Научная статья

Original article

УДК 632.9

DOI 10.55186/25876740_2024_8_3_7

BIOCONTROL OF PHYTOPHTHORA DISEASE IN CITRUS USING *BACILLUS PUMILUS* ISOLATED FROM DISEASE-SUPPRESSIVE CITRUS RHIZOSPHERE

БИОКОНТРОЛЬ ФИТОФТОРЫ ЦИТРУСОВЫХ С ИСПОЛЬЗОВАНИЕМ BACILLUS PUMILUS, ВЫДЕЛЕННЫХ ИЗ ПОДАВЛЯЮЩЕЙ БОЛЕЗНЯНУЮ РИЗОСФЕРУ ЦИТРУСОВЫХ РИЗОСФЕРЫ ЦИТРУСОВ

Чан Ван Куанг, Аспирант, Аграрно-технологического института, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (117198, Россия, г. Москва, ул. Миклухо-Маклая, д. 6), тел. 8(925) 007-84-80, ORCID: http://orcid.org/0000-0002-3359-7427, 1042185129@pfur.ru

Пакина Елена Николаевна, доктор сельскохозяйственных наук, доцент, директор агробиотехнологического департамента аграрно-технологического института, ФГАОУ ВО «Российский университет дружбы народов имени Патриса Лумумбы» (117198, Россия, г. Москва, ул. Миклухо-Маклая, д. 6), тел. 8(985) 347-27-54, ORCID: http://orcid.org/0000-0001-6493-6121, pakina-en@rudn.ru

Ха Вьет Кыонг, доктор сельскохозяйственных наук, доцент, кафедры патологии растений, Национальный сельскохозяйственный университет Вьетнама (ВНУА), (131000, Вьетнам, г. Ха Ной, Чау Куи, Гиа Лам) тел. (84) 978-056-254, ORCID: http://orcid.org/0009-0004-5699-1752, cuongvietha@gmail.com

Tran Van Quang, PhD student, Agrobiotechnological department, Agrarian and Technological Institute, RUDN University, 8 Miklukho- Maklaya st., Moscow, 117198, Russian Federation, tel. 8(925) 007-84-80, ORCID: http://orcid.org/0000-0002-3359-7427, 1042185129@pfur.ru

Pakina Elena Nikolaevna, Candidate of Biological Sciences, Associate Professor, Agrobiotechnological department, Agrarian and Technological Institute, RUDN University, 8 Miklukho- Maklaya st., Moscow, 117198, Russian Federation, tel. 8(985) 347-27-54, ORCID: http://orcid.org/0000-0001-6493-6121, pakina-en@rudn.ru

Ha Viet Cuong - Associate Professor, Plant Pathology department, Viet Nam of National University of Agriculture (VNUA), Ha Noi, 131000, Trau Quy, Gia Lam, VietNam, tel.(84) 978-056-254, ORCID: http://orcid.org/0009-0004-5699-1752, cuongvietha@gmail.com

Аннотация. *Phytophthora spp.* - известный патоген, вызывающий пагубное воздействие на цитрусовые культуры, включая корневую гниль, хлороз листьев и гуммоз, что в конечном итоге приводит к снижению урожайности и гибели деревьев. В настоящее время отсутствуют эффективные методы контроля за этим заболеванием. Цель данного исследования - выделить биологический агент контроля (БАК), обладающий антагонистической активностью против заболеваний, вызванных Phytophthora. В общей сложности из ризосферы здоровых цитрусовых деревьев было выделено 143 штамма бактерий. Среди них четыре штамма *Bacillus* проявили сильную антагонистическую активность против нескольких видов *Phytophthora*, включая *Phytophthora nicotianae*, *Phytophthora parvispora*, *Phytophthora palmivopa*, *Phytophthora citrophthora* и *Phytophthora mekongensis*, известных патогенов, поражающих цитрусовые деревья. Выделенные бактериальные эксудаты и летучие соединения, производимые этими штаммами, проявили сильное ингибирующее воздействие на рост *Phytophthora*, с уровнями ингибирования от 30.28% до 99.98% для всех тестированных видов *Phytophthora*. Изолированные бактериальные штаммы

были обозначены как VN-H5, VN-H8, VN-F8 и VN-K13 с помощью анализа сцепленных последовательностей 16S РРНК - *gyrB* - *PyrE*, все они относятся к виду *Bacillus pumilus* с 100% сходством. В заключение, данное исследование подчеркивает выделение и скрининг бактерий с антагонистической активностью против различных видов Phytophthora, подчеркивая потенциал четырех кандидатов в биологический контроль, VN-H5, VN-H8, VN-F8 и VN-K13, в управлении болезней, вызванных *Phytophthora*, в цитрусовых культурах. Кроме того, это знаменует собой новаторское использование *B. pumilus* в экспериментах по борьбе с фитофторой на цитрусовых деревьях во Вьетнаме и во всем мире.

Abstract. *Phytophthora spp.* is a notorious pathogen causing detrimental effects on citrus crops, including root rot, leaf chlorosis, and gummosis, ultimately resulting in diminished yields and tree mortality. Effective control methods for this disease are currently lacking. This study aimed to isolate a biological control agent (BCA) capable of antagonistic activity against Phytophthora disease. A total of 143 bacterial strains were isolated from the rhizosphere of healthy citrus trees. Among them, four *Bacillus* strains exhibited robust antagonistic activity against multiple *Phytophthora* species, including *Phytophthora nicotianae, Phytophthora parvispora, Phytophthora palmivopa*, *Phytophthora citrophthora,* and *Phytophthora mekongensis*, known pathogens affecting citrus trees. Both bacterial exudates and volatile compounds produced by these strains demonstrated potent inhibitory effects on Phytophthora growth, with inhibition rates ranging from 30.28% to 99.98% across all tested Phytophthora species. The isolated bacterial strains were denoted as VN-H5, VN-H8, VN-F8, and VN-K13 through 16S RNA - *GyrB* - *PyrE* concatenated sequence analysis, all belonging to the species Bacillus pumilus with 100% similarity. In summary, this study underscores the isolation and screening of bacteria with antagonistic activity against various *Phytophthora* strains, highlighting the potential of four biocontrol candidate strains, VN-H5, VN-H8, VN-F8, and VN-K13, in managing Phytophthora disease in citrus. Additionally, it marks the pioneering use of

B. pumilus in *Phytophthora* control experiments for citrus trees in Vietnam and globally.

Ключевые слова: *биологический контроль, почвы, подавляющие болезни, фитофтора, Вьетнам, противогрибковые соединения, антагонистические механизмы.*

Keywords: *biological control, disease-suppressive soils, Phytophthora disease, Vietnam, antifungal compounds, antagonistic mechanisms*

Introduction

Phytophthora disease in citrus is one of the most severe plant diseases worldwide. In Vietnam, there have been 5 *Phytophthora* species that are associated with Phytophthora disease in citrus, including *P. nicotianae, P. palmivora*, *P. parvispora, P. mekongensis* and *P. citrophthora* [14, 15]. To control this disease, chemical control is recognized as the primary and effective method [3]. Nonetheless, the adoption of chemical control modalities may potentially engender risks concerning safety, health, and the environment, and it may even contribute to the emergence of fungicide-resistant microorganisms (Droby et al., 2016). Thus, the development of alternative control strategies for this disease is necessary.

Bacillus pumilus is a Gram-positive, aerobic, rod-shaped bacterium commonly found in soil [11]. It is a member of the Bacillus pumilus group, which also includes *B. altitudinis, B. australimaris, B. safensis, B. xiamenensis, and B. zhangzhouensis* [4]. This species is notable for its robust resistance to various environmental stressors, such as low or limited nutrient availability, drought, irradiation, UV radiation, chemical disinfectants, and oxidizing enzymes [5]. *Bacillus pumilus* is considered to has the potential as part of a sustainable integrated disease management strategy for the control of soil-borne plant pathogens [1, 6]. For example, *B. pumilus* HR10, isolated from the rhizosphere of Pinus thunbergii, exhibited remarkable inhibitory activity against Rhizoctonia solani, the causative agent of pine seedling damping-off disease [17]. *Bacillus pumilus* W-7 has demonstrated the capacity to inhibit the growth of Phytophthora infestans mycelium [16]. Furthermore, *B. pumilus*

PTB180 has been reported for its robust in vitro antagonistic activity against seven plant pathogens, including *Fusarium oxysporum*, *Rhizoctonia solani, Sclerotinia sclerotiorum, Phytophthora capsici, Pythium ultimum,* and *Botrytis cinerea* [1]. In addition to its disease control capabilities, *Bacillus pumilus* has shown a direct impact on enhancing plant growth. For instance, *B. pumilus* TRS-3, isolated from the rhizosphere of tea plants, has been found to produce indole 3-acetic acid (IAA), siderophores, and engage in phosphate solubilization [2]. Research conducted using the Murashige and Skoog liquid medium has provided evidence that strain *B. pumilus* LZP02 promotes rice growth by enhancing parameters such as root length, root surface area, number of nodes, root tips, forks, and chlorophyll content [8]. Furthermore, the application of *B. pumilus* LZP02 has led to increased levels of nitrogen, phosphorus, calcium, and magnesium in rice roots [8]. Furthermore, the U.S. Food and Drug Administration (FDA) has granted the GRAS status (an abbreviation for "generally recognized as safe") to species such as *B. subtilis, B. licheniformis, and B. pumilus* [GRAS Notice Inventory|FDA].

In Vietnam, there is currently no documented application of biological control agents (BCAs) for the mitigation of Phytophthora infections in citrus plants. As a result, the primary objectives of this present research to isolate BCAs with antagonistic properties against Phytophthora from the rhizosphere soil of healthy citrus plants. This study comprises the following key elements: (1) the isolation and screening of Phytophthora-antagonistic bacteria, with a distinct emphasis on Bacillus Pumilus; (2) the assessment of the biological control efficacy demonstrated by selectively chosen BCAs under controlled in vitro; (3) the unequivocal identification of the isolated BCAs through the elucidation of their 16S rRNA, gyrB, and pyrE gene sequences, along with an exploration of their biological control mechanisms.mechanisms. Our study has revealed that the utilization of this Biological Control Agent (BCA) represents a viable and promising approach for the management of this disease.

Materials and methods

1. Plant pathogens

Five isolates of *Phytophthora* causing root rot and gummosis in citrus in northern Vietnam (*P. mekongensis* – VN- Oo48, *P. palmivora* – VN-Oo33, *P. parvispora* – VN-Oo10, *P. nicotianae* – VN-Oo65, *P. citrophthora* – VN-Oo78) were employed in this study [14, 15]. These isolates were cultured on Potato Dextrose Agar (PDA) medium at 25°C for 5 days before further use.

2. Bacterial isolates and culture conditions

Rhizosphere soils of pomelo and orange trees were collected in An Khang (21°47'20.1"N 105°15'04.6"E), situated in Tuyen Quang province, during the year 2023. It is imperative to note that the citrus trees observed in this locale have remained conspicuously free from manifestations such as leaf yellowing, stem gummosis, brown fruit rot, and root rot over the past few years. This stands in stark contrast to the pronounced disease incidence observed in the surrounding regions during the same period. The isolation of soil bacteria was performed utilizing the dilution method outlined by Han et al. (2022) [7]. One gram of soil sample was aseptically combined with 10 mL of sterile water in a 50 mL tube. Subsequently, this mixture was diluted 1:10 with distilled water, and 100 microliters of the dilution were uniformly spread onto nutrient agar (NA) medium supplemented with yeast extract (3 g), peptone (5 g), NaCl (5 g), agar (15 g), and 1000 mL of H_20. The inoculated plates were incubated at 27°C for 1-3 days, following which distinct bacterial colonies were selected and transferred to fresh NA medium for continued incubation at the same temperature. Pure bacterial cultures were obtained after several transfers and were preserved on NA plates at 4°C for future utilization.

3. Determination of antagonistic activity by plate confrontational culture

All bacterial isolates were subjected to a dual-culture assay to test their antagonism against Phytophthora on PDA as described by Han et al. (2022) [7]. A 5-mm plug of a *Phytophthora* culture that had been grown on potato dextrose agar (PDA) for 5 days was placed 2 cm from the edge of a new PDA plate with a diameter of 90 mm. A bacterial suspension obtained from a 24-hour culture was streaked as a broad line 2 cm away from the edge of the plate on the opposite side of the fungal plug. A control plate was prepared using distilled water. After incubating the plates at

25°C, the radius of colony growth for each fungus toward the side streaked with bacteria was measured when the control plate was completely covered with mycelium. The PIRG was then calculated using the formula PIRG = 100 × (C – T)/(C) (%), where C represents the radius of mycelium growth on the control plate and T represents the radius of mycelium growth toward the bacterial colonies (mm).

4. Determination of antagonistic activity by plate spreading

To evaluate the antagonistic activity of bacteria, an aliquot of 0.1 mL bacterial culture with a concentration of 1×10^8 cfu/ml was spread on the surface of a 9 cm diameter PDA plate. As a control, 0.1 mL of sterilized distilled water was also spread on a separate PDA plate. Next, a 5 mm diameter plug of agar, containing the Phytophthora colonies was placed at the center of each plate. The plates were then incubated at 28°C in the dark for 7 days. The size of the pathogen colony was measured and PIRG as described above was calculated. All treatments and controls were replicated in triplicates to ensure accuracy and reliability.

5. Antimicrobial test of bacterial VOCs

The Petri plates were utilized to investigate the effect of bacterial volatile organic compounds (VOCs) on pathogenic microorganisms as described by Han et al. (2022) [7], with minor modifications. Initially, Luria-Bertani (LB) medium was introduced into one lid of the Petri plate, followed by spreading antagonistic 0,1 mL bacteria (1×10^8 cfu/ml) over the surface of the medium one day before culturing the test pathogens. A 5-day-old plug of various fungi (*P. mekongensis, P. nicotianae, P. palmivora, P. citrophthora, P. parvispora*) was placed in the remaining lid containing Potato Dextrose Agar (PDA). Control plates consisted solely of fungal pathogens. Parafilm was used to seal the inoculated plates to observe normal pathogen growth. Incubation of plates containing pathogenic fungi and antagonistic bacteria was conducted at a temperature of 25°C. The growth of the fungi was evaluated by measuring the diameter of the mycelial growth. Subsequently, the percentage inhibition of fungal growth was calculated as a measure of the effectiveness of the antagonistic bacteria against the tested pathogens.

6. DNA extraction, purification, PCR and phylogenetic analysis of bacteria

Based on the screening of antagonistic bacteria against *Phytophthora spp*., four isolates labeled as VN-H5, VN-H8, VN-F8, and VN-K13 with robust antagonistic activity were selected for identification. Bacterial DNA extraction was conducted following the procedure described by Tsai et al. (1992) [13], with minor modifications. DNA isolated from bacterial colonies was dissolved in a 50 µl volume of TE buffer (Tris + EDTA) at pH 8.0 within 1.5-ml Eppendorf tubes. The bacterial aliquot was then heat-treated at 100°C for 10 minutes and utilized as the PCR template. The 16S rRNA, Gyrase B subunit (*gyrB)* and Orotate phosphoribosyltransferase (*pyrE)* genes of these bacteria were subjected to PCR amplification using universal primers 27F and 1525R [16], gyrBF and gyrBR, pyrBF and pyrBR, respectively [10].

The PCR reaction was conducted with a total volume of 25 µl, comprising 12.5 µl of 2x MytagMM, 0.4 µl of each primer, 0.5 µl of DNA, and 11.2 µl of H_2O. The PCR procedure was executed in a thermal cycler following these conditions: a single cycle of denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 15 seconds, extension at 72°C for 1 minute, and a final extension step at 72°C for 5 minutes. Subsequently, the reaction was halted and allowed to cool to room temperature. The PCR products were visualized by electrophoresis on an agarose gel, and the target band was excised and purified using the commercial PureLinkTM Quick Gel Extraction Kit (Invitrogen), according to the manufacturer's instructions. The purified PCR product was subsequently submitted to Institute of Biotechnology, (IBT, Hanoi, Vietnam) for DNA sequencing. The assembly and refinement of the DNA sequences were accomplished using Mega 11 software.

7. Statistical analysis

All experiments were performed in triplicate. Significant differences between treatments were analysed in the SPSS statistics v.26 software by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test ($P < 0.05$).

Results and discussion

1. Various rhizosphere bacteria from disease-suppressive soil with inhibitory activity against Phytophthora growth

Out of the 143 bacterial isolates obtained, four specific isolates denoted as VN-H5, VN-H8, VN-F8, and VN-K13 demonstrated significant antagonistic activity. This assessment entailed the observation of inhibition zones when these isolates were subjected to confrontations with five *Phytophthora* pathogenic isolates known to afflict citrus trees, as detailed in Table 1 and Fig. 1.

Table 1. Determination of antagonistic activity

Antagonistic activity	isolates	PIRG (%)*				
		P. palmivora	***P. mekongensis***	***P. nicotianae***	***P. citrophthora***	***P. parvispora***
Plate confrontational culture	VN-H5	43.70 a	57.55 ab	66.07 b	52.55 ab	43.54 a
	VN-H8	61.02 b	55.72 ab	65.94 b	55.30 ab	55.66 ab
	VN-F8	68.07 b	59.68 b	65.00 b	54.50 ab	54.39 ab
	VN-K13	68.31 b	59.06 ab	65.46 b	55.61 ab	53.13 ab
Plate spreading	VN-H5	90.43 g	79.29 d	99.59 kl	69.97 a	99.80 l
	VN-H8	95.83 h	80.52 e	98.56 ik	75.58 b	95.85 h
	VN-F8	99.98 l	82.44 f	97.59 i	77.58 c	90.96 g
	VN-K13	99.97 l	82.12 f	99.95 l	79.59 de	99.98 l
Organic volatile compounds	VN-H5	75.13 def	69.87 cd	53.11 b	80.23 fg	30.28 a
	VN-H8	64.03 c	72.82 de	57.01 b	83.36 g	31.21 a
	VN-F8	76.94 efg	78.38 efg	56.81 b	82.24 g	32.66 a
	VN-K13	90.72 h	81.62 fg	56.60 b	81.88 fg	31.27 a

Note: Means in both columns and rows in each method followed by the same letters are not significantly different ($p < .05$, Tukey's HSDtest). Data were recorded at 7 days.

Within the plate confrontational culture method, isolate VN-K13 stood out as the most efficacious, displaying percentage inhibition of radial growth (PIRG) values

ranging from 53.13% to 68.31%. These values are indicative of its exceptional capability to impede the radial expansion of *Phytophthora* species. Isolates VN-F8 and VN-H8 also exhibited substantial antagonistic activity, albeit slightly less potent compared to isolate VN-K13. In contrast, isolate VN-H5 presented the lowest level of antagonistic activity among the isolates, with PIRG values spanning from 43.54% to 66.07%. Nevertheless, it still demonstrated reasonable inhibitory effects in specific cases.

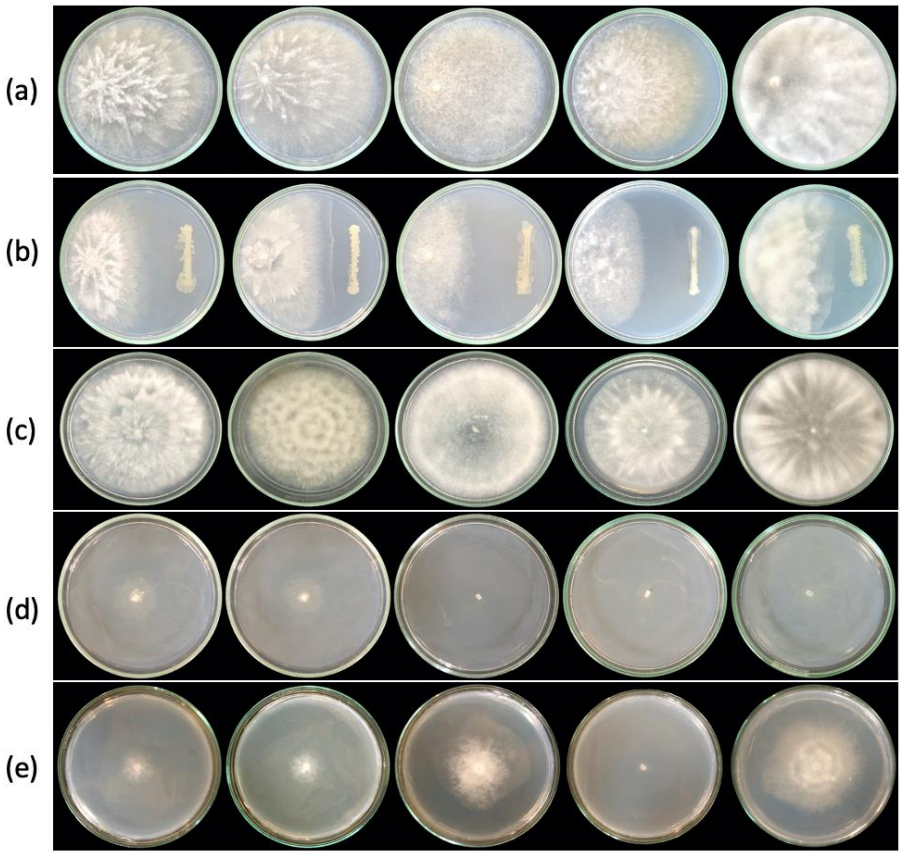

Figure 1. Evaluation of the antagonistic potential of bacterial isolate VN-K13 against five *Phytophthora* isolates. (a) and (c) Control conditions; (b) Antagonistic activity assessed via plate confrontational culture; (d) Antagonistic activity measured using plate spreading; (e) Detection of volatile organic compounds. From left to right, the Phytophthora species are as follows: *Mekongensis, citrophthora, nicotianae, palmivora,* and *parvispora*. Data was collected after a 10-day incubation period at 28°C.

In both the Plate Spreading and Organic Volatile Compounds Methods, VN-K13 consistently demonstrated the highest degree of antagonistic activity in limiting the

radial growth of Phytophthora species. This positions it as a promising candidate for incorporation into biocontrol strategies. VN-F8 also showcased noteworthy antagonistic activity, whereas VN-H8 and VN-H5 exhibited moderate to lower levels of antagonism. In summation, these findings underscore the consistent effectiveness of VN-K13 and VN-F8 in restraining the radial growth of diverse *Phytophthora* species through various assessment techniques. This highlights their potential for integration into biocontrol strategies aimed at combating these plant pathogens.

2. Identification of antagonistic bacteria

In our taxonomic investigation, the examination of species involved the application of polymerase chain reaction (PCR) to amplify the genetic sequences of 16S RNA and two housekeeping genes, *gyrB* and *pyrE*. These sequences were subsequently concatenated, with a specific order: 16S RNA - *GyrB* - *PyrE*. This genetic analysis was performed on four distinct *Bacillus* bacterial isolates, denoted as VN-H5, VN-H8, VN-F8, and VN-K13 (Table S1). The results of this analysis indicated a 100% sequence identity, positioning all four strains within the same phylogenetic cluster as *Bacillus pumilus*, a member of the pumilus group (Fig. 2). The findings from this genetic investigation strongly underscore the substantial antagonistic potential exhibited by these indigenous *Bacillus* strains, originating from the northern region of Vietnam, against *Phytophthora spp*., a group of plant pathogens. Additionally, the results reinforce the close genetic relationship between these strains and Bacillus pumilus within the pumilus group, which encompasses various related species such as *B. altitudinis, B. australimaris, B. safensis, B. xiamenensis, B. aerophilus* and *B. zhangzhouensis*. These observations notably differentiate these strains from those belonging to the *B. cereus* group.

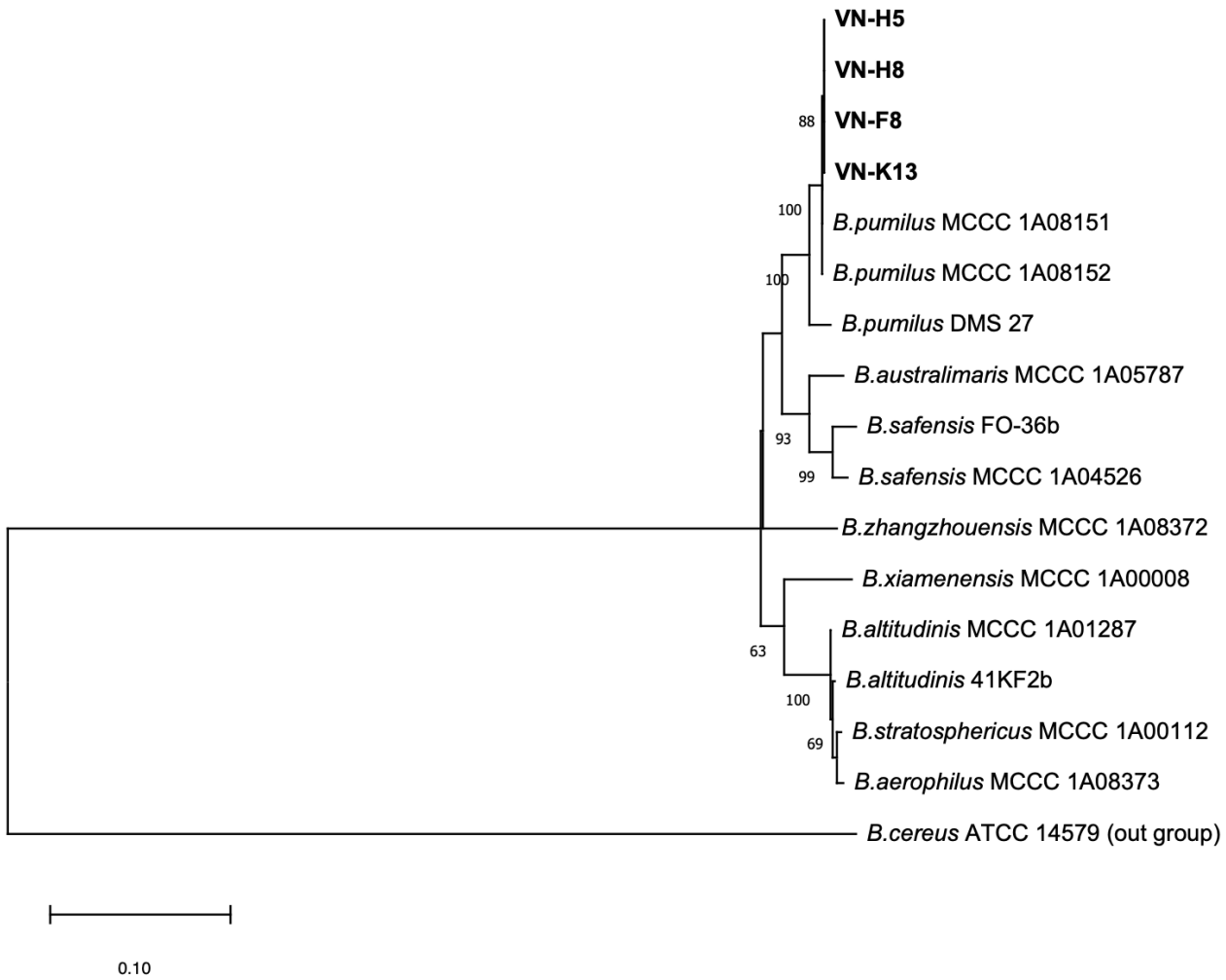

Figure 2. Maximum likelihood phylogenetic tree for 16 strains in the *Bacillus* group *pumilus* based on the TN 93 + G + I model using concatenated sequences of *16S rRNA, gyrB* and *pyrE*. The isolates from Vietnam are shown in bold. The referent (ex-type or well-authenticated) strains of the group *pumilus* are included (see Supplementary Table S1 for the strain identities). The bootstrap consensus tree is inferred from 1000 replicates. Bootstrap values for maximum likelihood are shown on the nodes. Only bootstrap support values greater than 50 % are shown. The scale bar represents substitutions per site. The tree is rooted to *Bacillus cereus* (isolate ATCC 14579).

Discussion

Phytophthora disease, a widespread concern in citrus agriculture with intricate management challenges, remains an understudied domain in the context of Biological Control Agents (BCAs) implementation for disease control. In current study, we have isolated four bacterial strains (designated as VN-H5, VN-H8, VN-F8, and VN-K13)

from the rhizosphere soils of healthy citrus plants. These isolates have exhibited the capability for biological control against five Phytophthora strains that contribute to citrus tree damage in the northern mountainous region of Vietnam.

The identified bacterial strains exhibited robust antagonistic activity against *Phytophthora* in vitro experimental conditions. Sequences of the 16S rRNA, *gyrB*, and *pyrE* genes were successfully amplified, sequenced, and deposited in the GenBank database. BLASTn searches revealed a close genetic relationship between these isolates and strains within the *Bacillus pumilus* group, which includes species like *B. pumilus, B. altitudinis*, *B. australimaris, B. safensis*, *B. xiamenensis*, and *B. zhangzhouensis* [4]. It's worth noting that distinguishing between bacteria within the *B. pumilus* group is challenging due to their high sequence similarity [10]. The identity of the three isolates was further validated by aligning the concatenated sequences of 16S rRNA, *gyrB* và *pyrE* with reference sequences of related *Bacillus* species in the *B. pumilus* group and conducting a maximum likelihood analysis. The phylogenetic tree grouped VN-H5, VN-H8, VN-F8 and VN-K13 with the ex-type strain (DMS 27) and other reference strains of *B. pumilus* with 100% support (Fig. 2). Taken together, these results confirmed the identity of VN-H5, VN-H8, VN-F8 and VN-K13 as *B. pumilus*.

B. pumilus has been reported as a biological control agent against several plant pathogens, including *Fusarium oxysporum , Rhizoctonia solani , Sclerotinia sclerotiorum , Phytophthora capsici, Pythium ultimum, Botrytis cinerea, Phytophthora infestans* [1, 16]. This study confirms that *B. pumilus* effectively controls five *Phytophthora* strains that impact citrus trees. Additionally, it marks the pioneering use of *B. pumilus* in *Phytophthora* control experiments for citrus trees in Vietnam and globally. In a co-culture setting, it's evident that *Phytophthora* mycelia inhibition occurs in the presence of *B. pumilus*, even without direct physical contact (Fig.2). This strongly suggests that our *Bacillus* isolate has the capacity to produce both diffusible and volatile compounds, leading to the suppression of *Phytophthora* (Fig.2). These findings are consistent with a body of literature demonstrating the

potent antifungal activity of Bacillus species through the production of secondary metabolites [7, 9].

While these strains are known to produce antifungal volatile organic compounds, our study was unable to identify them. As a result, propose further investigations to determine the precise antifungal volatile organic compounds produced by these strains.

In the current study, we have investigated a biocontrol strain that could be used as an alternative agent for controlling Phytophthora disease in citrus trees. Our experimental results have significantly enhanced our comprehensive understanding of the potential antifungal mechanisms of *B. pumilus*. To the best of our knowledge, this is the first study focused on citrus biocontrol using the antagonistic bacterium *B. pumilus*.

References

1. Bouchard-Rochette M, Machrafi Y, Cossus L, et al. Bacillus pumilus PTB180 and Bacillus subtilis PTB185: Production of lipopeptides, antifungal activity, and biocontrol ability against Botrytis cinerea. *Biological Control.* 2022;170:104925. doi: https://doi.org/10.1016/j.biocontrol.2022.104925

2. Chakraborty U, Chakraborty B, Roychowdhury P. Plant growth promoting activity of Bacillus pumilus in tea (Camellia sinensis) and its biocontrol potential against Poria hypobrunnea. *Indian Phytopathology.* 2013;66(4):387-96.

3. Chen K, Tian Z, He H, Long C-a, Jiang F. Bacillus species as potential biocontrol agents against citrus diseases. *Biological control.* 2020;151:104419. doi: https://doi.org/10.1016/j.biocontrol.2020.104419

4. Chen L, Liu Y, Wu G, et al. Induced maize salt tolerance by rhizosphere inoculation of Bacillus amyloliquefaciens SQR9. *Physiologia plantarum.* 2016;158(1):34-44. doi: https://doi.org/10.1111/ ppl.12441

5. Dobrzyński J, Jakubowska Z, Dybek B. Potential of Bacillus pumilus to directly promote plant growth. *Frontiers in Microbiology.* 2022;13:1069053. doi: 10.3389/fmicb.2022.1069053

6. Fu H, Li M, Tan G, Wang Z. Screening and identification of antagonistic strain against Phytophthora sojae. *Plant Diseases and Pests.* 2011;2(4):9-17.

7. Han V-C, Yu NH, Yoon H, et al. Identification, characterization, and efficacy evaluation of Bacillus velezensis for shot-hole disease biocontrol in flowering cherry. *The Plant Pathology Journal.* 2022;38(2):115-30. doi: 10.5423/PPJ.OA.01.2022.0004

8. Liu H, Wang Z, Xu W, et al. Bacillus pumilus LZP02 promotes rice root growth by improving carbohydrate metabolism and phenylpropanoid biosynthesis. *Molecular Plant-Microbe Interactions.* 2020;33(10):1222-31. doi: https://doi.org/10.1094/MPMI-04-20-0106-R

9. Liu W-w, Wei M, Zhu B-y, Du Y-c, Feng L. Antagonistic activities of volatiles from four strains of Bacillus spp. and Paenibacillus spp. against soil-borne plant pathogens. *Agricultural Sciences in China*. 2008;7(9):1104-14. doi: https://doi.org/10.1016/S1671-2927(08)60153-4

10. Liu Y, Lai Q, Dong C, et al. Phylogenetic diversity of the Bacillus pumilus group and the marine ecotype revealed by multilocus sequence analysis. *PloS one*. 2013;8(11):e80097. doi: https://doi.org/10.1371/journal.pone.0080097

11. Priest FG. Systematics and ecology of Bacillus. Bacillus subtilis and other gram-positive bacteria. *Biochemistry, physiology, and molecular genetics.* 1993:1-16. doi: https://doi.org/10.1128/ 9781555818388.ch1

12. Rainey FA, Dorsch M, Morgan HW, Stackebrandt E. 16S rDNA analysis of Spirochaeta thermophila: its phylogenetic position and implications for the systematics of the order Spirochaetales. *Systematic and applied microbiology*. 1992;15(2):197-202. doi: https://doi.org/10.1016/S0723-2020(11)80092-5

13. Tsai Y-L, Olson B. Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Applied and Environmental Microbiology*. 1992;58(7):2292-95. doi: https://doi.org/10.1128/aem.58.7.2292-2295.1992

14. Van Tran Q, Ha CV, Vvedensky VV, Han V-C. Current status and characterization of Phytophthora species associated with gummosis of citrus in

Northern Vietnam. *Journal of Phytopathology.* 2023; 171(9):178-188. doi: https://doi.org/10.1111/jph.13204

15. Van Tran Q, Ha CV, Vvedensky VV, Linh Le TT, Han V-C. Pathogenicity and fungicide sensitivity of Phytophthora parvispora, a new pathogen causing gummosis and root rot disease on citrus trees. *Microbial Pathogenesis.* 2023;175:105986. doi: https://doi.org/10.1016/j.micpath.2023.105986

16. Wang Y, Zhang C, Liang J, et al. Surfactin and fengycin B extracted from Bacillus pumilus W-7 provide protection against potato late blight via distinct and synergistic mechanisms. Applied *Microbiology and Biotechnology.* 2020;104:7467-81. doi: https://doi.org/10.1007/s00253-020-10773-y

17. Zhu M-L, Wu X-Q, Wang Y-H, Dai Y. Role of biofilm formation by Bacillus pumilus HR10 in biocontrol against pine seedling damping-off disease caused by Rhizoctonia solani. *Forests.* 2020;11(6):652. doi: https://doi.org/10.3390/f11060652

© Куанг В. Ч., Пакина Е. Н., Кыонг В. Х. *2024. International agricultural journal, 2024.№ 3, 828-843.*

Для цитирования: Куанг В. Ч., Пакина Е. Н., Кыонг В. Х. Biocontrol of phytophthora disease in citrus using *bacillus pumilus* isolated from disease-suppressive citrus rhizosphere // International agricultural journal. 2024. *№ 3, 828-843.*