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Antifungal activity of *Bacillus* sp. against *Colletotrichum* causing anthracnose in postharvest banana fruits

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ABSTRACT

One of the worst banana diseases is anthracnose due to many species of *Colletotrichum*, which influences fruit quality and decreases shelf life. Therefore, this study was conducted to elucidate the antagonistic activity of *Bacillus* sp. against *Colletotrichum* causing anthracnose on postharvest bananas. In this study, 10 fungal isolates were obtained from 12 samples of postharvest banana fruits infected with anthracnose. Bananas infected with strain COL4 (identified as *Colletotrichum gloeosporioides*) based on mycelium characteristics, conidial morphology, and internal transcribed spacer sequence analysis, satisfied Koch's postulates, with disease symptoms similar to those of naturally infected bananas. This study also isolated 25 strains of *Bacillus* from banana rhizosphere soils in Vinh Long Province, Mekong Delta. The findings demonstrated that 8 isolated strains exhibited spore inhibitory effects and that 9 isolates displayed mycelial antagonistic effects. Among them, strains M6, NA2, NA3, N5, N13, and L2 showed the strongest spore inhibition and mycelial antagonistic activities. Additionally, the bacterial strains studied also exhibited amylase, cellulase, protease, and chitinase activities. The results of 16S rRNA gene sequencing showed that NA2 and NA3 strains had a high identity with *Bacillus* in GenBank (>99%). The current findings show the potential application in post-harvest preservation of bananas of isolated bacterial strains in the future.

1. INTRODUCTION

Bananas (*Musa* spp.) are tropical fruit trees commonly grown in many countries because of their ease of cultivation, good growth, and development in many types of soil, and high nutritional value [1,2]. In Vietnam, bananas are one of the fruit trees commonly grown in the Red River Delta, the Southeast regions, and the Mekong Delta [3]. According to the Ministry of Industry and Trade (2024), bananas account for 19% of Vietnam's total fruit-growing area, with an estimated output of 1.4 million tons/year [4]. In the first half of 2024, fruit and vegetable exports bring Vietnam nearly 3.5 billion USD in revenue, with orders from many major markets such as China, Korea, and the U.S. increasing significantly [5]. Among them, bananas are 1 of the top 10 major export products (reference). The Ministry of Industry and Trade forecasts that, in 2024, banana exports will reach approximately 310–312 million USD, of which exports to the Chinese mark*et al*one will reach close to 200 million USD, accounting for nearly 30% of the country's total banana imports [5].

Anthracnose, caused by the fungus *Colletotrichum,* causes extensive damage to many valuable fruit trees both before and after postharvest stages [6]. In addition to being climacteric tropical commodities with storage and transportation constraints, bananas also have a high disease incidence, particularly after harvest [7,8]. Numerous investigations have identified *Colletotrichum musae* as the anthracnose's causative agent in postharvest bananas in many countries like Brazil [9], China [10], the Philippines [11], Vietnam [12], and Thailand [13]. This disease not only causes the fruit to ripen rapidly but also causes lesions on the fruit that reduce its sensory quality [11]. In Brazil, if *C. musae* is not managed, postharvest losses due to anthracnose can exceed 80%, especially in the early phases of harvest [9]. According to the review by Ciofini *et al*. [14], symptoms of anthracnose can lead to persistent deterioration and a decline in both quality and aesthetic standards, which can generate significant financial losses up to 30%–40% of the marketable items [14].

Currently, a variety of approaches, including traditional strategies and innovative methods, are used to control the anthracnose fungus (*C. musae)*, which causes anthracnose in postharvest fruits [14]. However, traditional treatments may have adverse effects on fruit quality, and this storage method is not suitable for most fruits [15,16]. Nonetheless, chemical approaches can effectively lower the amount of *C. inoculum* fungal growth that occurs during fruit storage [17]. However, inappropriate use of these drugs and subsequent use of similar therapies has been associated with decreased pathogen susceptibility and the emergence of resistance strains [18,19]. Furthermore, the use of fungicides in the agri-food industry poses serious risks to the environment and public health as they leave chemical residues on

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fruits and have contaminated soil, water, and non-target organisms [20,21]. Hence, many nations are paying attention to discovering sustainable ways, such as biocontrol, to reduce the drawbacks of conventional methods.

The sustainability of agriculture can be improved by applying biocontrol methods based on the inhibitory activity of yeast, fungal, or bacterial species. Among bacterial species used as biocontrol agents, *Bacillus* spp. is considered a potential candidate due to its widespread presence in many environments. Previous studies revealed that *Bacillus subtilis*, as well as its derivatives, was highly effective at reducing the severity of postharvest lesions and the incidence of anthracnose caused by the *Colletotrichum gloeosporioides* species complex and/or *Colletotrichum acutatum* [22,23]. Notably, *Bacillus* spp. have been shown to be potent contributors to the biocontrol of these pathogen species in various host species, as they are able to significantly reduce the incidence and severity of anthracnose disease [22,23]. Recently, the combination of edible coatings and bacteria such as *Bacillus* has been shown to be effective in significantly reducing fungal disease [24]. However, there are no reports on the inhibitory activity of *Bacillus* against *Colletotrichum* in postharvest bananas in Vietnam. Therefore, this study was conducted to isolate and select *Bacillus* spores that are resistant to the *Colletotrichum* that cause banana postharvest anthracnose.

2. MATERIALS AND METHODS

2.1. Isolation and Identification of *Colletotrichum*

A big, sunken, circular wound that was dark brown to black in color was one of the usual signs of the fungus *Colletotrichum*, which was identified from anthracnose banana fruits ("chuoi gia" cultivar) (Fig. 1A). The contaminated banana fruits (15 fruits) were gathered from various parts of Vietnam's Vinh Long Province, like Long Ho, Tra On, and Vung Liem districts, which are located in the Mekong Delta. As per the findings of Balendres *et al*. [11], the fungus was isolated. In summary, distilled water and flowing tap water were repeatedly used to rinse ailing banana fruit. Subsequently, the infected areas were divided into 1 × 1 cm segments and subjected to a 90-second 70% ethanol sterilization. After that, sterile distilled water was used to wash the samples three times. Afterward, these sections were put on a potato dextrose agar plate (PDA, Himedia, India) aseptically. Finally, the plates were incubated for 5–7 days at 30°C. A pure culture was obtained and kept in a PDA medium at room temperature prior to use.

2.2. Challenge Test

With a few minor adjustments, inoculation tests on healthy fruits were used to determine the pathogenicity of the isolated fungi [25]. In summary, fruits that were healthy and unharmed underwent multiple washings under running water. After that, 70% ethanol was used to sanitize the fruits' surfaces, and sterile distilled water was used to rinse them three times. The fungus *Colletotrichum* was then added to the fruits at a concentration of 10^6 spores/ml. The infected fruits were nurtured in the dark with moist tissue keeping them moist. To validate Koch's hypotheses, the pathogen was ultimately reisolated and identified from the affected region.

2.3. Fungal Identification

According to Jayawardena *et al*. [26], morphological traits, mycelial color, and features like spore shape and mycelial wall can

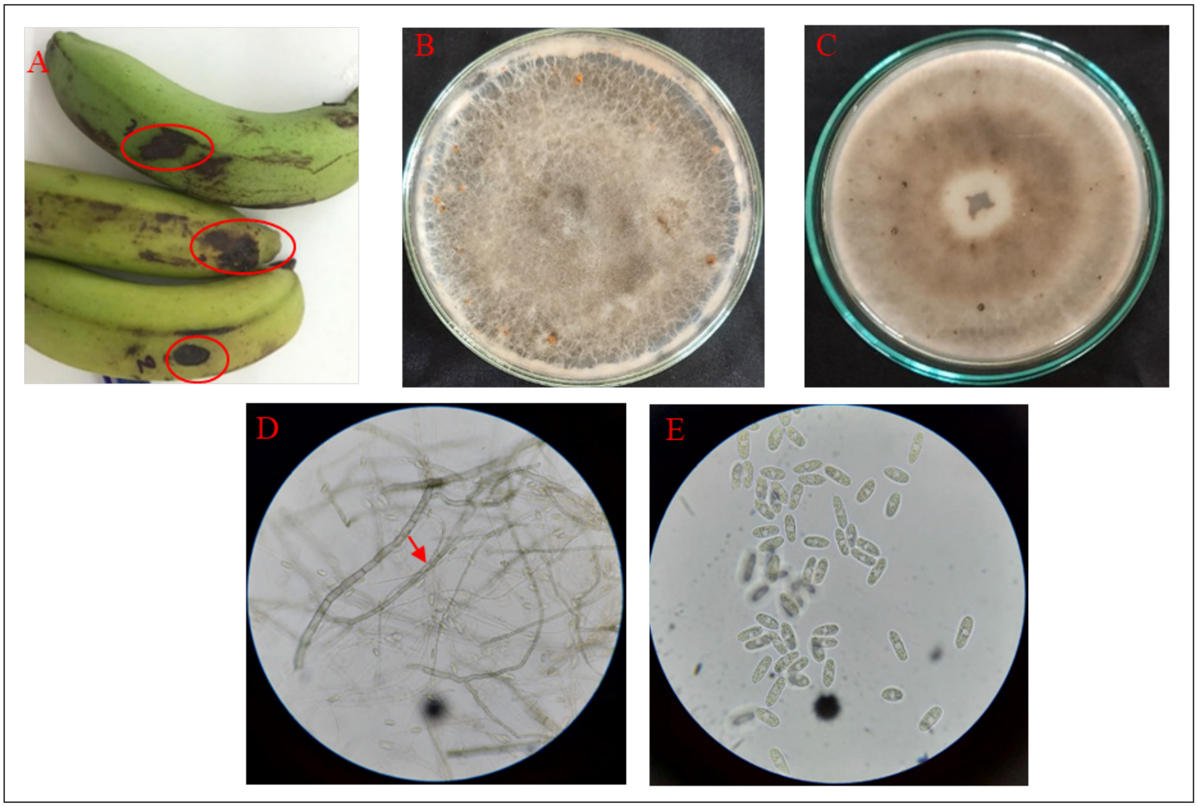

Figure 1. Morphological and spore characteristics of the fungus *Colletotrichum* isolated from Anthracnose disease bananas. A. Banana's Anthracnose Lesions (red circle); B. Upper surface of the fungus *Colletotrichum* isolate COL4; C. Reverse side of the fungus *Colletotrichum* isolate COL4; D. Fungal hyphae with septa (arrow) and branches; E. Spores of the fungus *Colletotrichum* isolate COL4.

all be used to tentatively identify *Collectotrichum*. Furthermore, using the ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer pairs [27], the isolated fungal isolates were identified by PCR. Using the Plant/Fungi DNA Isolation Kit (Norgen Biotek, Canada), fungal DNA was first isolated. When performing the extraction processes, the manufacturer's instructions were adhered to. After that, the PCR mixture (25 µl) was amplified using the following components: 2 µl of sample DNA, 8.5 µl of double-distilled water, 1.0 µl of primer ITS1 (20 pmol), and 1.0 µl of primer ITS4 (20 pmol) are combined with 12.5 µl of PCR Master Mix (Promega, USA). The initial denaturation at 95°C for 5 minutes was followed by 35 cycles of denaturation at 95°C for 50 seconds, primer annealing at 54°C for 45 seconds, extension at 72°C for 70 seconds, and a final extension at 72°C for 5 minutes. These were the conditions and cycles of the PCR. The PCR products were amplified, and then electrophoresed on a 2% agarose gel. The images were captured using Analytik Jena gel imaging equipment. Ultimately, 600 bp PCR results were forwarded to DNA Sequencing Company (Vietnam) for sequencing.

2.4. *Bacillus* Isolation

From banana rhizospheric soils, *Bacillus* was isolated according to the methods of Fan *et al*. [28], with some minor modifications. Briefly, 10 g of rhizospheric soil was heated to 80°C for 20 minutes after being diluted in 90 ml of sterile water. Next, the mixture was serially diluted to a 10^{-6} ratio. Following a streak on nutrient agar (NA, Himedia, India), a sample from each dilution was cultured for 24–48 hours at 37°C. After selecting and repeatedly cultivating suspect colonies on the same medium, the bacteria were purified. To prepare them for future research, the pure bacteria were kept at −40°C in 20% glycerol (v/v).

2.5. Inhibitory Activity of *Bacillus* Isolates

Utilizing the mycelium and spore antagonistic approach, *Bacillus*'s antifungal efficacy against *Colletotrichum* was evaluated. With respect to mycelium antagonistic activity, the dual culture method was used to evaluate mycelial growth inhibition [29]. In short, two 2 cm lines of bacteria were injected onto PDA agar plates. Subsequently, a mycelial plug measuring 0.5 mm by 0.5 mm was cut and inserted into the center of the plate's bacterial bands using a cork borer. After being incubated at 30°C for 5–7 days, the inhibitory activity was measured. As a negative control, plates containing solely mycelial plugs were employed. The percentage of mycelium inhibition relative to the control was determined according to Devi *et al*. [30]. The experiment was performed with three replicates.

The spore inhibitory activity of *Bacillus* was assessed in this work using the agar well diffusion method [31]. Briefly, *Bacillus* were cultivated in nutrient broth (NB, Himedia, India) for a full day. The culture was then centrifuged for 10 minutes at 10,000 rpm. The supernatant was then removed. A 10^6 spores/ml fungal suspension (100 µl) containing *Colletotrichum* spores was applied to the agar surface. Next, a well on PDA agar plates was punctured using a 5 mm cork borer. Subsequently, the plate was incubated at 30°C for 24 hours after the supernatant (80 µl) was added to the wells. To calculate the level of fungal inhibition, the diameters of the clear zones were measured, as was previously described. Three replicates were used in the experiment.

2.6. Extracellular Enzyme Activity

We evaluated the extracellular enzyme production capacity of *Bacillus* isolates according to Hossain *et al*. [32]. *Bacillus* isolates were cultured using liquid TSB medium (tryptone soy broth, Himedia, India) to enrich them for 24 hours at 37°C. After incubation, LB agar plates enriched with particular substrates were inoculated with the bacterial growth mixture to determine the presence of protease, cellulase, chitinase, amylase, and lipase. For the enzymes cellulase, amylase, protease, chitinase, and lipase, the substrates were 0.5% carboxymethyl cellulose, 1% starch, 1% gelatin, 5% colloidal chitin, and 1% (v/v) olive oil. After overnight incubation at 37°C, the colonies on the plates developed transparent halos, indicating the presence of enzyme activity. Three duplicates of the experiment were run.

2.7. Bacterial Identification

According to Abdullah-Al-Mamun *et al*. [33], *Bacillus* isolates were examined for morphological and biochemical traits such as spore and Gram staining, oxidase, and catalase reactions [33]. Additionally, using primers 27F (5'-AGA GTT TGA TCM TGC TCA G-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') for 16S rRNA gene fragment sequencing, they were detected by PCR [34]. Using the TopPURE® GENOMIC DNA EXTRACTION Kit (ABT, Vietnam), the isolated bacterial DNA was extracted. The manufacturer's instructions were followed when performing the extraction stages. The reaction mixture contained 10.0 µl of MyTaqTM DNA Polymerase (Bioline, Germany), 12.0 µl of double-distilled water, 0.5 µl of primer 27F (20 pmol), 0.5 µl of primer 1492R (20 pmol), and 2 µl of sample DNA were the reaction components (25 µl). The PCR cycle and conditions were as follows: 5 minutes of initial denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 1 minute, 1 minute of primer annealing at 58°C, 2 minutes of extension at 72°C, and a final 5 minutes of extension at 72°C. After amplification, the PCR products were electrophoresed on 2% agarose gel, and the images were captured using Analytik Jena gel imaging equipment. 1,500 bp PCR products were forwarded to the DNA Sequencing Company (Vietnam) for sequencing.

2.8. Statistical Analysis

Descriptive statistical were used to determine means and standard deviations (mean ± SD). Using Minitab 20 software, Tukey's multiple range tests and one-way analysis of variance (ANOVA) were utilized to assess the treatment differences with a 95% confidence level. The similarity of the sequencing results of many bacterial strains with reference *Bacillus* sequences in the NCBI database was assessed using the BLASTn program. Multiple alignments of *Bacillus* DNA sequences were performed using CLUSTAL W. A phylogenetic tree illustrating the genetic relationship between bacterial strains was constructed using MEGA X software, the neighbor-joining algorithm, and a bootstrap value of 1,000 replications [35,36].

3. RESULTS

3.1. Isolation and Identification of *Colletotrichum*

Ten *Colletotrichum* strains were isolated from banana anthracnose lesions. All the fungal strains grew quickly, covering the whole surface of the Petri dish in 5–7 days (Fig. 1). The majority of the isolates' fungal colonies produced flattened mycelia, gray-black, or milky white cottony colonies (Fig. 1B and C). The mycelium was septate, thin, and branched (Fig. 1D), and the surface of the culture medium was covered with numerous black or orange oil droplets (Fig. 1B and C, arrow). Under the microscope, the conidia of the isolates were aseptate, hyaline, straight, cylindrical, rounded at both ends, and septa free (Fig. 1E).

The selected fungal isolate, COL4, which is frequently present during isolation, passed pathogenicity tests and met Koch's postulates. Symptoms begin to appear on the fruit 5–7 days after inoculation. Banana fruits that were experimentally infected and showed typical lesions were subjected to re-isolation and identification. The necrotic and re-isolated fungus were comparable to the original ones, and the majority of the lesions were round, sunken, and dark brown in color. This isolate was selected for further study and sequencing. The sequencing results revealed that the fungal isolate COL4 was 99.75% homologous to the *C. acutatum* strain SUF147 (MT053112.1), the *Colletotrichum fructicola* isolate H29 (MT074117.1), the *C. gloeosporioides* isolate PM04 (MT071241.1), the *C. siamense* isolate DQZ3-3 (MT041653.1), and the *C. tropicale* isolate HPCS4 (PQ113781.1) in the NCBI database. The internal transcribed spacer (ITS) sequence-based phylogenetic tree revealed that they were distributed in the same cluster as the reference *C. gloeosporioides* isolate PM04 (MT071241.1) in the NCBI database (Fig. 2A and B).

3.2. Isolation of *Bacillus*

Twenty-five *Bacillus* isolates were found in soil samples collected from the rhizosphere of banana plants. The colony color was mainly milky white, and there were wrinkled large colonies, smooth small colonies, and smooth large colonies (Fig. 3A). Furthermore, the results showed that every isolate was gram-positive, long, rod-shaped (Fig. 3B), spore-forming (Fig. 3C), and positive for both catalase and oxidase (Fig. 3D and E).

3.3. Antifungal Activity of *Bacillus* Against *Colletotrichum*

Eleven out of the 25 *Bacillus* isolates exhibited spore antagonistic activity against *Colletotrichum*. Among them, three strains, NA2, NA21, and P2, showed the highest activity, with their respective inhibition zone diameters being 17.0 ± 0.0 mm, 17.0 ± 1.0 mm, and 17.0 ± 0.0 mm (Fig. 4). Of the 25 isolates of *Bacillus*, nine possessed mycelial antagonistic activity (Fig. 5), and four strains, NA3, N16, M6, and N12, showed the highest activity, with inhibitory effects

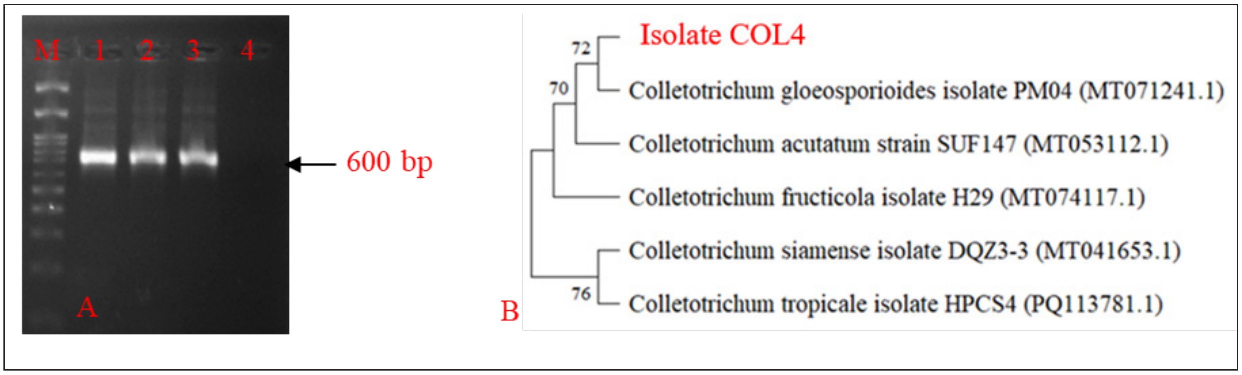

Figure 2. *Colletotrichum* identification A. Amplification results of its gene segment of fungal strains (M: 100 BP Plus standard ladder; Lanes 1–3: Fungal isolates COL4, COL4, and COL4; Lane 4: Negative control); B. The phylogenetic tree of *Colletotrichum* isolate COL4 is shown based on its sections of ribosomal dna sequences (Bootstrap values are given at branching sites).

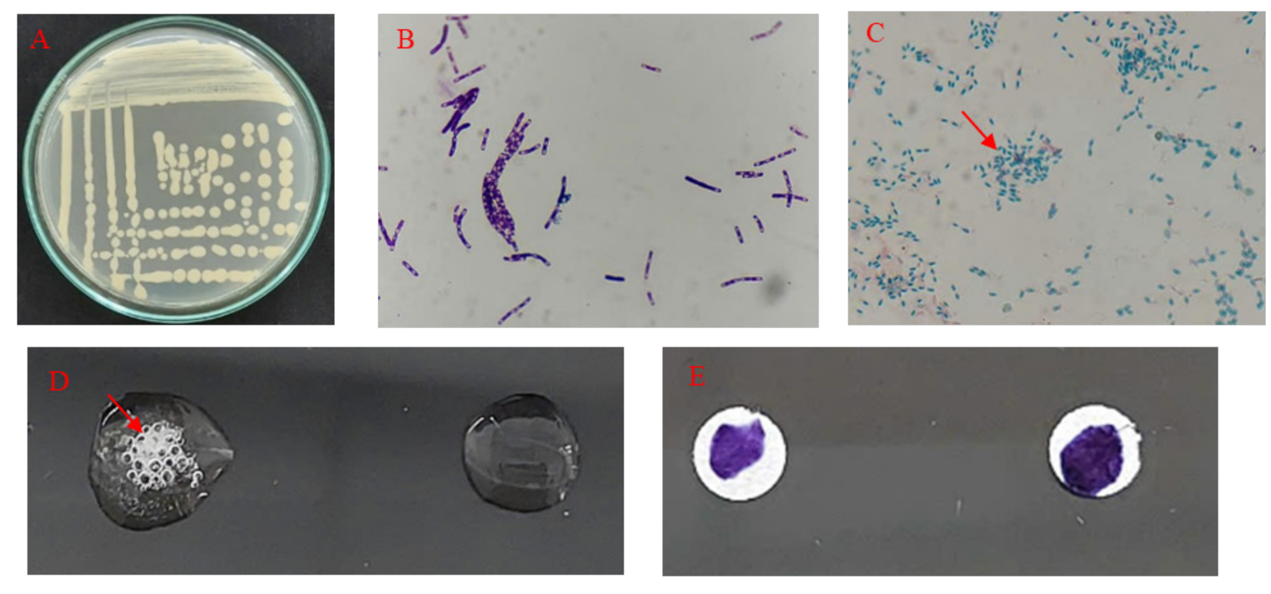

Figure 3. Morphological and biochemical characteristics of *Bacillus* derived from banana rhizospheric soils. A. *Bacillus* bacterial colonies on na medium; B. Gram staining (100X); C. Spore staining (Blue spores, arrow); D. Positive catalase activity (Bubble gas, arrow); E. Positive oxidase activity (Purple dics).

of 81.43%, 78.10%, 76.19%, and 76.19%, respectively, against *Colletotrichum*. Notably, the results also showed that six isolates, M6, NA2, NA3, N5, N13, and L2, exhibited inhibitory action against *Colletotrichum* in both spore and mycelial forms.

3.4. Extracellular Enzyme Activity

The results showed that six isolates of M6, NA2, NA3, N5, N13, and L2, which showed spore and mycelial inhibitory activity against *Colletotrichum*, possessed hydrolytic protease, cellulase, chitinase, and amylase enzyme activities. However, all these strains did not exhibit lipase enzyme activity (Table 1).

3.5. Identification of *Bacillus* Isolates From 16S rRNA Gene Fragments

The results showed that the 16S rRNA gene fragment was amplified in all of the chosen bacterial strains, with a 1,500-bp DNA band (Fig. 6A). Sequencing results showed that strain NA2 was 100% identical to *Bacillus amyloliquefaciens* (LC543399.1) and *B. subtilis* (LC543400.1), 99.89% similar to *B. amyloliquefaciens* strain B10 (OM756735.1) and *B. subtilis* strain 629 (JQ435867.1), 99.68% identical to *Bacillus* sp. strain Z80 (MG470731.1), and *Bacillus vallismortis* strain WHW30 (OK094126.1), 98.78% similar to *Bacillus velezensis* strain AB78 (MN100588.1), and 98.68% identical to *Bacillus siamensis* strain SB1001 (MG722818.2) in the NCBI database. On the other hand, strain NA3 had 100% identity with *Bacillus thuringiensis* strain TSA-10(2) (OP456643.1), *Bacillus cereus* strain ZR212 (OR294182.1), and *Bacillus pacificus* strain 2JA (MZ713004.1); 99.89% identity with *B. tropicus* strain 6TM-5 (ON000577.1), *B. toyonensis* strain FORT 40 (MG561358.1), and *B. wiedmannii* strain GB101 (OM267660.1); and 99.79% identical to *B. pacificus* strain A5 (ON819625.1), *B. albus* strain GFBI201 (ON231679.1), and *B. proteolyticus* strain D65_CV6R (MK883171.1). Strains NA2 and NA3 are classified into the same group as *B. thuringiensis* strain AB37-SW1 (MG890230.1) in GenBank according to the phylogenetic tree (Fig. 6B).

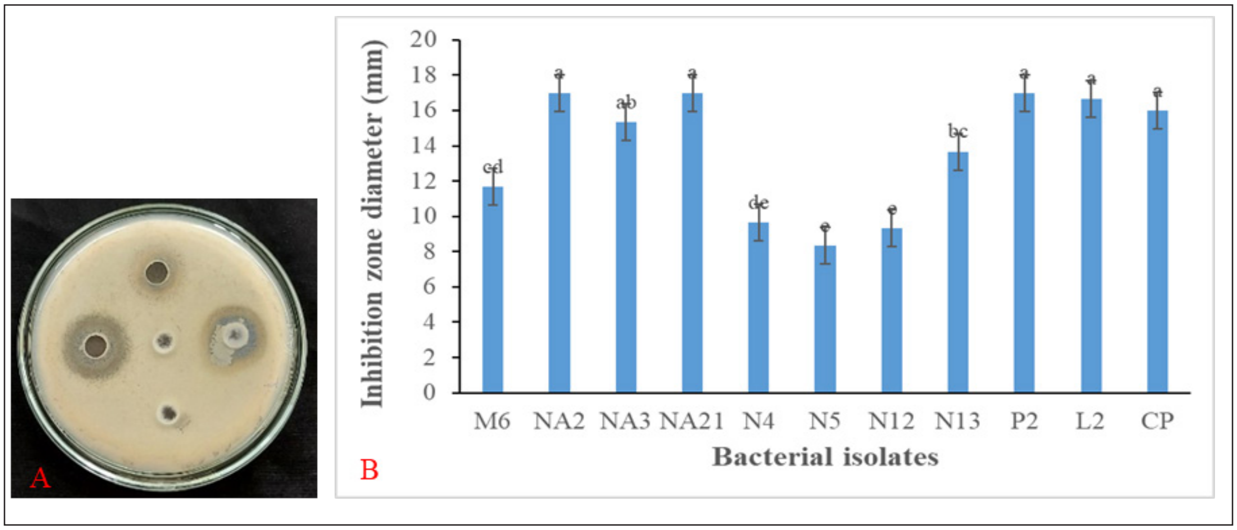

Figure 4. *Bacillus's* spore-inhibiting action against *Colletotrichum*. A. *Bacillus* isolates' antifungal activity; B. Differences in *Bacillus* isolates' inhibitory activity against *Colletotrichum* (Different letters on each bar indicate significant differences between treatments ($p < 0.05$, 95% confidence intervals).

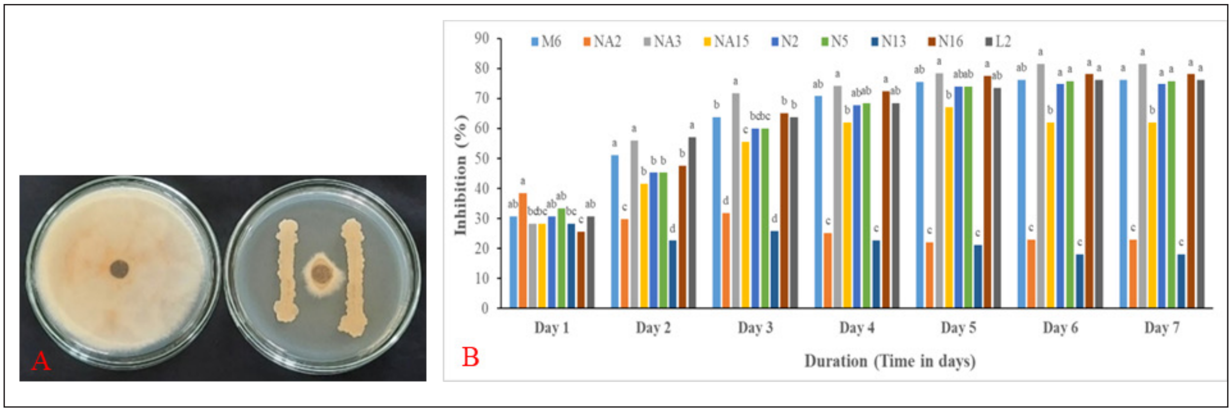

Figure 5. *Bacillus's* mycelial inhibition of *Colletotrichum*. A. *Bacillus* isolates' antifungal activity; B. Differences in *Bacillus* isolates' inhibitory activity against *Colletotrichum* (different letters on each bar indicate significant differences between treatments ($p < 0.05$, 95% confidence intervals).

Table 1. Activities of the extracellular enzymes of isolated *Bacillus* isolates.

Hydrolytic enzymes	Isolate M6	Isolate NA2	Isolate NA3	Isolate N5	Isolate N13	Isolate L2
Cellulase	+	+	+	+	−	+
Amylase	+	−	+	+	+	+
Protease	+	+	+	−	+	+
Chitinase	−	+	+	−	+	+
Lipase	−	−	−	−	−	−

*Note: +: Positive; -: Negative.

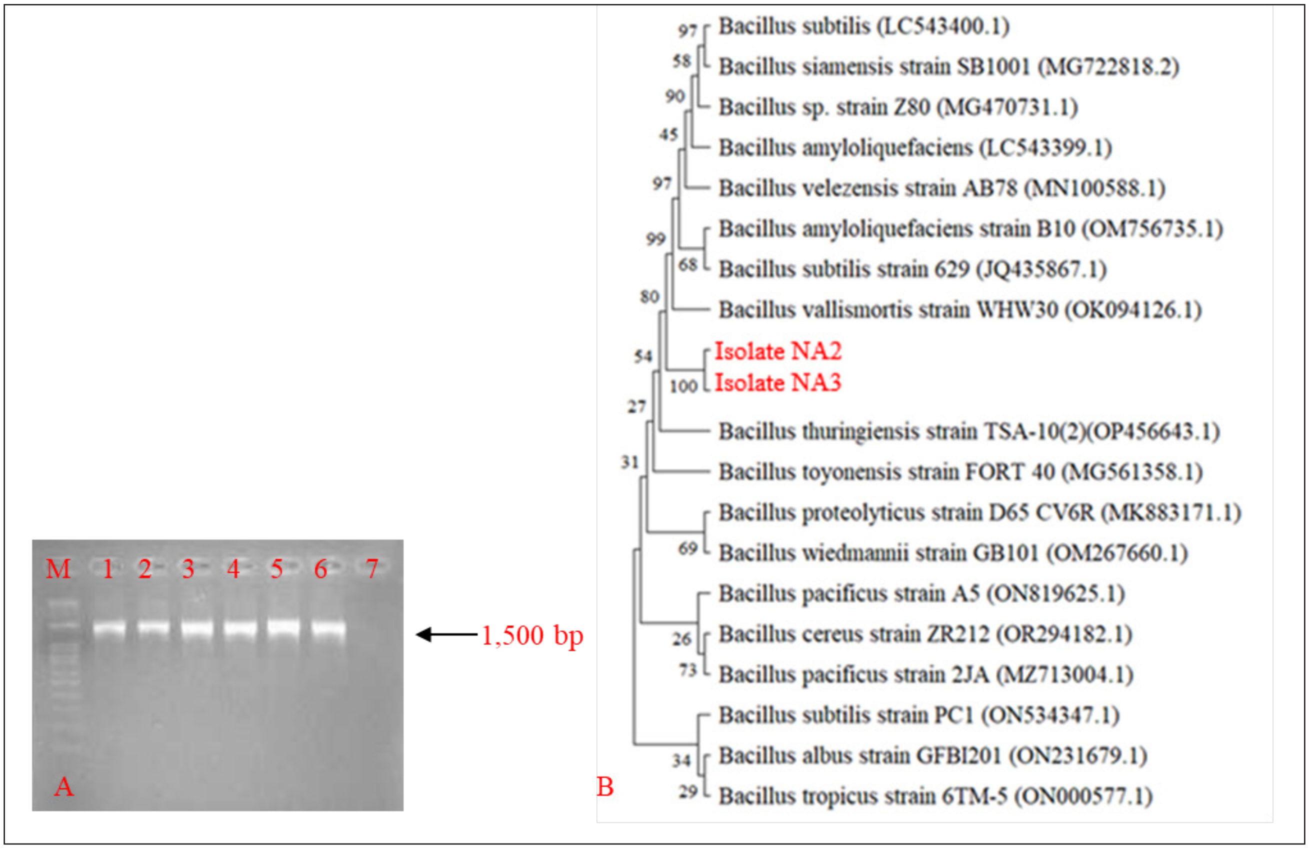

Figure 6. *Bacillus* identification by PCR reaction. A. Amplification results of the 16S rRNA gene segment of *Bacillus* (M: 100 bp standard ladder; Lanes 1-6: bacterial strains M6, NA2, NA3, N5, N13, and L2; Lane 7: Negative control); B. The 16S rRNA gene sequences of a bacterial isolate were used to construct a phylogenetic tree using reference *Bacillus* strains from GenBank (the numbers in the branches correspond to bootstrap values of 1,000 replicates).

4. DISCUSSION

In this study, 10 anthracnose isolates were isolated from banana anthracnose pathogens. This study is supported by many previous studies that showed that many climatic and nonclimatic fruits from temperate, tropical, and subtropical regions can be infected with *Colletotrichum* [6,37]. In the present findings, the isolated anthrax isolates had very diverse mycelial morphological characteristics, such as black, gray–black, or milky white cottony colonies and orange droplets on the surface of the culture medium. The mycelium is septate, thin, branched, and covered with numerous oil droplets. The conidia were aseptate, hyaline, straight, cylindrical, rounded at both ends, and septa free. These findings are consistent with the morphological characteristics of the fungus *Colletotrichum* isolated from bananas with previously described anthracnose disease. Balendres *et al*. [11] reported that the mycelia of *C. musae* from anthracnose-infected bananas were white, cottony, and aerial, which turned orange with age [11]. The conidia were cylindrical and hyaline, with round ends. According to studies conducted in Vietnam by Vy *et al*. [12], *Colletotrichum* spp. from anthracnose lesions of various banana cultivars displayed orange cottony colonies with flattened mycelia; some of them appeared as black or orange drops on the surface of the media. The mycelium had septa and several oil droplets and was hyaline, thin, and branching. The conidia lacked septa and were aseptate, hyaline, straight, cylindrical, and rounded at both ends.

Artificial COL4 strain-infected bananas presented disease symptoms similar to those of natural anthracnose disease bananas. The lesions of infected banana plants were dark brown, depressed, round, necrotic, and covered with white mycelia. These features are consistent with those mentioned by Damasceno *et al*. [9], which demonstrated that healthy bananas (*Musa paradisiaca* L.) developed black and sunken lesions with spore masses after inoculation with the pathogen *C.*

musae (Cm02). On the basis of the mycelium and spore morphological features and ITS gene sequence phylogenetic analysis, isolate COL4 was confirmed as *C. gloeosporioides*. These investigations were consistent with those of Sakinah *et al*. [38], who revealed that *C. gloeosporioides* was derived from bananas. Another study by Alam *et al*. [39] in Pakistan demonstrated that the anthracnose disease causative agent in banana is *C. gloeosporioides* [39]. However, this finding is not familiar with the document of Vy *et al*. [12], which identified *Colletotrichum* isolates from *C. musae* in Gia bananas in the Mekong Delta, Vietnam. It is thought that *C. musae* is the primary cause of most banana anthracnose cases [40]. In addition, numerous other investigations have demonstrated that the *Colletotrichum* species complex, which possesses many genetic and biological varieties, is the cause of banana anthracnose sickness, like *C. musae*, *C. cliviicola*, *C. fructicola*, *C. siamense*, and *C. karstii* [25].

In this study, 25 *Bacillus* strains were isolated from "Gia" banana rhizospheric soils. A similar report by Fan *et al*. [28] revealed that *Bacillus* was also derived from banana soils. Previous reports have shown that *Bacillus* are present in soil, especially in the rhizospheric soils of many plants [41]. Previous authors have isolated them from different crop rhizosphere soils, such as citrus [31], *Ageratina adenophora*, *Rabdosia amethystoides* [42], and wheat soils [43]. The current study's findings showed that the isolated *Bacillus's* morphological and biochemical properties match Ashwini and Srividya's [44] earlier findings. According to Kejela *et al*. [45], every *Bacillus* species that was isolated from *Coffea arabica* L.'s rhizosphere was rod-shaped, gram-positive, catalase-positive, spore-forming, and capable of withstanding temperatures as high as 80°C. Additionally, two isolates NA2 and NA3 were identified as *Bacillus,* and phylogenetic analysis revealed that this isolate clustered into the same reference *Bacillus* in the NCBI database.

In the current research, 14 *Bacillus* isolates that exhibited antagonistic activity against *Colletotrichum* were selected. According to Arroyave-Toro *et al*. [22], *Bacillus* spp. showed inhibitory effects against *Colletotrichum*, and these results are consistent with their findings. From the rhizospheric soil of an apple orchard, research by Heo *et al*. [46] revealed that *B. subtilis* isolate GYUN-2311 similarly has antagonistic effects on *Colletotrichum* [46]. In particular, the two bacterial strains in the present study have both spore and fungal mycelial inhibitory activity, with an inhibition circle diameter and inhibition efficiency of 18 mm and 67%, respectively. In Korea, *B. velezensis* CE 100 significantly suppressed the *C. gleosporioides*' growth, the causative agent of walnut anthracnose, according to the findings of Choub *et al*. [47], with spore germination and mycelial growth rates of 99.3% and 33.6%, respectively. Research by Rungjindamai [48] showed that *Bacillus* isolates extracted from healthy mango leaves and fruits could inhibit *C. gloeosporioides* by at least 60% in Thailand [48]. According to Thi *et al*. [49], two isolates, BHL21 and BHL23, showed the highest activity against papaya postharvest fruit anthracnose, with inhibition diameters of 20 mm and 18 mm, respectively. Six of the twelve *Bacillus* isolates from papaya rhizosphere soil showed antagonistic activity against spores and mycelia of the disease. Numerous studies have demonstrated the importance of various antifungal substances for biocontrol activities, especially antimicrobial lipopeptides produced by *Bacillus*, such as fengycin, surfactin, and iturin [50]. Chen *et al*. [50] discovered the genes of *Bacillus* strains from the tea rhizosphere that encode antimicrobial substances such as iturin, fengycin, subtilosin, and alkaline protease. These compounds significantly decreased the disease index of tea anthracnose and improved the growth of tea plants [51]. However, the efficiency of biocontrol is influenced by several factors like environmental circumstances (temperature, humidity, and storage conditions), host sensitivity, and pathogen virulence [50]. To better understand how these parameters affect the biocontrol efficiency of the *Bacillus* strains obtained in this study, more research must be done.

In addition to antifungal compounds, *Bacillus* also produces cell wall-degrading enzymes that have the ability to inhibit the growth or activity of other microbes [52,53]. The study also identified the hydrolytic enzymes present in the *Bacillus* strains, including protease, cellulase, chitinase, and amylase. These results are consistent with other previous studies that have shown that *Bacillus* is the source of these extracellular enzymes [54,55]. Because *C. gloeosporioides* was able to create proteases and cellulases, Wu *et al*. [56] showed that *B. altitudinis* strain GS-16 from healthy tea leaves exhibited strong antifungal activity against tea anthracnose [56]. Similarly, the phytopathogenic fungus *C. gloeosporioides*, which causes walnut anthracnose, was inhibited in its ability to produce spores and grow its mycelial growth by the crude enzyme of *B. velezensis* CE 100, which originated from the potting soil of a tomato plant and showed chitinase, protease, and β-l,3-glucanase activities [47]. Heo *et al*. [46] reported that *B. subtilis* GYUN-2311 had an antagonistic effect on *Colletotrichum*, demonstrating siderophore and phosphate solubility, as well as protease, cellulase, chitinase, and amylase [46]. Notably, *Bacillus* produces chitinases that have antifungal properties and can break down chitin, which is a component of the fungal cell wall structure of a number of pathogenic fungi, such as *Penicillium chrysogenum* [57], *Fusarium oxysporum* [58], and *Rhizoctonia solani* [59]. However, a variety of parameters, including the kind of pathogenic fungus, the bacterial strain, and environmental conditions, influence the antifungal activity of *Bacillus* species [46,60,61]. Therefore, more research is needed to elucidate the association between antifungal activity and the above variables.

5. CONCLUSION

In this study, *Bacillus* isolates with antifungal properties against *Colletotrichum* were isolated and identified from banana rhizospheric soils. Interestingly, NA2 and NA3 were the two isolates that showed the strongest antagonistic activity against *Colletotrichum*. Furthermore, the bacterial strains included in the study also exhibited amylase, chitinase, cellulase, and protease activities. According to the 16S rRNA fragment sequencing data, two strains of NA2 and NA3 were highly similar to the *Bacillus* in GenBank. The present results indicate that prospective isolated *Bacillus* strains may be used to preserve bananas after harvest.

6. 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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8. CONFLICTS OF INTEREST

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

9. ETHICAL APPROVALS

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

10. DATA AVAILABILITY

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

11. PUBLISHER'S NOTE

12. 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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