ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL ANTAGONISTS OF THE DRAGON FRUIT FUNGAL PATHOGEN *Neoscytalidium dimidiatum*

NGUYEN NGOC AN[1], HUA HUYNH MINH THAO[1], HO NGUYEN HOANG YEN[1], NGUYEN THI DIEU HANH[1], NGUYEN LE HIEN HOA[1], TRAN THI THANH TIEN[1], BUI THI LUYEN[2], PHAM TAN VIET[1*]

[1]*Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Viet Nam*

[2]*Faculty of Biology and Biotechnology, Vietnam National University Ho Chi Minh City - University of Science, Ho Chi Minh City, Vietnam*

phamtanviet@iuh.edu.vn

Abstract. Dragon fruit or pitahaya (*Hylocereus* spp.) are famous for their nutrient-rich favourable taste, which brings high economic value to subtropical and tropical countries. However, dragon fruit cultivation all over the world is threatened by fungal pathogens and among them, *Neoscytalidium dimidiatum* has recently been shown to be responsible for stem canker and fruit rot which cause big economic losses. In order to find an environmentally friendly way to control this pathogen, five out of sixty-nine bacterial isolates used in a screening test for antifungal activity were selected. All five strains appeared to be aerobic Gram positive spore forming bacteria suggesting that they all belong to the *Bacillus* genus. Cell-free culture supernatants of these strains were found to strongly inhibit both fungal spore germination and mycelia growth *in vitro* for at least 5 days. The strain D19 which possessed the highest antagonistic effect was further identified to be *Bacillus amyloliquefaciens*, a well-known species shown to have antifungal effect against several other pathogenic fungi. Thus, the results of this study opened a new promising perspective to prevent *Neoscytalidium dimidiatum* infection during cultivation of dragon fruit.

Keywords. Dragon fruit, *Neoscytalidium dimidiatum*, *Bacillus* antagonist, antifungal activity

1 INTRODUCTION

Dragon fruit or pitahaya (*Hylocereus* spp.) which belongs to the *Cactaceae* family, are cultivated in subtropical and tropical countries throughout the world. They are well-known and have high demand in not only national but also international markets of 40 countries and territories due to their favorable mildly sweet light sour taste and rich in linoleic acid, an essential fatty acid [1]. In Vietnam, the three provinces Tien Giang, Long An, and Binh Thuan account for more than 95% country's dragon fruit output, which makes the country one of the most famous and leading exporters of dragon fruit. Despite its high economic value, dragon fruit cultivation all over the world is currently threatened by insect pests, viruses, enterobacteria, nematodes, and especially fungal pathogens which cause mass yield losses [2].

The majority of dragon fruit fungal pathogens belongs to the *Colletotrichum*, *Bipolaris*, *Fusarium* genera and more recently, the emergence *Neoscytalidium* (*Scytalidium*) genus [3, 4]. The two ascomycetous fungi *Neoscytalidium dimidiatum* and *Scytalidium hyalinum* have been reported to be endemic opportunistic pathogens since it can cause nail, skin and lung infections in animal model as well as human in subtropical and tropical regions [5-7]. In addition, recent reports have raised great concerns about serious losses due to stem, wood canker and fruit rot caused by *Neoscytalidium dimidiatum* in not only dragon fruit but also grapevine and recently, almond tree cultivation [8-11]. Vietnam is a tropical country with high temperature and humidity and such conditions is very favourable for the growth and infection of this fungal pathogen which can persist for a long period.

Chemical fungicides have long been widely used in agriculture, which raises many concerns about their toxic residues which are the cause of rising of pathogen resistance, cross-species killing as well as potentially harmful to human health [12]. As a result, more and more projects have been carried out in order to control fungal pathogens by other environmentally friendly methods. One of the ideas is to take advantage of antagonistic bacteria and species belonging to the *Bacillus* genus has been shown to be good candidates [13, 14]. Therefore, this study aims to search for bacterial antagonists of *Neoscytalidium*

dimidiatum which could subsequently be used as one of the effectively safe alternative ways to control this dragon fruit pathogen.

2 MATERIALS AND METHODS

Isolation of dragon fruit fungal pathogen

Infected stem and fruit samples of *Hylocereus* spp. were collected from dragon fruit farms in Binh Thuan Province, Vietnam. All the experiments in this study were carried out at the Microbiotechnological Laboratory of the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City. The fungal pathogen was isolated by inoculating small pieces of infected stems and fruits (~1x1mm) in PDA (potato-dextrose agar) plates at room temperature for 3-4 days. Suspected fungal pathogen was subsequently further purified on PDA with the same conditions described above. The purified fungal pathogen was grown on PDA plate for 7 days at room temperature and spores was collected by adding to the plate 5 ml of sterilized NaCl 0.9%, gentle swirling a few times then recuperating the spore suspension. Spore concentration was determined using a Neubauer cell chamber. Healthy dragon fruits were prepared by soaking in chlorine 100 ppm solution for 5 minutes and the surface was subsequently cleaned with ethanol 70%. The fungal pathogen was re-checked for its pathogenicity by injecting 10 µl spore suspension (10^4 spores/ml) on the surface of prepared healthy fruit (not more than 1 mm in depth) then kept in a humidity-maintained plastic box at room temperature and the result was checked after 4 days of incubation. Sterilized distilled water was used as a negative control. The fungus which caused dragon fruit rot was re-isolated using sterilized pipette tips spotted on the lesion areas and spread on the potato-dextrose agar (PDA) dish. The cultured dishes were incubated at room temperature for 3 days and the growth and morphological characteristics of isolated fungi were subsequently recorded.

In vitro screening of fungal antagonistic bacteria

A total of 69 soil bacterial isolates were subjected to anti-fungal activity screening. Each strain was cultured overnight in Luria-Bertani (LB) medium at 37ºC. For this experiment, 10 µl of spore suspension (10^6 spores/ml) was spotted in the center of a PDA dish and 10 µl of each bacterial overnight culture was then streaked 2 cm away from the center. Fungal inhibitory activity was determined by comparing the length of the mycelial growth between parts with and without bacterial streak after 2 days of incubation at room temperature. Fungal colony development and inhibitory zones were subsequently monitored after 5-days and 10-days periods [15]

Effect of bacterial cell-free culture supernatant on the fungal spore germination

The fungal pathogen was cultured in PDA plates for 7 days at room temperature and spores were collected and suspended in potato-dextrose broth (PDB) (10^7 spores/ml). Each of the 5 antifungal strains were grown in 5 ml of LB broth at 37ºC with shaking at 180 rpm until the OD_{600nm} reach 0.6. The bacterial culture supernatants were recovered by centrifugation at 13,000 rpm for 20 minutes at 4ºC. The effect of culture supernatants on the spore germination was examined by incubating at 37ºC a mixture of equal volume (1 ml) of spore suspension and culture supernatant of each antifungal strain. The control was designed by using LB broth instead of bacterial supernatant. Conidia germination was examined under light microscope every 2 hours until germ tubes are observed.

Effect of bacterial cell-free culture supernatant on the mycelial growth

Fungal spores (10^7 spores/ml) were incubated in PDB in 8 hours at 37ºC for germination then 1 ml of germinated spores was mixed with 1 ml of the supernatant of each antifungal strain prepared as described above and incubated at 37ºC. Mycelial growth in PDB with or without bacterial supernatant was observed and compared under light microscope every 2 hours for a total of 6 hours. The mixture was subsequently spread on PDA plates and incubated at 37ºC for 5 days to observe further growth of mycelia.

Identification of fungal pathogen and Bacillus antagonists

The fungal pathogen was identified by examining its macroscopic and microscopic characteristics as well as sequencing the 18S rRNA gene using a couple of primer (F1A-5'-AACCTGGTTGATCCTGCCAGT-3' and R564-5'-GGCACCAGACTTGCCCTC-3') (Bioneer Corporation, Seoul, South Korea) [16]. The bacterial strain D19 which displayed highest antifungal activity were identified based on examined cultural and physiological characteristics, and further confirmed by 16S rRNA gene sequencing using the bacterial universal primer pair: 27mF (5'-AGAGTTTGTTTGATCMTGGCTCAG-3') and 1492mR (5'-GGYTACCTTGTTACGACTT-3') (Bioneer Corporation, Seoul, South Korea) [17]. PCR was done for

both 18S and 16S rRNA amplifications at 95°C-5 minutes, 30 cycles of (95°C-30 seconds; 55°C-40 second; 72°C-90 second), and 72°C-5 minutes using Bio-Rad MyCyler Thermal Cycler PI-MC. Amplified products were Sanger sequenced by Animal biotechnology laboratory, Konkuk university, South Korea. Sequencing results were compared with nucleotide databases on National Center for Biotechnology Information (NCBI) by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

3 RESULTS AND DISCUSSION

Isolation and confirmation of the dragon fruit fungal pathogen

The isolated fungi were re-inspected by injecting the fungal spores into the healthy dragon fruit and monitoring the occurrence of disease symptoms. The brown spots, fruit rot was observed on the site injected with fungal spores, whereas this symptom did not show in the dragon fruit treated with sterilized distilled water (Figure 1). This indicated that the isolated fungus was one of the causes of the fruit rot disease. This fungus strain was re-purified on the PDA medium and used for further studies.

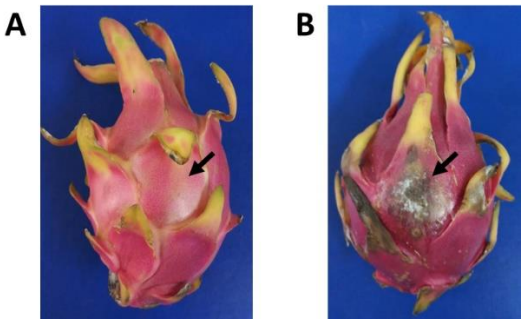

Figure 1. Pathogenicity of isolated fungus from infected dragon fruit. (A) Healthy dragon fruit injected sterilized distilled water. (B) Healthy dragon fruit injected with the fungus isolated from infected rots (arrows show the injected sites)

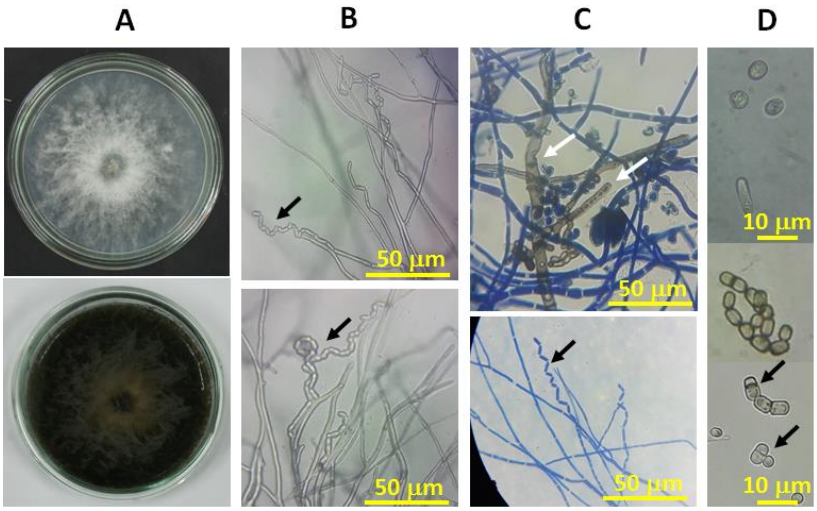

Figure 2. Morphological characteristics of the isolated *Neoscytalidium dimidiatum*. (A) Fungal colony on PDA medium after 3 days (above) and 6 days (below). (B) Arthroconidia chains forming at the head of hyaline hyphae (black arrows). (C) The fungal mycelia stained with Methylene blue and ascospores forming from brownish hyphae (white arrow), arthroconidia chains (black arrows). (D) The difference of spore shapes which were orbicular, straight, thick-walled, and 0-1-septate (black arrows).

Identification of the fungus pathogen

Colony morphology of pathogenic fungus on PDA medium was observed during 6 days of incubation. The colony was white, and gradually became black, hairy and wooly. The colony diameter was reached up to 7.0±0.5 cm at room temperature after 3 days and filling a 90 mm Petri dish after 6 days of incubation (Figure

2A). Microscopic features of the fungal pathogen stained with Methylene blue solution were observed under light microscope. Fungal microscopic features showed that the mycelia were branched, septate, hyaline and brownish. The hyaline hyphae were constricted into spore chains (Figure 2B) and separated to become arthroconidia whereas the brownish hyphae produced ascospores (Figure 2C). The conidia were orbicular, straight, ellipsoidal or fusiform, thick-walled, and 0-1-septate (Figure 2D). All these morphological characteristics showed that the isolated fungus possesses the same features with *Neoscytalidium* [8, 9, 11]. The result of 18S rRNA gene sequencing has confirmed that this fungus is *Neoscytalidium dimidiatum* (Table 1). Indeed, *Neoscytalidium dimidiatum* has been shown to be responsible for not only dragon fruit rot but also stem canker [8, 11]. This once again confirmed that we have successfully isolated the target pathogen.

Table 1. Sequencing result of fungal pathogen *Neoscytalidium dimidiatum*

Primer	Partial 18S rRNA sequence	Species	Ident (%)
F1A	GCCAGAAAGCCATGCATGTCTAAGAAAAGCAATCTATA CTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTT TATTCGATAGTACCTTACTACTTGGATAACCGTGGTAAT TCTAGAGCTAATACATGCTAAAAACCCCGACTTCGGGA GGGGTGTATTTATTAGATAAAAAACCAATGCCCTTCGG GGCTCCTTGGTGATTCATGATAACTTAACGAATCGCATG GCCTTGCGCCGGCGATGGTTCATTCAAATTTCTGCCCTA TCAACTTTCGATGGTAGGATAGTGGCCTACCATGGTATC AACGGGTAACGGGGAATTAGGGTTCTATTCCGGAGAGG GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGC AGGCGCGCAAATTACCCAATCCCGACGCGGGGAGGTAG TGACAATAAATACTGATACAGGGCTCTTTTGGGTCTTGT AATTGGAATGAGTACAATTTAAATACCTTAACGAGGAA CAATTGGAGGGT	*Neoscytalidium dimidiatum* CBS 251.49	99.41
R564	CGACACTCGGATCCTTTCCATTCAACGGGAACCCAAAA GAGCCCTGTATCAGTATTTATTGTCACTACCCCTCGCGT CGGGATTGGGTAATTCCGCGCGCCTGCTGCCTTCCTTGG ATGCGGTAGCCGTTTCTCAGGCTCCCTCTCCGGAATAGA ACCCTAATTCCCCGTTACCCGTTGATACCATGGTAGGCC ACTATCCTACCATCGAAAGTTGATAGGGCAGAAATTTG AATGAACCATCGCCGGCGCAAGGCCATGCGATTCGTTA AGTTATCATGAATCACCAAGGAGCCCCGAAGGGCATTY GGTTTTTTATCTAATAAATACACCCCTCCCGAAGTCGGG GTTTTTAGCATGTATTAGCTCTAGAATTACCACGGTTAT CCAAGTAGTAAGGTACTATCAAATAAACGATAACTGAT TTAATGAGCCATTCGCAGTTTCACAGTATAGATTGCTTA TACTTAGACATGCATGGCTTAATCTTTGAGACAAGCATA TGACTACTGGCAC	*Neoscytalidium dimidiatum* CBS 251.49	98.75

In vitro screening of antifungal bacteria

The screening of antifungal bacteria against *N. dimidiatum* from 69 bacterial isolates showed that there are 6 isolates named D5, D7, D11, D19, TL1, and TL2 displayed a varied inhibitory activity ranging from 62.5 ± 0.4% to 88.0 ± 1.1% inhibition rates for the mycelial growth after 2 days of incubation (Figure 3A and 3C). Moreover, after 5 days and 10 days of incubation, all the 6 bacterial isolates showed clear mycelial inhibitory zones. The biggest inhibitory zone was formed by D19 (Figure 3B and 3D) while TL2 performed weaker antifungal activity (62.5 ± 0.4%) after 2 days of incubation and formed small inhibitory zone (0.4 cm) after 5 days and 10 days of incubation. Therefore, the TL2 strain was removed from further studies. The five isolates D7, D7, D11, D19 and TL1 which showed over 70% antifungal activity will be used for biological characterization and biocontrol activity assay.

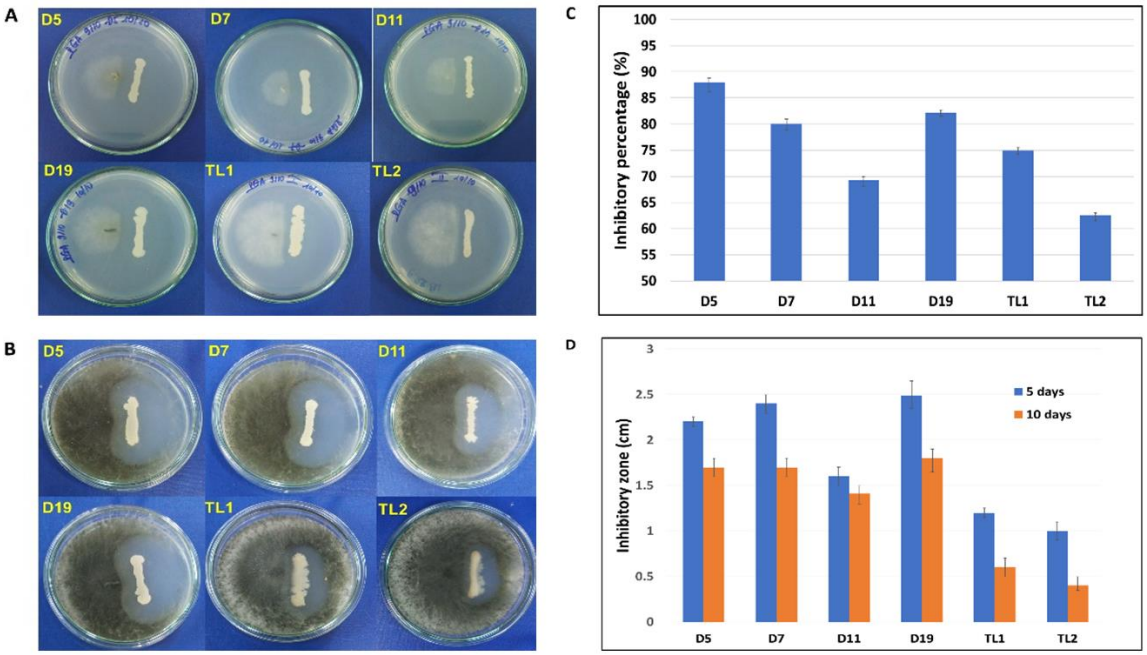

Figure 3. Antifungal activities of bacterial isolates. The inhibitory activity of isolated bacteria on the mycelial growth after 2 days (A) and 5 days (B) of incubation on PDA medium. The inhibitory percentage (C) and inhibitory zone after 5 days and 10 days of incubation (D).

Identification of antifungal bacteria

The colony features of 5 bacterial isolates on LB agar after 48 hours of incubation were dry, white for D19 to cream-colored for D5, D7, D11, TL1. The colony of D19 were irregular shape, raised, undulate margins; D5 and D7 were irregular shapes, smooth margin. The colonies of D11 showed the round shape, smooth margin, raised with spreading edge, whereas the TL1 was irregular shape, lobate margin, raised with spreading edge (Figures 4A). Microscopic features showed that the 5 bacterial isolates were endospore-forming Gram positive (Figures 4B and 4C). Moreover, the 5 bacterial isolates also possess catalase activity (data not shown). Based on the examined characteristics and the Bergey's manual of systematic bacteriology, *Bacillus* genus are Gram positive, form endospore and produce catalase. Therefore, we concluded that these isolates belong to the *Bacillus* genus.

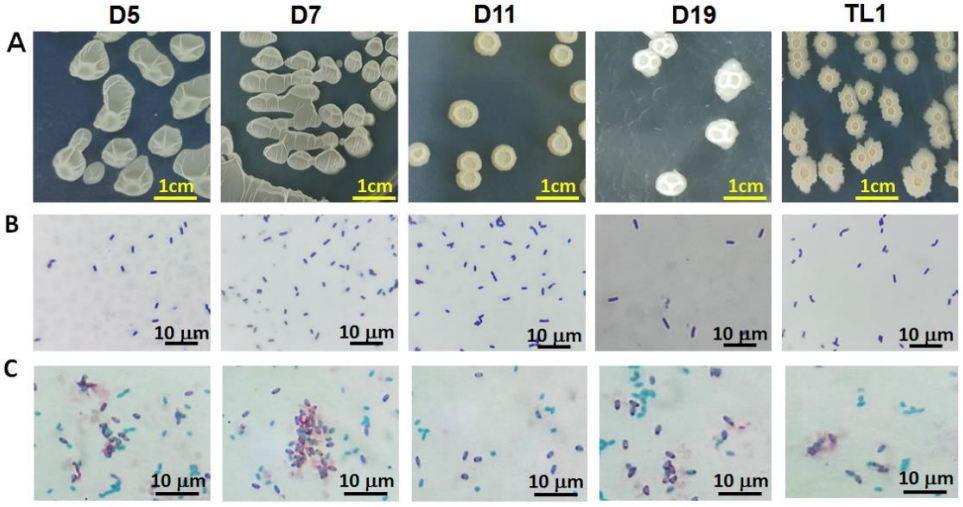

Figure 4. Morphological characteristics of selected antifungal isolates. Colony morphology (A), Gram staining (B) and endospore staining (C).

Analysis of 16S rRNA gene sequence of D19 showed very high homology (99,5%) with several *B. amyloliquefaciens* strains ANA25, MPRN2, Ba13 and YP6 (Genebank accession numbers MT122819.1, MT107118.1, MG846076.1, CP032146.1, respectively) (Table 2) Therefore, the isolate D19 was now

identified as *B. amyloliquefaciens* D19 and this is one of the first isolated *B. amyloliquefaciens* strains that was proved here with very high antifungal activity against *N. dimidiatum*. Besides, the two other *Bacillus* strains which are *Bacillus vezenensis* and *Bacillus atrophaeus* have also recently been shown to have similar effect on *N. dimidiatum* pathogenic to dragon fruit [18, 19].

Table 2. Sequencing result of D19 isolate

Primer	Partial 16S rRNA sequence	Species
27mF	GCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGC GGACGGGTGAGTAACACGTGGGTAACCWGCCTGTAAGACTGGGA TAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAACCG CATGGTTCARACATAAAAGGTGGCTTCGGCTACCACTTACAGATG GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG GCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGA ATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAG TGATGAAGGTTTTCGGATCGTAAAGCWCTGTTGTTAGGGAAGAAC AAGTGCCGTTCAAATAGGGCGGCACCTTGACGGTACCTAACCAGA AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT GGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCG GTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGG TCATTGGAAACTGGGGAACTTGAGTGCA	*B. amyloliquefaciens* strains ANA25 *B. amyloliquefaciens* strains MPRN2
1492mR	CGGCTGGCTCCAAAAGGTTACCTCACCGACTTCGGGTGTTACAAA CTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCAC GCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTG GGATTGGCTTAACCTCGCGGTCTCGCTGCCCTTTGTTCTGCCCATT GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGAC GTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAG TGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTT GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCA TGCACCACCTGTCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAG GATTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTT CGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT TCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCT TAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACT TAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTG TTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAG AGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCAC CGCTACACGTGGAATTCACTCTCTCTTCTGCACTCAAGTTCCCCAG TTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTT	*B. amyloliquefaciens* strains Ba13 *B. amyloliquefaciens* strains YP6

Bacterial isolates suppressed spore germination of N. dimidiatum

The inhibitory effect of bacterial isolates on spore germination was determined by incubating the pathogenic spores with cell-free bacterial cultured supernatants. The observation under light microscope after 8 hours of incubation showed the inhibition of spore germination in the presence of bacterial cultured supernatants. The four isolates D5, D7, D11 and D19 displayed significant spore germination suppression. Although the weak germination of conidia was observed in the case of TL1, the germ tubes were different from those of the non-treated control indicated by the much shorter and aberrant shape. The conidia in the non-treated control with LB broth showed long clear germ tubes growing from conidia, whereas the conidia treated with bacterial cultured supernatants showed the absence of germ tubes (D5, D19) or very tiny germ tubes (D7, D11) with swelling and large vesicles inside (Figure 5).

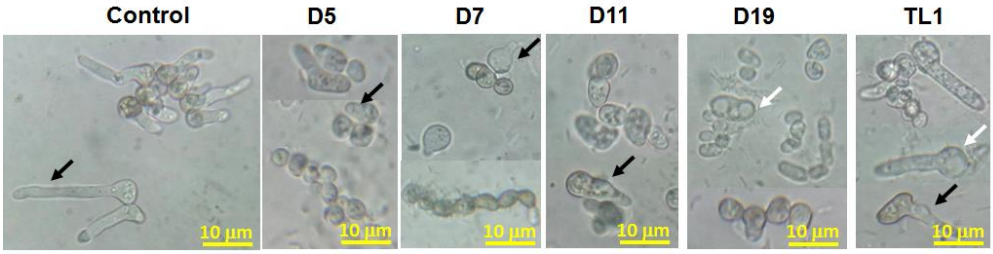

Figure 5. Inhibitory effect of bacterial isolates on fungal spore germination. Black arrows indicate the germ tubes (Control) and aberrant spore germination (D5, D7, D11, TL1). White arrows indicate the occurrence of large vesicles in the germinating spores (D19, TL1).

This indicates the inhibitory effect of these isolates on various stages of spore germination and therefore demonstrates the antifungal activity of the yet unknown substances in the bacterial cultured supernatants. Similar to the *B. amyloliquefaciens* D19 strain in this study, there are few others which display anti-spore germination effect such as the PPCB004 strain against *Penicillium crustosum* causing food spoilage [20], the AG4-4 strain against *Bipolaris cactivora* pathogenic to dragon fruit [21], the CNU114001 strain against various plant pathogenic molds [22], the SQR9 strain against the wheat and barley production thread *Fusarium graminearum* [15], and the SD-32 strain against cucumber pathogen *Podosphaera fusca* [23].

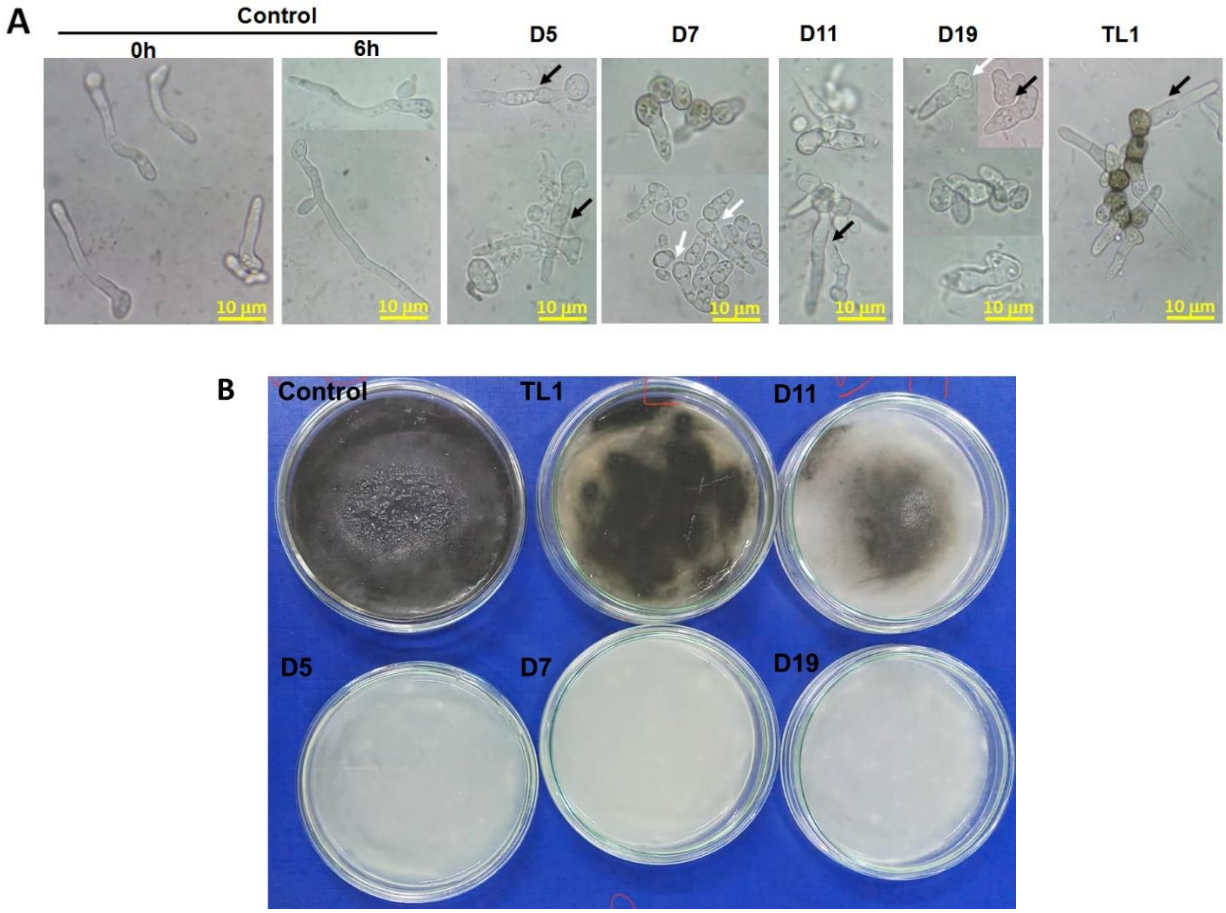

Figure 6. Effect of bacterial isolates on the mycelial growth. (A) Microscopic features of germ tubes with or without the present of bacterial cultured supernatants; black arrows indicate the swollen germ tubes and white arrows indicate the occurrence of large vesicles in the germ tubes. (B) The growth of mycelia in the present of bacterial cultured supernatants on PDA.

Bacterial isolates suppressed growth of N. dimidiatum mycelia

The effect of bacterial isolates on the mycelial growth was examined by incubating the germinated spores and cell-free bacterial cultured supernatants s. In the control case, observation under light microscope after

6 hours of incubation showed the growth of long, branched mycelia, while in the case of germinated spores treated with bacterial cultured supernatants, the mycelia growth were inhibited with the occurrence of swollen mycelia (D5, D19), numerous large vesicles (D7, D19), aberrant grown mycelia (D11, TL1) (Figure 6A). The inhibition of bacteria on the mycelial growth was further demonstrated when the mixture of germinated treated spores was inoculated on PDA medium. After 5 days of incubation, mycelia development and pigment forming were observed in the non-treated control, whereas no fungal growth in the present of D5, D7, D19 cultured supernatants s, and very weak mycelial growth in the present of D11 and TL1 cultured supernatants s (Figure 6B).

Therefore, we concluded that the antifungal activity of these strains could be arranged in the following order D19>D7>D5>D11>TL1 and the antifungal mechanism against *N. dimidiatum* was via inhibition of spore germination and mycelial growth by yet to be identified compounds existing in the cultured supernatant. Recent studies have reported that *B. amyloliquefaciens* is able to synthesize several natural compounds including prumycin and cyclic lipopeptides such as surfactin, fengycin, and iturin-like compounds with antimicrobial and especially antifungal activities against various *Colletotrichum*, *Bipolaris* and *Fusarium* fungal genera [15, 20, 22-24]. Additionally, as mentioned above, various negative effects on pathogenic fungal germ tubes have also been observed in the presence of cultured supernatant of different other *B. amyloliquefaciens* strains such as PPCB004, AG4-4, CNU114001 and SQR9 [15, 20-22]. Last but not least, *B. amyloliquefaciens* has been shown to be better than common chemical fungicides since it also has positive effect on leaf-length growth in pepper *Capsicum annum* L. [25].

4 CONCLUSIONS

The fungal pathogen from infected stem and fruit samples of *Hylocereus* spp. collected from many dragon fruit farms in Binh Thuan province was sucessfully isolated and its pathogenicity was re-checked on healthy fruit. Preliminary examination on cultural and morphological characteristics as well as 18S rRNA gene sequencing led us to determine that it belongs to the *Neoscytalidium* genus, *dimidiatum* species. In order to find a safe biological method to control this pathogen, we isolated 69 bacterial strains from soil and screened for their antifungal activity. Five strains, identified to be *Bacillus* spp., displayed high antagonistic effect against *N. dimidiatum.* Among these strains, the *Bacillus amyloliquefaciens* D19 possesses the highest antifungal activity by synthesize and secrete bioactive substances that can inhibit spore germination and germ tube normal growth development. Further studies are undergoing with the aims to identify precise secreted compounds from *B. amyloliquefaciens* D19 with inhibitory effect against *N. dimidiatum* pathogenic to dragon fruit as well as determine the optimal cultural conditions for production of these compounds. In conclusion, *B. amyloliquefaciens* D19 and the other 4 *Bacillus* have been proved to be very potential bio-control agents for not only dragon fruit cultivation, but also other plants and crops in a promising sustainable perspective.

ACKNOWLEDGEMENT

The authors would like to give special thanks to Industrial University of Ho Chi Minh City and the Vietnamese Mycological Association VMA for warm supports and advice on the project.

REFERENCES

1. Ariffin, A., et al., *Essential fatty acids of pitaya (dragon fruit) seed oil.* Food Chemistry, 2009. 114(2): p. 561-564.
2. Valencia-Botín, A.J., H. Kokubu, and Y.D. Ortíz-Hernández, *A brief overview on pitahaya (Hylocereus spp.) diseases.* Australasian Plant Pathology, 2012. 42(4): p. 437-440.
3. Mizrahi, Y., *Thirty-one years of research and development in the vine cacti pitaya in Israel*. In *Improving Pitaya Production and Marketing*. 2015. Kaoshiung, Taiwan.
4. Oeurn, S., Jitjak, W., & Sanoamuang, N., *Fungi on dragon fruit in Loei province, Thailand and the ability of Bipolaris cactivora to cause post-harvest fruit rot.* Asia-Pacific Journal of Science and Technology, 2015. 20(4): p. 405–418.

5. da Silva, R.T., et al., *Cutaneous murine model of infection caused by Neoscytalidium dimidiatum: a preliminary study of an emerging human pathogen.* Medical Mycology, 2016. 54(8): p. 890-898.
6. Dionne, B., et al., *Pulmonary fungal infection caused by Neoscytalidium dimidiatum.* Journal of Clinical Microbiology, 2015. 53(7): p. 2381-4.
7. Machouart, M., et al., *Scytalidium and scytalidiosis: What's new in 2012?* Journal de Mycologie Médicale, 2013. 23(1): p. 40-46.
8. Mohd, M.H., B. Salleh, and L. Zakaria, *Identification and molecular mharacterizations of Neoscytalidium dimidiatum causing stem canker of Red-fleshed Dragon fruit (Hylocereus polyrhizus) in Malaysia.* Journal of Phytopathology, 2013. 161(11-12): p. 841-849.
9. Nouri, M.T., et al., *Neoscytalidium dimidiatum causing canker, choot clight and fruit rot of Almond in California.* Plant Disease, 2018. 102(8): p. 1638-1647.
10. Rolshausen, P.E., et al., *First report of wood canker caused by Neoscytalidium dimidiatum on Grapevine in California.* Plant Disease, 2013. 97(11): p. 1511-1511.
11. Yi, R.H., et al., *Fruit internal brown rot caused by Neoscytalidium dimidiatum on pitahaya in Guangdong province, China.* Australasian Plant Disease Notes, 2015. 10(1).
12. Oliver, R.P., & Hewitt, H. G., *Fungicides in crop protection*, 2014, Wallingford: CABI Publishing, United Kingdom.
13. Frey-Klett, P., et al., *Bacterial-Fungal Interactions: Hyphens between Agricultural, Clinical, Environmental, and Food Microbiologists.* Microbiology and Molecular Biology Reviews, 2011. 75(4): p. 583-609.
14. Lopes, R., et al., *A look into a multifunctional toolbox: endophytic Bacillus species provide broad and underexploited benefits for plants.* World Journal of Microbiology and Biotechnology, 2018. 34(7).
15. Gu, Q., et al., *Bacillomycin D Produced by Bacillus amyloliquefaciens Is Involved in the Antagonistic Interaction with the Plant-Pathogenic Fungus Fusarium graminearum.* Applied and Environmental Microbiology, 2017. 83(19).
16. Medlin L, E.H., Stickel S, Sogin ML, *The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions.* Genes, 1988. 71: p. 491–499.
17. Weisburg, W.G., et al., *16S ribosomal DNA amplification for phylogenetic study.* Journal of Bacteriology, 1991. 173(2): p. 697-703.
18. Đỗ Thị Thanh Dung, L.T.B., Viên Thị Thanh Trúc, Võ Đình Quang, *Khả năng đối kháng của vi khuẩn bacillus sp. với vi nấm Neoscytalidium dimidiatum gây bệnh đốm trắng trên Thanh long*. Journal of Science-Natural Sciences And Technology, 2018. 15(12): p. 32-42.
19. Nguyễn Văn Giang, P.T.L.Q., Nguyễn Văn Thành, *Đặc điểm sinh học của chủng vi khuẩn đối kháng nấm Neoscytalidium dimidiatum gây bệnh đốm nâu trên cây thanh long*. Vietnam Journal of Agricultural Sciences, 2018. 10(95).
20. Arrebola, E., R. Jacobs, and L. Korsten, *Iturin A is the principal inhibitor in the biocontrol activity of Bacillus amyloliquefaciens PPCB004 against postharvest fungal pathogens.* Journal of Applied Microbiology, 2010. 108(2): p. 386-395.
21. Bae, S., S.G. Kim, and Y.H. Kim, *Biocontrol Characteristics of Bacillus Species in Suppressing Stem Rot of Grafted Cactus Caused by Bipolaris cactivora.* The Plant Pathology Journal, 2013. 29(1): p. 42-51.

22. Ji, S.H., et al., *Biocontrol Activity of Bacillus amyloliquefaciens CNU114001 against Fungal Plant Diseases.* Mycobiology, 2013. 41(4): p. 234-242.
23. Tanaka, K., et al., *Importance of prumycin produced by Bacillus amyloliquefaciens SD-32 in biocontrol against cucumber powdery mildew disease.* Pest Management Science, 2017. 73(12): p. 2419-2428.
24. M. Balhara, S.R., Sandeep Dhankhar and A.K. Chhillar, *Bioactive Compounds Hold Up - Bacillus amyloliquefaciens as a Potent Bio-control agent.* The Natural Products Journal, 2011: p. 20-28.
25. CHUNG, S.A.S.-D.K., *Biological Control of Phytopathogenic Fungi by Bacillus amyloliquefaciens 7079; Suppression Rates are Better Than Popular Chemical Fungicides.* The Journal of Microbiology and Biotechnology, 2005. 15(5): p. 1011–1021.

PHÂN LẬP, ĐỊNH DANH VÀ XÁC ĐỊNH ĐẶC TÍNH CỦA VI KHẨN ĐỐI KHÁNG VỚI MỐC *Neoscytalidium dimidiatum* GÂY BỆNH TRÊN CÂY THANH LONG

Tóm tắt: Cây thanh long (*Hylocereus* spp.) là loại cây phổ biến cho quả có hàm lượng dinh dưỡng cao, mùi vị thơm ngon và có giá trị kinh tế cao ở các nước nhiệt đới và cận nhiệt đới. Tuy nhiên, cây thanh long đã và đang bị đe dọa bởi nhiều nấm gây bệnh, đặc biệt là *Neoscytalidium dimidiatum* gây bệnh đốm trắng làm thiệt hại kinh tế lớn cho nông dân. Với mục tiêu tìm được một phương pháp tiết kiệm và thân thiện với môi trường để kiểm soát tác nhân gây bệnh này, 69 chủng vi khuẩn khác nhau đã được phân lập và trong đó, 5 chủng có khả năng kháng mốc *N. dimidiatum* đã được chọn lọc. Cả 5 chủng vi khuẩn được xác định là Gram dương, hiếu khí, có khả năng sinh bào tử và thuộc chi *Bacillus*. Dịch nuôi cấy của 5 chủng này cho thấy có khả năng ức chế invitro mạnh lên sự nảy mầm của bào tử cũng như sự phát triển của hệ khuẩn ty trong ít nhất 5 ngày. Đặc biệt, chủng D19 có khả năng đối kháng mốc mạnh nhất được định danh ở mức hình thái lẫn phân tử thuộc loài *Bacillus amyloliquefaciens*, một loài thường được biết đến với khả năng ức chế nhiều loại vi nấm khác nhau. Vì vậy, các chủng vi khuẩn đối kháng được chọn lọc trong nghiên cứu này cho thấy nhiều tiềm năng trong việc ứng dụng ngăn ngừa nhiễm nấm bệnh *N. dimidiatum* trên cây thanh long.

Từ khóa: Thanh long, *Neoscytalidium dimidiatum*, *Bacillus* đối kháng, Đặc tính kháng mốc

Ngày nhận bài: 20/03/2020

Ngày chấp nhận đăng: 05/06/2020