antifungal activity every 3 hours over a 36-hour period. The growth curves and percentage of inhibition revealed that *Bacillus* sp. isolate SCFPSU17 began to inhibit fungal growth starting at 12 hours (Fig. 6), indicating that the bacterium starts producing inhibitory compounds during the exponential phase and shows increased antifungal efficacy during the stationary phase. This suggests that the compounds produced are secondary metabolites, such as lipopeptides, including surfactin, iturin, and fengycin. At 36 hours, the inhibition percentage of *F. moniliforme* was highest, reaching 68.33% ± 1.92% ($p < 0.05$) (Fig. 7).

4. DISCUSSION

Biological control involves using naturally occurring microorganisms from the soil or plants to prevent plant pathogenic fungi [23,24]. These microorganisms can produce beneficial biological control agents, with the advantages of being environmentally friendly and representing a first step toward achieving biological impact [25]. In this study, 11 bacterial isolates were selected from soil in which corn was cultivated, and it was found that the SCFPSU17 isolate exhibited morphological characteristics and biochemical properties similar to those of *Bacillus* sp. This isolate demonstrated an ability to inhibit the growth of the fungus *F. moniliforme*, and the 16S rDNA sequencing analysis confirmed that SCFPSU17 belongs to the *Bacillus* sp. group. Consistent with previous research in earlier studies, bacteria isolated from plant root soils in Bangladesh were preliminarily identified through morphological and biochemical tests as belonging to the *Bacillus* sp., capable of inhibiting both bacteria and fungi. Additionally, these isolates inhibited pathogenic bacteria in aquatic animals, such as *Pseudomonas* spp. and *Xanthomonas* spp. [18]. Other research also isolated bacteria from the roots of healthy apple trees, identifying *B. amyloliquefaciens* based on morphological and biochemical analyses. This bacterium exhibited inhibitory effects against *F. proliferatum*, *F. solani, F. verticillioides, F. oxysporum, Alternaria alternata, Aspergillus flavus, Rhizoctonia solani, Penicillium brasilianum,* and *Albifimbria verrucaria*. It also demonstrated broad-spectrum antibacterial properties, with mechanisms that include inhibiting fungal mycelial growth and spore germination, causing abnormal swelling, wilting, rupturing, and cytoplasmic leakage in fungal hyphae [26]. Given that *Bacillus* sp. can produce inhibitory substances for controlling fungal growth, it has been reported that *B. vallismortis* can produce volatile organic compounds that effectively inhibit *F. oxysporum, F. moniliforme, F. proliferatum*, and *F. solani* while also promoting apple tree growth [27]. Additionally, *Bacillus* sp. BM2 showed an efficacy of 79.6% in controlling *F. moniliforme*, a cause of corn diseases. The chitinase enzyme test from *Bacillus* sp. isolates BM2 and BM3 showed that they produced the highest levels of chitinase, which can degrade the components of fungal hyphae [28]. Additionally, the isolate SCFPSU17 exhibits properties similar to *B. subtilis* N3 and *Bacillus* sp. strain M10, which were isolated from soil in Kanchanaburi province, Thailand. These bacteria can inhibit the growth of *Curvularia lunata* the causative agent of rust spots in orchids, and *Colletotrichum capsici*, which causes anthracnose in chili and tomato [21,29]. The *Bacillus* sp. WB, which was isolated from soil around watermelon roots, produces biocontrol agents capable of inhibiting the growth of *F. oxysporum* [5]. The previous research also isolated *Bacillus* sp. strain VIMSH06 from mangrove areas in Nakhon Si Thammarat province, Thailand, which was found to inhibit *F. solani* and *Phytophthora palmivora*, exhibiting similar properties to the isolate SCFPSU17 [7]. As mentioned above, *Bacillus* sp. has been observed to inhibit a wide range of plant pathogenic fungi and is commonly found in natural environments. This demonstrates that using this type of bacteria is safe for producers, consumers, and the environment, as it does not leave harmful residues [30]. *Bacillus* strains can be isolated from soil in naturally cultivated cornfields [18]. This study discovered *Bacillus* strains effective against fungi in the *Fusarium* sp. group, which cause diseases in corn. In Thailand, corn is widely cultivated as a key ingredient in animal feed production and

seed production, with significant exports to international markets. Thailand's ability to produce high-quality, sweet-tasting corn and its continuous development of corn varieties have gained global recognition. Therefore, using *Bacillus* sp. to address fungal diseases in corn offers a promising solution for farmers [8,9].

Optimizing the conditions for bacterial production of biological control agents to inhibit plant pathogenic fungi can enhance the effectiveness of these bacteria. This study found that cultivating *Bacillus* sp. in NB and LB media at pH 8 and a temperature of 30°C resulted in the best production of inhibitory substances against *F. moniliforme*. This aligns with previous research, which reported that *B. licheniformis* B307 cultured in the LB medium at 30°C and pH 6 produced chitinase enzymes effectively [31]. Additionally, it has been reported that cell-free supernatants of *B. subtilis* cultured in liquid media such as TSB, DeMan Rogosa and Sharpe and AM2ab can inhibit the germination of *F. oxysporum* spores, causing abnormalities such as swelling and cell wall leakage in the hyphae and spores [16]. It was also found that *Bacillus* sp. isolated from mangrove soil, when cultured in the NB medium at pH 7, was able to effectively produce substances that control the growth of *Fusarium* spp., a plant pathogen [7]. Studies on the optimal conditions for *Bacillus* sp. in producing antifungal agents generally indicate that a temperature of 30°C and pH 7–8 can increase antifungal activity by 1.2 times [32]. Many studies have reported the optimal conditions for *Bacillus* sp. to produce inhibitory substances, such as *B. cereus*, which was cultured in TSB at pH 7 and incubated at 37°C for 48 hours, effectively inhibiting the growth of *Aspergillus niger* and *Aspergillus flavus*, which cause black mold in plants [33]. Additionally, *B. amyloliquefaciens* cultured in the LB or TSB medium at 37°C for 48 hours was able to produce protease enzymes that effectively inhibited fungal growth [34]. Additionally, it has been reported that culturing *Penicillium* sp. to efficiently produce metabolites requires optimal conditions, similar to the cultivation of bacteria for use as biological control agents [35]. Optimizing the conditions for cultivating bacteria to produce inhibitory compounds can serve as a guideline for enhancing production efficiency by adjusting parameters and using predictive modeling; the most optimal values can be achieved. Applying computational techniques can improve accuracy and significantly reduce the time required for experimentation [36].

The *Bacillus* sp. group is used as a biological control agent because it can inhibit plant pathogenic fungi by producing enzymes such as chitinase, glucanase, and cellulase. These enzymes can degrade the components of fungal hyphae, thereby inhibiting fungal growth [30]. Additionally, it has been reported that *B. subtilis* can produce biological control substances such as bacillomycin, bacilycin, tasaA, and mersacidin, which can inhibit fungi of the Fusarium sp. group [37]. It can also produce surfactin A, iturin A, and fengycin, which effectively inhibit fungi such as *C. gloeosporioides*, *F. solani* KCTC 6328, *F. oxysporum* KACC 40037, and *R. solani* KACC 40151 [38]. Previous studies have reported that *B. subtilis* strain YB-05 causes significant alterations in the mycelial hyphae of pathogenic fungi, leading to abnormal enlargement, deformation, or the absence of cytoplasm within the cells. Additionally, it has been documented that *Bacillus* sp. strains can enhance plant growth. These remarkable abilities have made bacteria of the genus *Bacillus* sp., a continuous focus of scientific research and investigation [39]. Each strain of *Bacillus* sp. has different optimal conditions for producing antifungal substances. Therefore, for newly isolated strains, it is necessary to determine the optimal conditions through laboratory testing [13]. The isolation and optimization of *Bacillus* sp. for inhibiting *F. moniliforme* have a direct impact on the prevention and control of plant diseases, such as controlling *F. moniliforme*. However, there are limitations in practical application because it may not be effective in all soil types and environmental conditions. Therefore, in the future, deep learning could be utilized to enhance the efficiency of monitoring processes and identify suitable areas for applying microorganisms, leveraging deep learning techniques [10].

Although this study demonstrates the potential of *Bacillus* sp. SCFPSU17 in inhibiting *F. moniliforme* and optimizing conditions to produce bioactive compounds, further research is essential to maximize its scientific and practical impact. Future study directions include molecular mechanisms of inhibition, isolation and characterization of bioactive compounds, bioproduct development, and field applications. The study should focus on elucidating the precise molecular mechanisms through which *Bacillus* sp. SCFPSU17 exerts its antifungal effects. This includes identifying and characterizing the bioactive compounds, such as surfactins, iturins, and fengycins [39], which are known to disrupt fungal cell membranes and inhibit mycelial growth. Advanced techniques such as metabolomics and proteomics can provide deeper insights into these mechanisms. Although this study highlights the antifungal activity of *Bacillus* sp. SCFPSU17, isolating and purifying the specific compounds responsible for this activity is a crucial next step. These compounds could then be tested individually for their efficacy and stability under various environmental conditions, which would be vital for developing commercial biocontrol products [40]. To bridge the gap between laboratory research and practical implementation, field trials should be conducted to evaluate the performance of *Bacillus* sp. SCFPSU17 under real-world agricultural conditions [39]. These trials would assess the strain's effectiveness in controlling *F. moniliforme* and other plant pathogens in diverse soil types, climates, and crop systems. Additionally, studies should explore the potential of combining *Bacillus* sp. SCFPSU17 with other biocontrol agents or technologies to enhance its overall efficacy. Scaling up the production of *Bacillus* sp. SCFPSU17 and its bioactive compounds for commercial use is another critical area of research. This includes optimizing fermentation processes, developing formulations with long shelf lives, and ensuring the safety and environmental compatibility of the final product [41]. These future directions will not only expand our understanding of *Bacillus* sp. SCFPSU17 but also facilitate its adoption as a sustainable and effective alternative to chemical fungicides in agriculture. By addressing these aspects, the research can significantly contribute to the advancement of environmentally friendly crop protection strategies and support global efforts toward sustainable farming.

5. CONCLUSION

In this study, bacteria were studied and isolated from soil samples collected from a corn-growing area at the agricultural farm of Prince of Songkla University, Surat Thani Campus; a total of 17 bacterial isolates were obtained. Gram-staining and morphological studies revealed that only one isolate resembled the *Bacillus* group, identified as isolate SCFPSU17. Classification through 16S rDNA sequencing confirmed that it was *Bacillus* sp. Testing for inhibition of plant pathogenic fungi using the dual-culture method showed that this isolate could inhibit *F. moniliforme*. Further experiments to determine the optimal conditions for *Bacillus* sp. isolate SCFPSU17 in producing bioactive substances to inhibit the growth of *F. moniliforme* revealed that culturing in the NB medium at pH 8 and incubating at 30°C for 36 hours effectively inhibited the growth of *F. moniliforme*, which causes stalk rot in corn, with an inhibition rate of over 68%. Therefore, studying the optimal conditions for producing biological control substances from *Bacillus* sp. can be further developed by investigating the mechanisms of fungal inhibition,

conducting experiments to identify the types of bioactive compounds produced by *Bacillus* sp. for antifungal activity, and assessing the effectiveness of using *Bacillus* sp. SCFPSU17 on test plants under real-world conditions. Further research is essential to maximize its scientific and practical impact: expanding the application of *Bacillus* sp. to other economically important crops affected by fungal diseases; testing the stability and shelf life of the product under various environmental conditions; and analyzing the genome of *Bacillus* sp. to identify genes associated with the production of antifungal compounds. This would enhance practical application efficiency and provide a foundation for developing agricultural products for commercial markets. Such products aim to reduce the use of chemical fungicides, ensuring safety for producers and consumers, leaving no residues in the produce, and being environmentally friendly. This development not only reduces reliance on synthetic chemicals in agricultural systems but also increases the value of agricultural products and promotes long-term sustainable farming. It aligns with the growing trend of consumer preference for safe and eco-friendly products. Additionally, it presents a novel solution for sustainable agriculture in Thailand, supporting the country's future agricultural advancements.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

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13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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