Isolation and Selection of Antagonistic Bacteria against *Cercospora arachidicola* Causing Brown Spot on Peanut

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ABSTRACT

This study was conducted with the aim to isolation bacterial strains capable of antagonism to *Cercospora arachidicola* (*Passalora arachidicola*) causing brown spot in peanut. From 3 soil samples collected in the rhizosphere of peanut grown in Tay Ninh province, Vietnam, 10 bacterial strains were found having antagonistic action with *Cercospora arachidicola*. After 5 days of testing, the antagonistic efficiency of all isolates ranged from 33.33±1.28 to 60.183±0.927%. Studying the antagonistic mechanism showed that 5/10 isolates produced siderophore, 5/10 isolates were capable of decomposing chitin, 7/10 isolates capable of decomposing cellulase and 8/10 isolates proteolytic. Basing on the results of 16S rRNA gene sequence combined with morphological and biochemical characteristics, TN-TB 4, TN-TB 6 and TN-TB 12 were identified as *Bacillus*, representing the genera *Bacillus velezensis*, *Bacillus amyloliquefaciens*, and *Bacillus pasterurii*, respectively. These strains of antagonistic bacteria have the potential to produce probiotics.

1. Introduction

The short-lived industrial plant known as the peanut (*Arachis hypogaea* L.) is indigenous to Central and South America and is a member of the Fabaceae family of legumes. Peanut is native to more than a hundred nations and contributes significantly to agricultural productivity in numerous nations, including Vietnam. In terms of planting area, yield, and export volume, peanut has traditionally ranked as the top oilseed crop. It significantly raises the total value of Vietnam's agricultural exports each year. The leaves of the peanut plant are used as cattle fodder, and peanuts are used as industrial raw materials. Additionally, because peanut can fix nitrogen, peanuts are employed as soil supplements (Dakora and Keya 1997; Mokgehle *et al.* 2014). Numerous high-yielding peanut cultivars have been developed recently, and a variety of technological techniques have been implemented, including fertilization, irrigation, planting density, pest and disease control. Although Viet Nam has many advantages in terms of natural conditions productivity and yield of peanuts have been inconsistent throughout the years. The primary cause is that, in most regions, dangerous illnesses are frequently present in peanuts. A set of foliar diseases, including black spot, brown spot, rust, leaf scorch, and others are significant pathogens on peanuts. *Cercospora arachidicola* is a widespread fungus that affects peanut leaves globally, causes peanut brown spot, which manifests itself across the field of production (Yu *et al.* 2019). Brown spot disease is a prevalent, very virulent illness. The disease primarily affects leaves and appears in the early and middle phases of peanut growth. The harm is fairly substantial. When the illness manifests, numerous round or irregular lesions with a distinct yellow halo form on the leaves. This causes early defoliation and negatively impacts the plant's capacity for photosynthetic activity. The photosynthesis of plants can be significantly reduced by brown spot, which can also encourage defoliation, drastically lowering plant production. Depending on the area, season, and time of year, as well as the stage of the plant's growth, leaf disease can cut yields by 10 to 50 percent (Ghewande *et al.* 2002).

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Using of antagonistic bacteria against fungal disease depends on the nature of the antagonistic properties and on the mechanisms of action of the organism. Bacteria are able to synthesize a wide range of metabolites to control the growth of fungi. Screening of potential antagonistic strains was made by many kinds of agar diffusion technique. Groups of bacteria are capable for resistance to pathogens on plants due to the competition about nutrition or produce some chemical substances which affect badly with fungi (Siddiqui 2006). Thus, the host plant often requires biological control to resist with pathogens (Compant *et al.* 2005). Plant Growth–Promoting Bacteria (PGPB) was recorded the ability to produce some antagonistic substances and the antibiotic release from this bacteria group often have broad spectrum effect (Siddiqui and Akhtar 2009).

As a result, the finest management practices must be implemented promptly. Preliminary study must now be done in order to tackle brown spot disease in a practical and environmentally sustainable manner. Then, to identify the strains that are most effective in containing the disease, an isolation and screening study of *Cercospora arachidicola* resistance should be conducted.

2. Materials and Methods

2.1. Materials

Soil samples were randomly collected from healthy peanut farm of the Trang Bang dictrict, Tay Ninh province, Vietnam for isolation of bacteria.

Plant explant (peanut leaves) was infected Cercospora arachidicola form some farm in Trang Bang dictrict, Tay Ninh province, Vietnam.

2.2. Methods

2.2.1. Isolation of Soil Bacteria and Fungal Pathogen

The infected peanut leaves sample might be present in the deep seated tissues. Peanut leaves specimens were sterilized in 70% alcohol for 3 minutes, followed by 5 minutes of soaking in 3% peroxide. Rinse the samples once again, three to five times, using sterile water. The infected tissue was cut into thin slices (1 × 1 cm) in PDA with purity 97%, Merck, Germany (potato - dextrose agar) plates. After incubation at 28–30°C in the dark for 2–3 days, samples had actively growing mycelium were transferred to new agar-petri dishes for determining characterized of *Cercospora arachidicola*-pathogenic fungus.

Ten grams of each sample were dissolved in 90 ml sterile distilled water then isolation of bacteria from the suspensions was carried out by using 30 µL of each bacterial suspension spreaded on nutrient agar petri dishes. Nutrient agar medium composition contains 10 g/L peptone, 5 g/L beef extract, 5 g/L NaCl, 10 g/L D-glucose, 20 g/L agar and distilled water at just pH 6.8 (Shivaji *et al.* 2006). After 24 hours of incubation at 30°C, each sample which has individual colonies developed was used for isolation until pure.

2.2.2. Antagonistic Effects against *Cercospora arachidicola* of Soil Bacteria

Biocontrol assay was done by using visual agar plate assay. The antagonistic activities to *Cercospora arachidicola* of soil bacteria on nutrient agar medium were tested to determine bacterial strains that inhibit the growth of fungal pathogen. The *Cercospora arachidicola* conidial sample was inoculated in the center of petridish, in the three places, 2 cm away from the center. The width of the clear zone around individual colony was measured every day and the significance of the inhibition ratio of each bacterial strain was calculated by this following formula (Wang *et al.* 1999):

$$I = \frac{R - r}{R} \times 100$$

Where:
I = is the inhibition ratio
R = is the average diameter of colonies in the control
r = is the average diameter of clear zone around colonies in the experimental group.

2.2.3. Enzymetic Assay of Antagonistic Bacteria

All isolates of antagonistic bacteria were submitted for screening for the production of cellulase, chitinase and protease. The petri plate was divided into four parts and 10 µL of each isolate suspension was plated on three parts in specific media. The isolates showed positive reaction which was determined by the clear zone around the colony then used for the quantitative assays.

Checking for cellulase activity: The Kasana *et al.* (2008) approach was used to evaluate the generation of cellulases qualitatively (Kasana *et al.* 2008).

Screening for chitinase activity: the activity of chitinase of antagonistic bacteria was determined based on method of Hsu and Lockwood (1975) (Hsu and Lockwood 1975).

Screening for protease activity: the bacterial isolate was streaked on skim milk agar medium which was prepared by Saran (2007) (Saran *et al.* 2007). These plates were incubated for 2 days at 37°C and detected of protease enzyme secreting organism on the agar plates. Those with halos of less than 5.0 mm were rated as weak producers (+), those with halos of 5.0 mm or more were rated as strong producers (++), and those with halos of 10.0 mm or more were rated as very strong producers (+++). The lack of a halo indicated non-producers (-).

2.2.4. Production of Siderophores by Competing Bacteria

The production of siderophores-iron chelates produced by microbes in response to low iron level in the surrounding environment (Loper and Henkels 1997) was determined using CAS-blue agar. Therefore, antagonistic efficacy of bacteria are normally related with the competition of these compounds with fungal pathogens (Loper and Henkels 1997). Determination of siderophore production assay was done following dual culture method (nutrient agar medium overlaid with CAS-blue agar) which described by Pérez *et al.* (Pérez-Miranda *et al.* 2007). A single colony of each antagonistic strain was incubated in 10 ml LB broth at 37°C for 24 hours with shaking at 250 rpm. Ten μL of the activated bacterial fluid was inoculated into each petridish containing dual culture. The sample were incubated 37°C and collected the data after 72 hours intervals. Changing color zones surrounding siderophore-producer strain would be measured every 24 hours then analyse data by statistic software.

2.2.5. Identification of Antagonistic Bacteria

Using conventional techniques based on Bergey taxonomy, three strains with potential antagonistic activities against *Cercospora arachis* were chosen and classified at the taxonomic level. Gram staining, which identifies the spore's form, size, and number, is the first stage of identification. The strains to be identified were then subjected to physiological and biochemical tests.

Antagonistic bacteria which showed highest antagonistic efficiency were selected for amplification of the 16S rRNA gene as described by Pereira (Pereira *et al.* 2006). Primers used to the target gene were 27F (5'-AGAGTTTGATCCTGGCTC-3') and 1492R (5'-TACGGTTACCTTGTTACGACT-3') (Lane 1991). The PCR reaction cycle was started at 94°C/5 min; 35 times × (94°C/90s; 52°C/1 min; 72°C/90s); 72°C/5 min The presence of PCR products was confirmed by electrophoresis on a 1.5% (w/v) agarose gel. After being checked through electrophoresis analysis, DNA extract was purified before sequencing. The resulting 16s rRNA gene sequence was blasted in NCBI Gene bank by using BLAST tool. The identification of bacteria was based on the identifying percent and query cover and some specific experiments for differentiation.

2.2.6. Statistics Analysis

All measured data (inhibition zone, enzyme activity and siderophore production) were statistic analyzed by using software–MiniTab version 16.0.

3. Results

3.1. Isolation and Confirmation of the Brown Spot Pathogen

The isolated fungi were re-inspected by injecting the fungal spores into the healthy peanut leaf and monitoring the occurrence of disease symptoms. The brown spots was observed on the site injected with fungal spores, whereas this symptom did not show in the peanut leaf treated with sterilized distilled water. This indicated that the isolated fungus was one of the causes of the brown spot disease. This fungus strain was re-purified on the PDA medium and used for further studies (Figure 1A). The morphological characters were compared with the pathogen investigated and they were found to be identical, hence the pathogen was identified as *Cercospora arachidicola*.

3.2. Identification of the Fungus Pathogen

During the course of six days of incubation, the pathogenic fungus' colony shape on PDA medium was observed. Initially white, the colony eventually turned dark, hairy, and woolly. After 3 days of incubation, the colony's diameter increased to 5.0±0.3 cm at ambient temperature, and after 6 days, it had filled a 90 mm petri dish (Figure 1A). With a light microscope, the fungal pathogen's microscopic characteristics were seen. The mycelia were branching, septate, hyaline, and brownish, according to fungus microscopic characteristics. The conidia were ellipsoidal or fusiform, orbicular, straight, thick-walled, and 0–1-septate (Figure 1B and C). *Cercospora arachis* is the fungus in question,

according to the results of the ITS gene sequencing (Figure 2). This served as more proof that the target pathogen was effectively isolated.

3.3. Antagonistic Effiency against *Cercospora arachis* of Soil Bacteria

A total of 52 bacterial isolates with different colony morphotypes isolated from 3 soil samples, collected from infected peanut farms in the Tay Ninh province, Vietnam. Among 131 isolates, 10 isolates were found to be antagonists against *Cercospora arachis* by dual-culture tests but some isolates became weaker then no longer expressed antagonistic ability. Antagonistic efficiencies of 10 selected isolates were presented in Table 1. After 5 days of testing, the result indicated that two isolates TN-TB 6 and TN-TB 4 reduced mycelia growth of *Cercospora arachis* fungus with the inhibition zones reached 26 mm and 28.5 mm and fungal growth inhibition of 58.52±1.96% and 60.18±0.93%, respectively (Figure 3). The antagonistic effectiveness of various biological control agents, and even diverse strains of the same species, has

generally been shown to vary significantly. Hence, preliminary screening is a crucial first step in the identification of effective biological control agents for the treatment of plant diseases. In the current investigation, we isolated soil bacteria with the goal of finding effective *Cercospora arachis* antagonists. As a first step in the screening process, a dual culture assay was used to determine antagonism.

3.4. The Involvement of Enzyme Activities and Siderophore Production in the Antagonistic Effects of Bacteria

The 10 isolates were tested for protease, chitinase and cellulase production. There were 8 isolates showed the capacity to produce protease, 7 isolates had strong cellulase activities in the conditions tested, and 5 isolates produced chitinase (Table 2). Seventy percent of the isolates generated cellulase. Because cellulase and chitinase are required for infection, these microbes can aid in the plant's infection. Chitinase activity and subsequently cellulase production are necessary for the initial infection. Thus, some microorganisms observed
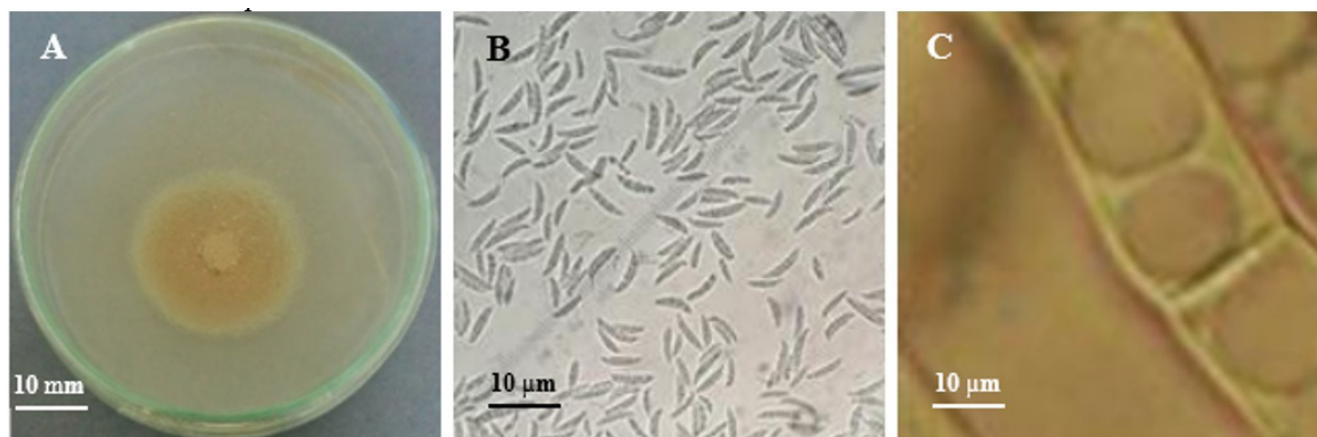

Figure 1. Morphological characteristics of the isolated *Cercospora arachis*. (A) fungal colony on PDA medium after 3 days, (B) spore shapes (400 times magnification), (c) spore shapes which were orbicular, straight, thick-walled

Figure 2. Results of a search using the BLAST tool (NCBI)

in this study can act multiple enzymatic activity rather than they only produce one kind of enzyme. The chitinase and cellulase production were lower than the protease production.

In general, the assay showed that isolate which have strongest antagonist effects, was also had highest enzymatic activities. Of the 10 isolates, the 3 isolates exhibited antifungal effects and performed clearly in enzymes and siderophore production at all four assessment, namely TN-TB 4, TN-TB 6 and TN-TB 12. These three isolates (TN-TB 4 TN-TB 6 and TN-TB 12) were among the top five that showed the largest halo zone with the radii of over 10 mm at all four experiments (Figure 4). Therefore, they were selected for further identification. The findings of this study indicated that *Cercospora arachis* biocontrol most likely involves a variety of mechanisms.

3.5. Identification of the Isolates TN-TB 4, TN-TB 6 and TN-TB 12

The colony features of 3 bacterial isolates on nutrient agar after 24 hours of incubation were dry,

white for TN-TB 4 and TN-TB 12 to orange for TN-TB 6. The colony of TN-TB 4 were irregular shape, raised, undulate margins; TN-TB 6 and TN-TB 7 were irregular shapes, smooth margin (Figure 5). The 16S rRNA gene of the two isolates (TN-TB 4, TN-TB 6 and TN-TB 12) was sequenced and aligned to other bacterial 16S rRNA genes in the GenBank database (NCBI).

Sequence analysis of the 16S rRNA genes showed that all three isolates TN-TB 4, TN-TB 6 and TN-TB 12 were identified as *Bacillus*, representing the genera *Bacillus velezensis*, *Bacillus amyloliquefaciens*, and *Bacillus pasterurii*, respectively. To reflect their origin, these strains were designated *Bacillus velezensis* TN-TB 4, *Bacillus amyloliquefaciens* TN-TB 6, and *Bacillus pasterurii* TN-TB 12 (Table 3).

For the ultimate species-level identification, morphological and biochemical studies were carried out and summarized in (Table 4 and Figure 6). Thus, the isolate TN-TB 4 was recognized as *Bacillus velezensis* based on morphological, biochemical, and 16S rRNA gene similarities.

Table 1. Fungal growth inhibition among 10 isolates (%)

Isolates	Fungal growth inhibition		
	3 days	5 days	7 days
TN-TB 4	60.18±0.93[a]	60.18±0.93[a]	58.56±0.9[a*]
TN-TB 5	61.48±0.74[a*]	37.04±0.74[d*]	27.41±0.74[d*]
TN-TB 6	65.18±0.74[a*]	58.52±1.96[b*]	39.26±1.96[c*]
TN-TB 7	37.04±2.14[d]	35.63±2.3[d]	32.29±1.04[c]
TN-TB 12	65.18±0.74[a*]	54.07±0.74[b*]	33.56±1.28[c*]
TN-TB 13	55.56±0.00[b*]	49.17±0.83[c*]	39.26±1.48[c*]
TN-TB 15	45.56±1.11[c]	44.79±1.04[c]	40.4±1.09[b]
TN-TB 22	43.7±0.74[c*]	33.33±1.28[d*]	14.07±0.74[e*]
TN-TB 26	37.04±2.14[d]	37.93±2.00[d]	38.24±0.00[c]
TN-TB 31	1.59±1.59[e*]	43.33±6.94[c*]	51.43±5.95[a*]

The letters that follow the values in the same row are statistically significant at 5%. (*) is a statistically significant difference with a confidence level >95% in each survey point

Table 2. Bacterial isolate (10 isolates of total) that showed enzyme activities and siderophore production

Isolates	Protease production	Cellulase production	Chitinase production	Siderophore production
TN-TB 4	+++	+++	+++	+++
TN-TB 5	-	-	-	++
TN-TB 6	+++	+++	+	+++
TN-TB 7	-	+	-	+
TN-TB 12	+	++	++	+++
TN-TB 13	++	+	++	-
TN-TB 15	+++	+	-	-
TN-TB 22	++	-	-	-
TN-TB 26	+	-	++	-
TN-TB 31	++	+	-	-

Symbols (+) represents effect antagonist ability and (−) no effect of antagonistic ability
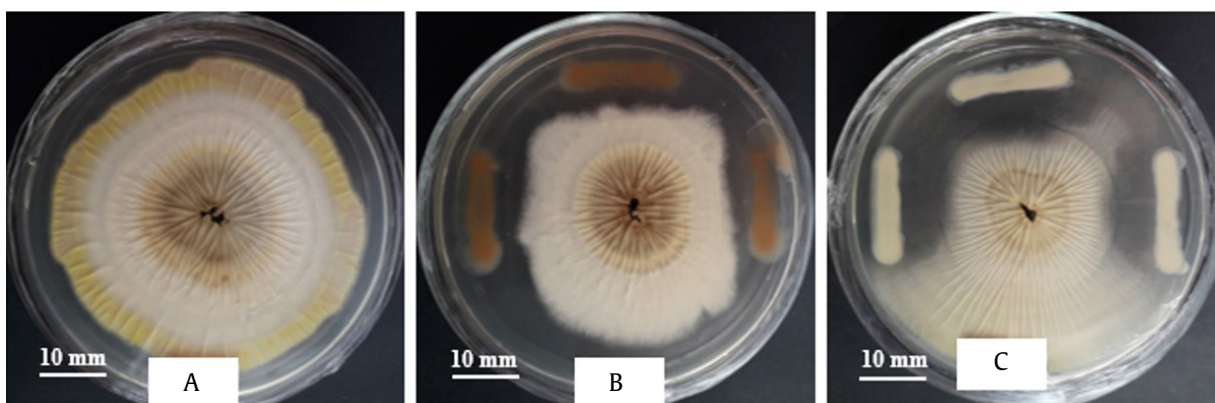

Figure 3. Antagonistic assay of (A) isolate have no antagonistic ability (control sample), (B) antagonistic efficiency of isolate TN-TB 6, (C) antagonistic efficiency of isolate TN-TB 4
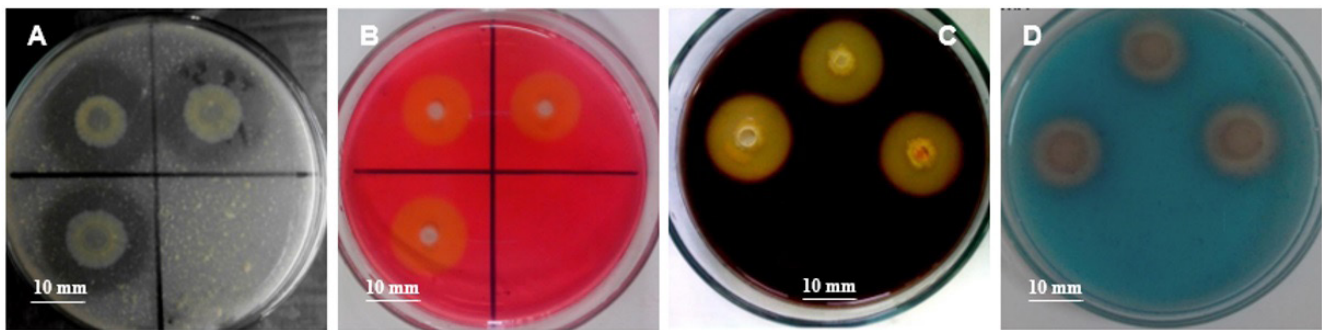
Figure 4. (A) Proteolytic ability of isolate TN-TB 4, (B) cellulose degradation ability of bacteria strain TN-TB 4, (C) chitin degradation ability of isolate TN-TB 4, (D) the halo ring represents the siderophore–producing ability of the TN-TB 4 strain
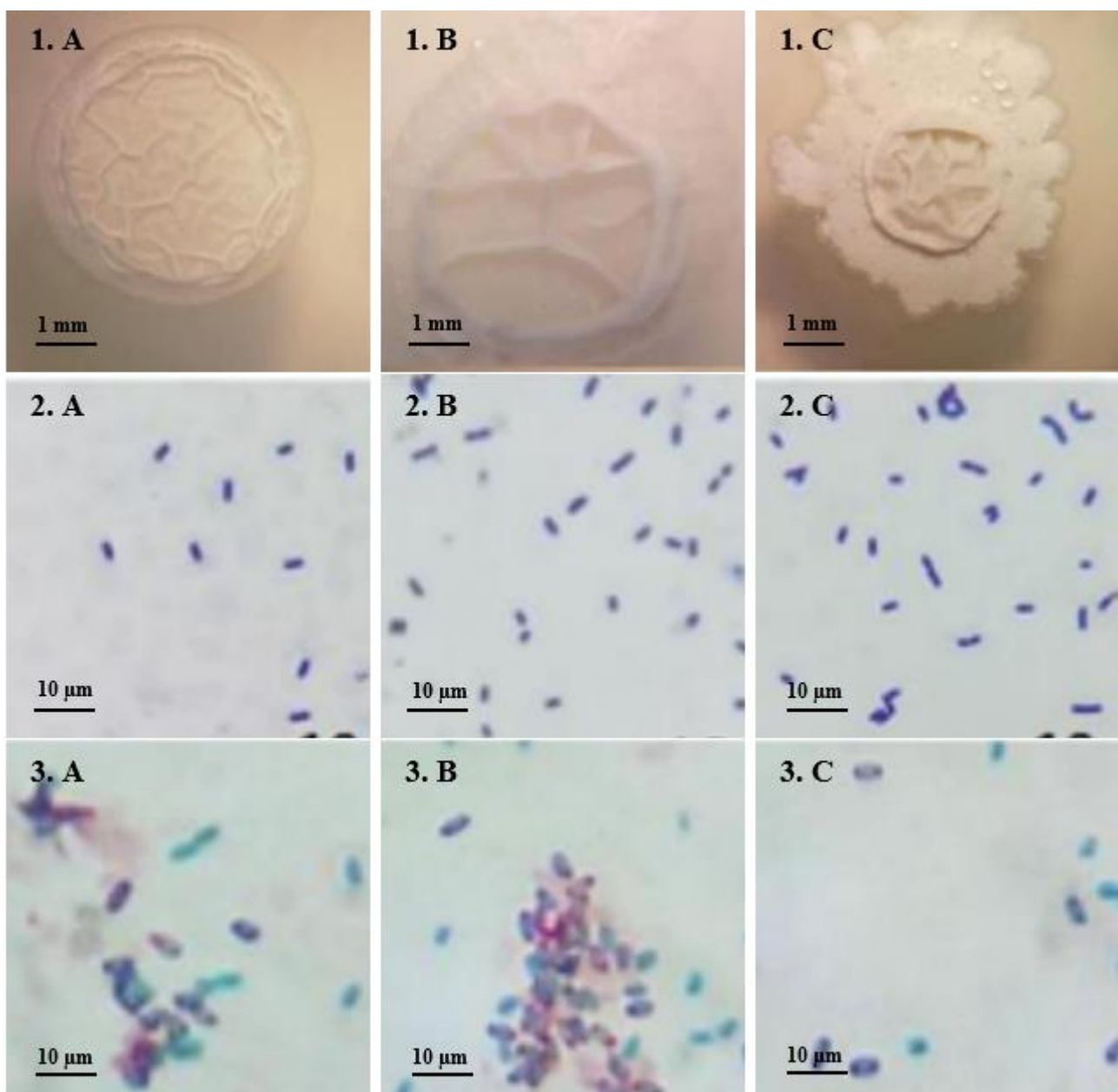

Figure 5. The morphological features of a few antifungal isolates. (A) Colony morphology, (B) gram staining, (C) endospore staining

Table 3. Preliminary identification of antagonism to *Cercospora arachidicola* strains isolated from healthy peanut farm of the Tay Ninh province, Vietnam based on 16S-rRNA gene sequences

Isolates	Identification	Homology				E-value
		Organism	Origin	Identity (%)	Accession no	
TN-TB 4	*Bacillus velezensis* TN-TB 4	*Bacillus velezensis* strain EN01	Soil, Taiwan	100	CP053377.1	0.0
TN-TB 6	*Bacillus amyloliquefaciens* TN-TB 6	*Bacillus amyloliquefaciens* strain 3820	Soil, China	100	MT538668.1	0.0
TN-TB 12	*Bacillus pasteurii* TN-TB 12	*Bacillus pasteurii*	Soil, Canada	100	U56479.1	0.0

Table 4. Biochemical and morphological characteristics of TN-TB 4

Test	TN-TB 4
Gram staining	+
Spores	Rods
Lactose Fermentation	-
Starch hydrolysis	+
Voges proskauer (VP) test	+
Cell diameter >= 1 μm	-
Citrate	+
6.5% NaCl Growth	+

Worldwide, there has been intensive research on the creation and application of *Bacillus* for biological control. There are no reports, yet, on the management of peanut brown spot by *Bacillus* species. *Cercospora arachis* was severely inhibited by *Bacillus velezensis* TN-TB 4, which was screened from a healthy peanut farm for this study. These active ingredients are crucial in suppressing bacteria and fungus.

4. Discussion

Early brown spot is one of the serious diseases that cause great economic losses to peanut production. The current control measures are mainly to select disease-resistant varieties and use chemical control agents. Biological control is considered as one of the most potential control methods because of its non-pollution and long-term effects (Dai *et al.* 2016). The way a peanut plant responds to a *Cercospora* infection depends on the host species, the pathogen biotype or species, the environmental factors, and the host's developmental stage. A sensitive plant typically produces brown to black circular lesions
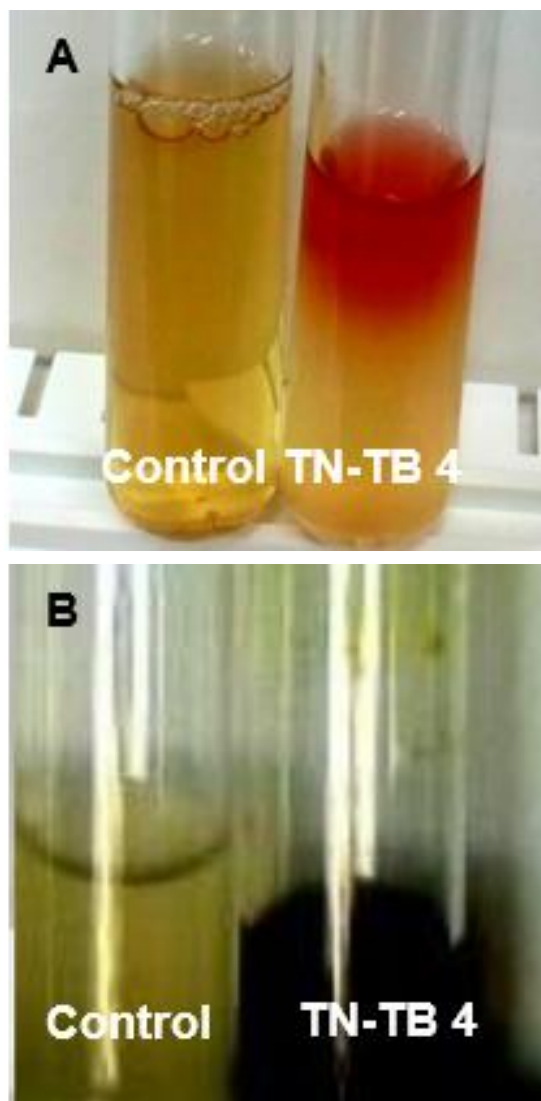

Figure 6. VP and amylase test results of isolate TN-TB 4. (A) test VP (+), (B) amylase test

that are 2 to 3 mm in diameter and frequently have a yellow tissue halo around them as a response to infection. If there are many lesions, the leaflets frequently fall off in 2 to 3 weeks (Jenkins 1938; Miller 1953; Hemingway 1957; Abdou 1966; Jackson and Bell 1969). The isolated fungus shared many of the same morphological traits as *Cercospora*, as evidenced by these morphological characteristics (Gangopadhyay *et al.* 1996; Nutsugah *et al.* 2007; Liu *et al.* 2010). Several studies have shown that many competing bacteria can prevent illnesses brought on by fungi that attack plants (Weller and Cook 1983; Fridlender *et al.* 1993; Emmert and Handelsman 1999). The production of hydrolytic enzymes and antibiotics, niche colonization and competition for host nutrients, induction of plant host defense systems, and interference with pathogenicity factors are just a few of the mechanisms used by species of *Bacillus, Pseudomonas, and Paenibacillus* to inhibit a wide variety of phytopathogenic fungi (Raupach and Kloepper 2000; McSpadden 2004; Zhang *et al.* 2004; Ongena *et al.* 2007; Larte 2022). The isolate HG54 was Gram-positive and yielded positive results in Voges-Proskauer, Starch hydrolysis, Citrate) and 6.5% NaCl Growth tests (Madhaiyan *et al.* 2010), which were in agreement with characteristics of *Bacillus velezensis* described by Ruiz-García *et al.* (2005) (Ruiz-García *et al.* 2005). On the other hand *Bacillus velezensis* give negative lactose fermentation (Fan *et al.* 2017; Adeniji and Babalola 2019) and the cell diameter lower than 1 μm (Cao *et al.* 2018). The antagonistic proteinase and chitinase are the two main active ingredients in *Bacillus velezensis's* wide range of inhibitory action (Chen *et al.* 2018). The development and use of *Bacillus* has been extensively studied in Vietnam and around the world. However, there is no report on the prevention and treatment of peanut brown spot by *Bacillus*. In this study, *Bacillus velezensis* TN-TB 4 was screened from soil, and it strongly inhibited *Cercospora arachis*. After 5 days of testing, the result indicated that *Bacillus velezensis* TN-TB 4 reduced mycelia growth of *Cercospora arachis* fungus with the inhibition zones reached 28.5 mm and fungal growth inhibition of 60.18±0.93%.

Bacillus velezensis is an aerobic, Gram-positive, endospore-forming bacterium that promotes plant growth. Many strains of this species have been reported to inhibit the growth of microbial pathogens including bacteria, fungi, and nematodes. Genome analysis revealed that *B. velezensis* possesses strain-specific gene clusters related to the biosynthesis of secondary metabolites that play important roles in pathogen suppression and plant growth promotion. In particular, *B. velezensis* displays a high heritability for the synthesis of cyclic lipopeptides (i.e., surfactin, bacitracin-D, fengmycin, and bacillibactin) and polyketides (i.e., macrolides, bacillaene, and difficidin). Secondary metabolites produced by *B. velezensis* can also trigger plant-induced systemic resistance, the process by which plants resist repeated attacks by virulent microorganisms. *B. velezensis* has been formulated into the commercial fungicide Botrybel (Agricaldes, Spain) because of its activity against *Botrytis cinerea*, a *Botrytis cinerea* pathogen reported to affect more than 200 plant species worldwide (Romanazzi and Feliziani 2014).

In this study, we investigated the properties of *Bacillusvelezensis* TN-TB 4 asaplantgrowth-promoting rhizobacteria. *B. velezensis* TN-TB 4 can effectively inhibit *Cercosporium arachis*, produce siderophores, and degrade proteins. It was previously reported that *B. velezensis* LM2303 disrupts cell membrane permeability of *Fusarium graminearum* via cyclic lipopeptides (fengycin, iturin, surfactin) (Chen *et al.* 2018). Several studies have shown that *B. velezensis* can produce a variety of metabolites associated with disease resistance, including antimicrobial proteins, lipopeptide antibiotics, polyketides, siderophores, and NH3 (Stein 2005; Chen *et al.* 2007; Meng *et al.* 2016; Kim *et al.* 2017; Adeniji and Babalola 2019). *B. velezensis* FZB42 has been reported to produce high levels of siderophores, which inhibit fungi by depleting essential iron (Rabbee *et al.* 2019).

Increased use of chemical fertilizers and pesticides has led to the accumulation of chemical residues in the environment, and disease-causing microorganisms have begun to develop resistance. In order to avoid these adverse effects, the most important thing is to use biological agents, such as biological fertilizers and biological pesticides. Among the closely related *Bacillus* species, *B. velezensis* has attracted much attention as a valuable biocontrol agent. Therefore, understanding the antibacterial potential of *B. velezensis* biosynthesis is becoming increasingly important in order to develop and formulate biobased products. A further crucial step for boosting the synthesis of metabolites by advantageous microorganisms and allowing metabolic engineering is the elucidation of the genes responsible for bioactive secondary metabolites and

the capability to control such genes. *B. velezensis* may serve as a useful and potent biocontrol agent that can be utilized in place of synthetic agro-chemicals as an efficient substitute.

After identification, the results showed that strain TN-TB 4 was *Bacillus velezensis*. The use of *Bacillus velezensis* as a biocontrol agent with an antagonistic impact on a fungus pathogen on peanut leaves appears to be new. The first and most important phase in biological control is the selection of potent antagonistic organisms. According to the findings of this investigation, the *Bacillus velezensis* TN-TB 4 isolate exhibits antagonistic behavior, likely as a result of enzymatic activity, which has been shown to be an efficient strategy in regulating the fungal disease *Cercospora arachis*. These findings and additional research will aid in the development of *Bacillus velezensis* isolates as a viable biological control agent against *Cercospora arachis*, which *in vivo* conditions causes brown spots disease on peanuts.

In conclusion, we isolated and identified the strain named TN-TB 4 which were a *Bacillus velezensis*. This appears to be the first report of *Bacillus velezensis* as a biocontrol agent which can antagonist effect on fungal pathogen on peanut. The selection of effective antagonistic organisms is the first and foremost step in biological control. In this study, it is concluded that the *Bacillus velezensis* TN-TB 4 isolate is showing antagonistic property probably through the enzymatic activities, which has been proved to be an effective mechanism in controlling the fungal pathogens-*Cercospora arachis*. These observations and further studies will help in developing *Bacillus velezensis* isolate as a potential biological control agent against *Cercospora arachis* which cause brown spots disease on peanut in *in vivo* condition.

Acknowledgments

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