World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

# Study on organic matter decomposition and siderophore production of rhizobacteria isolated from black pepper grown in Loc Ninh, Binh Phuoc province, Vietnam

Ha Bao Son [1], Dang Thi Ngoc Thanh [2], Huynh Nguyen Van Anh [2], Pham Thi Thu Ly [3] and Hoang Minh Tam [2,*]

[1] *Nguyen Huu Tho High School, District 7, Ho Chi Minh City, Vietnam.*
[2] *Faculty of Natural Science Pedagogy, Sai Gon University, Ho Chi Minh City, Vietnam.*
[3] *Department of Facility Management, Sai Gon University, Ho Chi Minh City, Vietnam.*

World Journal of Advanced Research and Reviews, 2023, 17(03), 009–017

Publication history: Received on 14 January 2023; revised on 24 February 2023; accepted on 27 February 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.3.0317

## Abstract

This study was carried out determining capability of organic compound decomposition and siderophore synthesis of 39 plant growth promotion rhizobacteria strains isolated from rhizosphere of black pepper (*Piper nigrum* L.) in a previous study. The ability to degrade organic compounds including proteins, lipids, cellulose and chitin was investigated by agar well diffusion method. The ability to produce siderophore was qualified by liquid CAS method and strains with good results were further investigated by PS-CAS agar plate method. The results showed that the number of bacterial strains capable of degrading protein, cellulose, chitin and lipid were 37, 27, 16 and 6, respectively. Twenty-two strains changed color of CAS agar, of which 6 strains had a band width of 4.5 cm. The seven best strains identified were *Proteus mirabilis* (LD2), *Bacillus megaterium* (LT4), *B. subtilis* (LT2), *B. cereus* (LD7, LD8, LD16, LT19) by Matrix Assisted Laser Desorption/Ionization-Time of Flight method. These were plant growth-promoting rhizobacteria that had been reported on potential applications in agriculture.

**Keywords:** Agar well diffusion method; Binh Phuoc province; Biodegradation; Black pepper (*Piper nigrum* L.); Plant Growth Promoting Rhizobacteria; Siderophore

## 1. Introduction

Black pepper or pepper was a spice with long-standing economic value, and for many years, Vietnam had been a major producer and exporter of pepper in the world. In 2022, Vietnam's pepper exports were estimated at 220,000 tons, accounting for 55% of the total world pepper production [1]. However, the biggest difficulty of Vietnam's pepper export industry was the high chemical residue that hindered the trade process through difficult markets such as the US and Europe. Organic farming was an inevitable choice in the production of pepper as well as other agricultural products. Organic agriculture emphasized the role of bio-fertilizers and bio-pesticides, and one of the potential applications was Plant Growth-Promoting Rhizobacteria (PGPR).

PGPR were considered to be biological antagonists, as well as an indirect way to stimulate plant growth, because they had ability to produce antibiotics, produce hydrolytic enzymes, secrete siderophore, and stimulate Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR) of plant, etc. [2, 3]. The study of *Pseudomonas* isolated from the black pepper rhizosphere in Vietnam to produce biosurfactants with zoosporicidal activities provided ability to control foot and root rot of black pepper and promote growth of plants [4]. Other studies on PGPR pepper plants grown in Binh Phuoc, Vietnam showed ability to supplement nutrients through fortification of macronutrients such as N and P, production of IAA and siderophore, decomposition of organic matter, and antifungal activity [5, 6]. The citations showed potential of rhizobacteria in pepper plants to promote plant growth either directly through nutrition or indirectly through promoting healthy plants as well as the basis for this research.

* Corresponding author: Hoang Minh Tam

Copyright © 2023 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

Endogenous bacterial strains were selected from the vegetative parts of wheat, sunflower, and maize grown in Russia were the subjects of study on enzymatic activity for starch degradation, proteolysis, lipolysis and cellulose degradation, and production capacity of catalase and chitinase. The isolates also exhibited antagonistic activity against the toxic mold *Fusarium sporotrichioides*. Notably, 3 out of 18 isolates were identified as *Bacillus subtilis* [7]. The ability to produce siderophore of PGPR was also screened in addition to the ability to make enzymes to degrade substances [8]. In an aerobic environment, $Fe^{2+}$ was easily oxidized to $Fe^{3+}$ existing in the form of insoluble hydroxide and oxyhydroxide which was difficult to absorb. Therefore, bacteria that were able to produce siderophores to help absorb iron also meant that their competitiveness was enhanced [9-11]. Hydrolytic enzymes such as chitinases, chitosanases, glucanases, cellulases, lipases, and proteases were also extensively produced by *Bacillus* strains. Several *Bacillus* had been involved in the biocontrol of plant pathogens, for example BacGM17 produced by *B. clausii* GM17 was active against tumor plant pathogens *Agrobacterium tumefaciens*. Thus, a wide range of microbial Biological Control Agents (BCAs) had been discovered over the past decades as promising alternatives for the management of fungal and bacterial diseases [12].

The purpose of this study was to investigate the degradation of protein, lipid, cellulose, chitin and the production of siderophore of 39 strains of rhizobacteria isolated from pepper grown in Loc Ninh district, Binh Phuoc province included in the collection. The selected strains would be the premise for studies on the application of beneficial bacteria in pepper cultivation.

## 2. Material and methods

### 2.1. Activation of bacteria and preparation of bacterial suspensions

Prior to inclusion in the study, all 39 PGPR strains were streaked on isolation medium to check for purity. Next, a loop filled with the biomass of each bacterial strain was added to a test tube containing 5 mL of liquid LB medium (10 g Bacto tryptone; 5 g Bacto yeast extract; 10 g NaCl; pH 7) [13] medium and subjected to a 120 rpm (rounds per minute) shaker, at 28 ± 2 ℃. After 48 hours of culture, bacterial suspensions of each strain were obtained. Cell density was measured by spectrophotometer at 600 nm and adjusted to McFarland standard 0.5 corresponding to 1.5×10^8 CFU/mL [14]. This standard density would be applied to all subsequent experiments.

### 2.2. Investigation of the decomposition of protein, lipid, cellulose and chitin

Formulas of culture media and reference sources were presented in Table 1 below. Chemicals were mixed in water and settled to 1,000 mL with distilled water. The culture media were adjusted to the required pH and then solidified by the addition of agar (15 g per L).

**Table 1** Formulas of culture media and experimental purposes

| Media | Experimental purposes | Formulas | References |
|---|---|---|---|
| Skim milk | Decomposition of protein | 5 g peptone; 3 g meat extract; 1 g yeast extract; 300 mL skim milk; pH 6.5 | [15] |
| TBA - olive oil | Decomposition of lipid | 5 g peptone; 3 g yeast extract; 10 mL olive oil; 0.05 g Rhomadine B; pH 7.2 | [16] |
| CMC | Decomposition of cellulose | 1 g $(NH_4)_2SO_4$; 1g $K_2HPO_4$;<br>0.5 g $MgSO_4$; 9 mg NaCl; 10 g CMC;<br>10 mL Congo red; pH 6.8 | [17] |
| Colloidal chitin | Decomposition of chitin | 0.7 g $K_2HPO_4$; 0.3 g $KH_2PO_4$;<br>0.5 g $MgSO_4.5H_2O$; 0.01 g $FeSO_4.7H_2O$; 1 mg $ZnSO_4$; 0.1 mg $MnCl_2$;<br>0.5 g colloidal chitin; pH 6-7 | [18] |

Agar well diffusion method was used to evaluate decompositions of organic substances [19]. Wells with a diameter of 5 mm were formed on the agar plate and each well was injected with 50 mL of the suspension (density 1.5×10^8 CFU/mL) of each bacterial strain. The agar plates were incubated at 28 ± 2 ℃ for 48 hours, then observed the formation of halo rings to evaluate the results. The abilities of bacterial strains to decompose organic substances were evaluated through the sizes of the halo zones according to the formula of Azman et al. [8]. The decomposition was assessed by measuring

the clear halo zone. The halo zone was calculated by subtracting bacterial colony diameter from the combined halo zone and bacterial colony diameter (or well diameter).

For cellulose and chitin degradation, to clarify the presence of halo rings, the agar plate should be stained with Congo red dye 0.1% for 15 min, then washed with NaCl 1 M [20]. For the lipolysis assay, fluorescence of the lipid complex and Rhomadine B under UV light at 365 nm would clarify the appearance of the halo rings [6].

### 2.3. Investigation of siderophore production

Siderophore detection was performed using a liquid CAS assay according to the method of Bharucha et al. [21]. The CAS solution was prepared according to the formula of Alexander and Zuberer [22]. One mL of bacterial suspension (density 1.5×10^8 CFU/mL) was centrifuged at 10,000 rpm for 3 minutes. The supernatant was obtained and transferred to a fresh tube, then mixed with an equal volume of CAS solution. The mixture was incubated in the dark for 30 minutes and then observed the color changed. The color change of blue CAS reagent to another color such as orange, purple, green indicates the presence of siderophore.

Strains with good qualitative results were selected to investigate the ability to produce siderophore on PS - CAS agar medium [23]. PS medium (10 g peptone; 20 g sucrose; pH 7) was poured into half of the Petri dish and served as a substrate for bacterial culture. After 48 hours of culture, if the bacteria produce siderophores, the discoloration of the CAS agar in the other half of the agar plate would show this possibility. The width of the discolored band would indicate the level of siderophore production.

### 2.4. Identification of selected bacterial strains

This experiment was limited to the strains with the best experimental results. Classification according to the MALDI-TOF method (Matrix-assisted laser desorption ionization/Time of flight) was performed by the Science and Biotechnology Center, Faculty of Biology and Biotechnology, HCMC University of Natural Sciences, Vietnam.

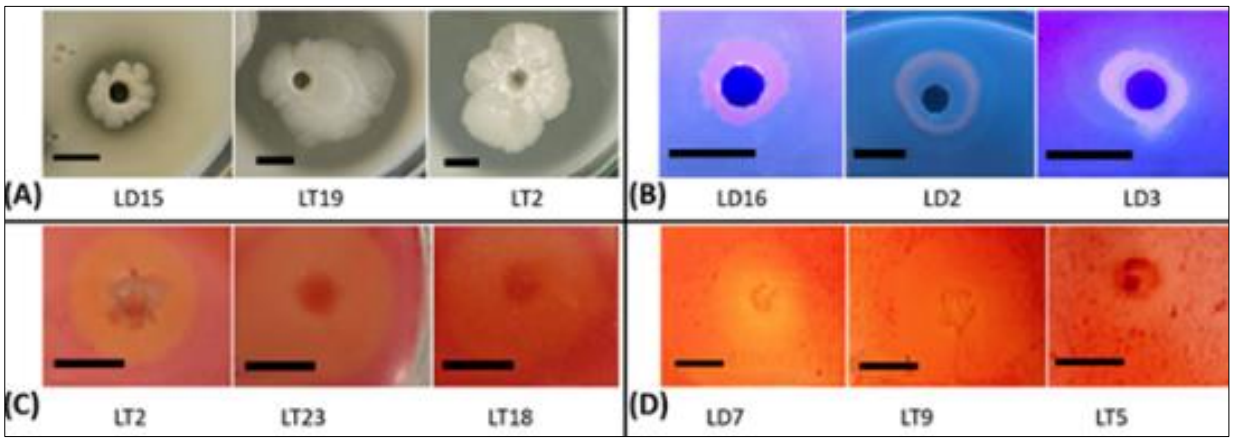
### 2.5. Experiment design and data analysis

The quantitative experiment was arranged in a completely randomized design with 3 replications. One-factor analysis of variance and Duncan's test with the value α=0.05 with the support of IBM SPSS Statistics software version 20.0.

## 3. Results and discussion

### 3.1. Ability of PGPR to decompositions of protein, lipid, cellulose and chitin

The results showed that, among 39 PGPR strains of pepper plants, the number of strains capable of degrading protein, lipid, cellulose and chitin were 37, 6, 27 and 16, respectively. Some of the best halo ring forming strains were shown in Figure 1 below.




(A): protein; (B): lipid; (C): cellulose; (D): chitin; Scale bar = 1 cm

**Figure 1** Halo rings of some PGPR strains in organic matter decomposition experiments

Regarding protein decomposition, except for 2 strains LD9 and LT6 which did not have this ability, the remaining 37 strains (accounting for 94.9% of the total number of strains surveyed) all produced halo rings. The number of strains with decomposition values greater than 1, from 1 to less than 2 and over 2 (cm) were 16, 18 and 3 strains, respectively. The best strain was LT2 with a measured value of 2.53 cm. According to some studies in the same field, the number of bacterial strains with proteolytic ability could account for 50% to 69.2% of the surveyed strains [24-26]. The diameter of the largest halo zone was measured from 1.6 to 1.9 cm [8, 27].

Regarding the ability to degrade lipids, only 6 strains of PGPR in the black pepper rhizosphere had this ability, accounting for 13.4%. The measured values ranged from 0.2 to 1.2 cm with strain LD16 being the best. The number of bacterial strains capable of lipolysis in general accounted for a small proportion in studies in the same field, for example 2 strains out of 140 strains (1.4%) or 8 strains out of 250 strains (3.2 %) [28, 29]. The values obtained for the halo zones also ranged from 0.3 to 1.6 cm [8].

The results on the ability to degrade cellulose showed that there were 27 PGPR strains (accounting for 69.2%) with halo formation. The number of strains with cellulose decomposition values greater than 1, from 1 to less than 2 and over 2 (cm) were 9, 16 and 2 strains, respectively. The two strains with the best results were LT23 and LD8 with measured values of 2.07 and 2.13 cm, respectively, but this difference was not statistically significant. According to results of previous studies, the sizes of the halo zones ranged from 0.2 to 0.9 cm [8, 30], but the number of strains capable of degrading cellulose could be up to 59.8% (61 strains out of 102 isolates) [31].

For the ability to degrade chitin, there were 16 strains out of 39 PGPR strains capable of forming halo rings on the culture medium containing the colloidal chitin substrate, accounting for 41%. This result showed the abundance of bacteria with this ability in the rhizosphere of black pepper grown in Loc Ninh, Binh Phuoc, Vietnam. Chitin was a major component in fungal cell wall, the ability to secrete the enzyme chitinase had been considered a key factor in detecting antifungal properties of bacteria in many studies. The percentage of bacterial strains capable of degrading chitin was 0.7% (3 out of 420 strains) [32], or 12.5% (9 out of 72 strains) [21]. The chitinolytic ability of 16 PGPR strains of pepper in the present study ranged from 0.2 to 1.2 cm. Only strain LT19 showed a halo zone of more than one cm (1.2 cm), the remaining 15 strains all had halo size less than 1 cm. The ability to degrade chitin through ring size was generally not high, even below 0.1 cm [33].

The bacteria did not possess individual plant growth-promoting properties, but rather multiple properties combined (additive hypothesis) [9, 34]. The ability to secrete enzymes such as protease, lipase, chitinase helped PGPR break down harmful fungal cell walls. Furthermore, when secreting extracellular enzymes into the environment, rhizobacteria also contributed metabolize a large number of organic compounds available in the soil into a simple form that plants could absorb. The rhizospheric enzyme was more active and important enzymes present in the bulk soil for the breakdown of carbon substrates and other organic substances containing N, P and S [6]. In contrast, organic matter such as sewage sludge or composted sewage sludge also had a selective effect on bacterial population and activated the synthesis of suitable enzymes to break down substrates that had a positive effect on plants [35, 36].

### 3.2. Ability of PGPR to produce siderophore

The qualitative results of siderophore production by liquid CAS method showed that all 39 strains had this ability. Based on the color spectrum of Nielsen et al. [37], there were 34 strains (accounting for 87.2%) that produced good siderophores, corresponding to the reagent color transition from blue to yellow and orange, and 5 strains (accounting for 22.8%) that were less able to produce siderophores, corresponding to green color. In some studies in the same field, the percentage of strains capable of producing siderophore accounted for only 0.7% (9 out of 72 strains) [32] or 9.2% (9 out of 72 strains) [38].

For the experiment on PS - CAS agar plate, out of a total of 22 strains with liquid CAS that changed color from green to orange (producing many siderophores), 6 strains (LD5, LD13, LT4, LT5, LT8, LT16) completely changed color 1/2 of CAS agar plate, the width of discolored band was greater than or equal to the radius of the Petri dish (4.5 cm). The remaining fourteen strains showed the width of discolored bands (yellow or green) ranging from 1.0 to 3.7 cm. Cultivation of bacteria on agar containing CAS would cause death of some strains due to the toxicity of this medium. The method proposed by Srivastava et al. allowed bacteria grown on the nutrient medium (PS) half plate and the siderophore in the exudate (if any) readily diffuse to the half plate of CAS and discolor the reagent. Sometimes the color of the CAS reagent changed from blue to purple, pink or other colors because the siderophores was composed of many different chemical radicals bound to iron(III) [39, 40]. Because of the wide variety of siderophores, there was currently no standard curve for all, so colorimetric siderophore quantification was very rarely performed.

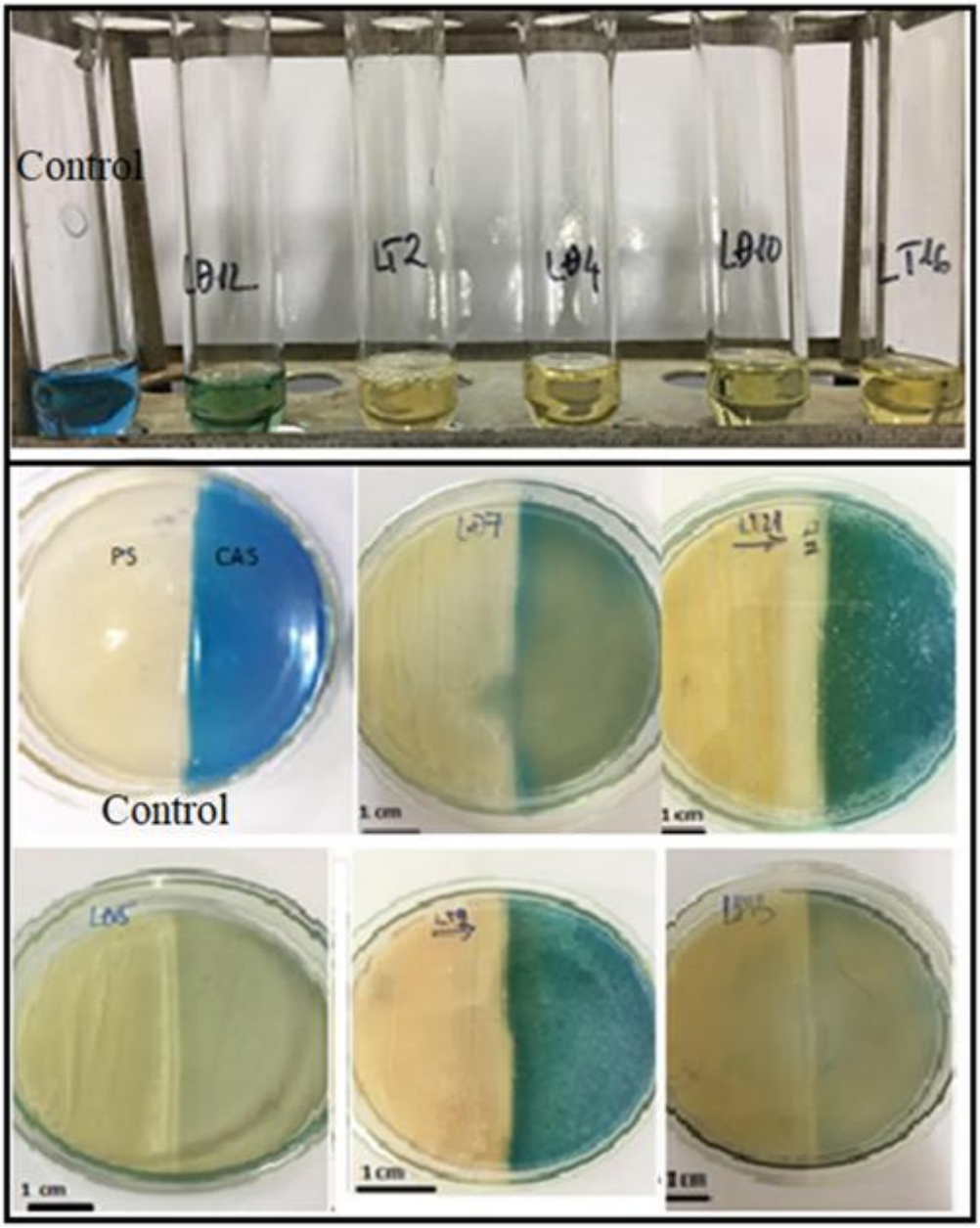



**Figure 2** Color change of CAS reagents in liquid CAS (above) and PS-CAS agar (below) experiments under the influence of siderophore produced by some strains of PGPR

### 3.3. Identification results of selected isolates

Seven strains were selected to be identified by the MALDI-TOF technique. This method had been used by authors [41-43] to identify PGPR. Characteristics of organic matter decomposition, siderophore production and species names of these 7 strains were presented in Table 2 after checking the suitability of the identification results with their cell and colony morphological characteristics. Strain LD2 was identified as *Proteus mirabilis*. The remaining six strains belonged to the genus *Bacillus*. In which LD8, LT2 and LT4 had been identified as *B. cereus*, *B. subtilis* and *B. megaterium*, respectively [5]. Three strains LD7, LD16, LT19 were identified as *B. cereus*.

**Table 2** Organic matter degradation, siderophore production and identification results of selected strains

| Strains | Decomposition of organic matter | | | | Siderophore production | Identification result |
|---|---|---|---|---|---|---|
| | Protein | Lipid | Cellulose | Chitin | | |
| LD2 | 0.9 $^{jkl}$ | 0.7 $^{b}$ | 1.9 $^{b}$ | - | - | *Proteus mirabilis* |
| LD7 | 1.1 $^{hij}$ | - | 0.3 $^{p}$ | 0.6 $^{b}$ | 3.8 $^{b}$ | *Bacillus cereus* |
| LD8 | 1.3 $^{efg}$ | - | 2.1 $^{a}$ | 0.2 $^{d}$ | - | *B. cereus** |
| LD16 | 1.2 $^{fgh}$ | 1.2 $^{a}$ | 0.5 $^{o}$ | - | 2.2 $^{f}$ | *B. cereus* |
| LT2 | 2.8 $^{a}$ | - | 1.3 $^{i}$ | 0.2 $^{d}$ | 2.9 $^{d}$ | *B. subtilis** |
| LT4 | 0.53 $^{pq}$ | - | 0.5$^{o}$ | - | 4.5 $^{a}$ | *B. megaterium** |
| LT19 | 1.07$^{hij}$ | - | 1.5 $^{gh}$ | 1.2 $^{a}$ | - | *B. cereus* |

In the same column, values followed by the same letter were not statistically significant according to Duncan's test. (*) These strains had been identified previously [5] because of their good ability in N fixation, P solubilization and IAA production.

*Proteus mirabilis*, a Gram-negative bacterium that had been isolated from the rhizosphere soil of maize (*Zea mays*) grown in Pakistan. The study showed that this bacterium had the ability to produce IAA and siderophore, and limit the toxicity of zinc residues in soil to maize [44]. *Bacillus cereus* and *B. megaterium* were also found in that study. *Bacillus* were Gram-positive bacteria common in soil. They had many plant growth-promoting properties, such as nitrogen fixation, phosphate solubilization, IAA biosynthesis, secretion of organic matter-degrading enzymes, and siderophore production [45]. *B. cereus* had the ability to secrete chitinase [33]. They were also resistant to fungi, produced siderophores, and enhanced the plant's ability to absorb mineral elements [46]. While *B. subtilis* was very well known and had many applications [45]. They were isolated from the soil of tomato rhizosphere in Kazan (Russia), and were resistant to some fungal diseases, produced siderophores, secreted protease, cellulase and HCN [47].

## 4. Conclusion

The ability to degrade protein and cellulose was more common in the rhizobacterial strains of black pepper grown in Loc Ninh, Binh Phuoc. The number of strains that degraded protein, cellulose, chitin, and lipid were 37, 27, 16, and 6, respectively, out of a total of 39 strains investigated. Qualitative experiments showed that all 39 strains were able to change color of liquid CAS demonstrating their ability to produce siderophore, but only 22 strains were able to change color of CAS agar. The six best strains completely changed the color of half of the CAS agar plate (4.5 cm or more). The seven best strains in terms of abilities investigated were identified as *Proteus mirabilis* (LD2), *Bacillus megaterium* (LT4), *B. subtilis* (LT2), *B. cereus* (LD7, LD8, LD16, LT19) showing additional application potential for black pepper cultivation as biosafety strains.

## Compliance with ethical standards

### *Acknowledgments*

The authors would like to thank for the support of Saigon University.

### *Disclosure of conflict of interest*

There is no conflict of interest.

## References

[1] Hoai Thu. Vietnam is still the largest pepper producer and exporter [Internet]. Vietnam News Agency/Vietnamplus. © 2022 Nov 10 [cited 2023 Feb 7]. Available from: https://link.gov.vn/RUPJ6CmV.

[2] Gupta G, Pairhar SS, Ahirwar NK, Snehi SK and Singh V. Plant–growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. Journal of Microbial and Biochemical Technology. 2015; 7(2): 96-102.

[3] Rawat S and Mushtaq A. Plant growth promoting rhizobacteria, a formula for sustainable agriculture: A review. Asian Journal of Plant Science and Research. 2015; 5(4):43-46.

[4] Tran H, Kruijt M, Raaijmakers JM. Diversity and activity of biosurfactant-producing Pseudomonas in the rhizosphere of black pepper in Vietnam. Journal of Applied Microbiology. 2008; 104(3): 839-51. Doi: 10.1111/j.1365-2672.2007.03618.x.

[5] Thanh, D.T.N. and Tram, D.T.T. Isolation and characterization of plant growth promoting rhizobacteria in black pepper (*Piper nigrum* L.) cultivated in Chon Thanh and Loc Ninh districts of Binh Phuoc province, Vietnam. International Journal of Innovations in Engineering and Technology. 2018; 10(1): 1-10.

[6] Dang Thi Ngoc Thanh, Ha Bao Son, and Chau Kim Xuyen. The ability of degrading protein, lipid, starch, chitin, and fungal inhibiting of rhizobacteria isolated from black pepper plants (*Piper nigrum* L.) cultivated in Chon Thanh district, Binh Phuoc province. Can Tho University Scientific Journal. 2020; 56(1B): 95-103.

[7] Idiyatov II, Khammadov NI, Eroshin AI, Tremasova AM, Tremasov YM, Potekhina RM, Khabirova SR, Smolentsev SY, Biryulya VV, Erokhondina MA, Khuzin DA and Faizov TH. Study of Antagonism of Endophytic Bacterial Isolates Against *Fusarium sporotrichioides*. Natural Volatiles & Essential Oils, 2021; 8(4): 3550-3565.

[8] Azman NA, Sijam K, Hata EM, Othman R and Saud HM. Screening of Bacteria as Antagonist against *Xanthomonas oryzae* pv. oryzae, the Causal Agent of Bacterial Leaf Blight of Paddy and as Plant Growth Promoter. Journal of Experimental Agriculture International. 2017; 16(4): 1-15.

[9] Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University. 2013.

[10] Rajkumar M and Freitas H. Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere. 2008; 71(5): 834–842.

[11] Saharan BS and Nehra V. Plant Growth Promoting Rhizobacteria: A critical review. Life Sciences and Medicine Research. 2011; 21:1-30.

[12] Bonaterra A, Badosa E, Daranas N, Francés J, Roselló G and Montesinos E. Bacteria as Biological Control Agents of Plant Diseases. Microorganisms. 2022; 10(9): 1759.

[13] Bertani G. Studies on Lysogenesis I: The mode of phage liberation by lysogenic *Escherichia coli*. Journal of Bacteriology. 1951; 62(3): 293-300.

[14] Sutton S. Determination of Inoculum for Microbiological Testing. Journal of GXP Compliance. 2011; 15: 49-53.

[15] Sokol PA, Ohman DE and Iglewski BH. A more sensitive plate assay for detection of protease production by *Pseudomonas aeruginosa*. J. Clin. Microbiol. 1979; 9: 538.

[16] Lechuga EGO, Zapata IQ and Nino KA. Detection of extracellular enzymatic activity in microorganisms isolated from waste vegetable oil-contaminated soil using plate methodologies. African Journal of Biotechnology. 2016; pp. 408-416.

[17] Teather RM and Wood PJ. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl. Environ. Microbiol. 1982; 43:777-780.

[18] Hsu S and Lockwood J. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. J. Appl. Microbiol. 1975; 29:422-426.

[19] Balouiri M, Sadiki M and Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6(2): 7-79.

[20] Ashwini N, Srividya S. Potentiality of Bacillus subtilis as biocontrol agent for management of anthracnose disease of chili caused by *Colletotrichum gloeosporioides* OGCI. Original Article. 2014; 4:127-136.

[21] Bharucha UD, Patel KC and Trivedi UB. In vitro screening of isolates for its plant growth-promoting activities from the rhizosphere of Alfalfa (*Medicago sativa*). Journal of Microbiology and Biotechnology Research. 2013; 3(5): 79-88.

[22] Alexander DB and Zuberer D A. Use of chrome azurol S agents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil Soils. 1991; 12: 39-45.

[23] Srivastava MP, Tiwari R, Sharma N. Effect of different cultural variables on siderophores produced by *Trichoderma* spp. International Journal of Advanced Research. 2012; 1(7): 1-6.

[24] Farooq U, Bano A. Screening of indigenous bacteria from rhizosphere of maize (*Zea Mays* L.) for their growth promotion ability and antagonism against fungal and bacterial pathogens. The Journal of animal and plant Sciences. 2013; 23(6): 1642-1652.

[25] Dutta J and Thakur D. Evaluation of multifarious plant growth promoting traits, antagonistic potential and phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling, India. PLoS One. 2017; 12(8): e0182302.

[26] Rasool A, Mir MI, Zulfajri M, Hanafiah MM, Unnisa S, Mahboob M. Plant growth promoting and antifungal asset of indigenous rhizobacteria secluded from saffron (*Crocus sativus* L.) rhizosphere. Microbial Pathogenesis. 2021; 150: 104734.

[27] Nguyen Thi Lien, Nguyen Thi Yen Nhu, Tran Thi Xuan Mai, and Nguyen Thi Pha. Isolation and selection of antagonistic bacteria from rhizosphere soil against *Colletotrichum* sp. causing anthracnose on chilli. Can Tho University Scientific Journal. 2016; 47b: 16-23.

[28] Duong Minh Lam, Vu Thi Ly. Research and selection of alkaline lipase-producing *Bacillus* strains from mangroves. Journal of Science and Technology. 2012; 50(2): 259-266.

[29] Geetha K, Venkatesham E, Hindumathi A and Bhadraiah B. Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vigna Radita* (L.) R. Wilczek. Original Research Artide. 2014; 6:799-809.

[30] Nguyen Thi Thanh Truc. Isolation and selection of cellulose-degrading bacteria from dragon fruit branches. Vietnam Academy of Agricultural Sciences. 2015.

[31] Fahmy H, Zohri AEA, Mohamed GE, Hafez E. Sugar beet is a suitable source for cellulase-producing bacteria and actinomycetes. Egyptian Sugar Journal. 2022; 18: 40-52.

[32] Han J H, Shim H, Shinand JH, Kim KS. Antagonistic Activities of *Bacillus* spp. Strains Isolated from Tidal Flat Sediment Towards Anthracnose Pathogens *Colletotrichum acutatum* and *C. gloeosporioides* in South Korea. The Plant Pathology Journal. 2015; 31(2):165 - 175.

[33] Mubarik RS, Mahagiani I, Anindyaputri A, Santoso S and Rusmana I. Chitinolytic Bacteria Isolated from Chili Rhizosphere: Chitinase Characterization and Its Application as Biocontrol for Whitefly (*Bemisia tabaci* Genn.). American Journal of Agricultural and Biological Sciences. 2010; 5 (4): 430-435.

[34] Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G and Mora ML. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr. 2010; 10(3): 293-319.

[35] Liu X, Guo K, Huang L, Ji Z, Jiang H, Li H and Zhang J. Responses of absolute and specific enzyme activity to consecutive application of composted sewage sludge in a Fluventic Ustochrept. PLoS One. 2017; 12(5): e0177796.

[36] Lloret E, Pascual, JA, Elm B, et al. Sewage sludge addition modifies soil microbial communities and plant performance depending on the sludge stabilization process. Applied Soil Ecology. 2016; 101: 37-46.

[37] Nielsen A, Mansson M, Wietz M, Varming AN, Phipps RK, Larsen TO, Gram L, Ingmer H. Nigribactin, a novel siderophore from *Vibrio nigripulchritudo*, modulates *Staphylococcus aureus* virulence gene expression. Journal List – Mar Drugs. 2012; (10)11: 2584-2595.

[38] Tran Thi Giang, Nguyen Thi Quyen, Cao Ngoc Diep. Isolation and identification of growth-promoting rhizosphere bacteria (PGPR) from some leafy vegetables grown in Can Tho City. Can Tho University Journal of Science. 2014; 35:65-73.

[39] Dang Thi Ngoc Thanh, Nguyen Thi Xuan My and Cao Ngoc Diep. Indole acetic acid and siderophore production by selected isolates of plant associated bacteria and their effects on growth of maize (*Zea mays* L.) in pot experiments. Can Tho University Journal of Science. 2016; 47a (2016): 59-67.

[40] Tarah SS., Seema R, Virginia H G, Adash R, Janice ET. Siderophore production of African dust microorganisms over Trinidad and Tobago. Aerobiologia. 2012; 28(3): 391-401.

[41] Stets MI, Pinto Jr AS, Huergo LF, de Souza EM, Guimaraes VF, et al. Rapid identification of bacterial isolates from wheat roots by high resolution whole cell MALDI-TOF MS analysis", Journal of Biotechnology. 2013; 165: 167-174.

[42] Tani A., Sahin N, Fujitani Y, Kato A, Sato K, Kimbara K. Methylobacterium Species Promoting Rice and Barley Growth and Interaction Specificity Revealed with Whole-Cell Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) Analysis. PLOS ONE. 2015; e0129509.

[43] Urquiza CC, Hernández IA, Medina JA., López MAR, Cruz JP, Zarazúa RLR et al. Identification by MALDI-TOF Mass Spectrometry of Mercury-resistant Bacteria Associated with the Rhizosphere of an Apple Orchard. Geomicrobiology Journal. 2017; 33: 176-182.

[44] Islam F, Yasmeen T, Riaz M, Arif SM. Proteus mirabilis alleviates zinc toxicity by preventing oxidative stress in maize (*Zea mays*) plants. Ecotoxicology and Environmental Safety. 2014; 110:143-152.

[45] Saharan BS and Nehra V. Plant Growth Promoting Rhizobacteria: A critical review. Life Sciences and Medicine Research. 2011; 21:1-30.

[46] Ying M, Rajkumar M, Helena F. Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. Journal Hazardous Materials. 2008; 166(2-3):1154-1161

[47] Mardanova, AM, Hadieva, GF, Lutfullin, MT, Khilyas, IV, Minnullina, LF, Gilyazeva, AG, Bogomolnaya LM and Sharipova MR. *Bacillus subtilis* Strains with Antifungal Activity against the Phytopathogenic Fungi. Agricultural Sciences. 2017; 8: 1-20.