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A snapshot of bacterial endophytes isolated from the roots of Vetiver grass (*Chrysopogon zizanioides*) grown at Bien Hoa airbase, Dong Nai province

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Abstract. Vetiver grass (*Chrysopogon zizanioides*) with a strong root system has been widely used for environmental pollution treatment. Recently, this grass has been cultivated at Bien Hoa airbase to mitigate dioxin concentration in the contaminated soil. Bacterial endophytes have been recognized to play key roles in plant growth promotion and responses of plant hosts to environmental factors. However, an inspection of endophytic bacteria from the roots of Vetiver grass grown in dioxin-contaminated soil at Bien Hoa airbase has not been elucidated. In the present study, we successfully isolated sixteen endophytic bacterial strains from the roots of Vetiver grass. Results of 16S rRNA gene sequencing revealed that these culturable bacterial endophytes belong to 9 species of different genera. Four strains, including *Klebsiella variicola* B1, *Enterobacter cloacae* B4, *Enterobacter cloacae* B6, and *Enterobacter asburiae* B11, produce high amounts of indole acetic acid (IAA). Strains *K. variicola* B1 and *E. cloacae* B4 also produce phytase and phosphatase to dissolve phytate and phosphate, respectively. These strains were selected to evaluate their abilities in promoting the growth of tomato plants as a model. After 28 days of inoculation with the tested strains through the roots, tomato plants grew 19 % to 22 % faster than the control plants. Conclusively, this study shows for the first time the

successful isolation of endophytic bacteria from the roots of Vetiver grass cultivated in dioxin-contaminated soil, and some culturable bacterial strains exhibit as promising candidates for plant growth promotion.

Keywords: endophytic bacteria, dioxin-contaminated soil, indole acetic acid, plant growth promotion, Vetiver grass.

Classification numbers: 3.1.1, 3.1.2, 3.4.3.

1. INTRODUCTION

Vetiver grass (*Chrysopogon zizanioides*) is well-known for various applications, such as producing essential oils, making crafts, preventing soil erosion and landslides, and environmental pollution treatment. This plant can grow well in extreme conditions or soils with low nutrient content. Vetiver grass is remarkably exploited for phytoremediation to remove toxic substances, including persistent organic pollutants, heavy metals, herbicides, etc [1-5]. Bien Hoa airbase is a hotspot of dioxin contamination in Vietnam [6]. In previous work, Vetiver grass has been applied to mitigate dioxin pollution at Bien Hoa airbase. The results showed a significant decrease in dioxin content in the contaminated soil. Notably, Vetiver grass grew very well in the soil of this location [7]. The growth of this grass is promoted by the expanded root system. Plant growth may benefit from soilborne and endophytic microorganisms [3, 8, 9]. Endophytic bacteria living inside plant root tissues have been reported to play vital roles in the growth and development of plant hosts. They help plants to absorb nutrients and produce biostimulants to promote the plant growth. These microbial endophytes can also assist crops in elevating the tolerance to salinity and drought stress [10 - 15].

In this study, we report for the first time sixteen endophytic bacterial strains isolated from the root tissues of Vetiver grass grown in dioxin-contaminated soil at Bien Hoa airbase, Dong Nai province. These strains could grow on the basic medium in the laboratory. Phenotypic analyses and 16S rRNA gene sequencing revealed that the isolated strains belong to different bacterial species. Notably, some of them could produce high amounts of indole acetic acid as a potential plant growth stimulant. Assays using tomato seedlings inoculated with two selected strains indicated that these endophytes were able to promote the growth of the tested plants.

2. MATERIALS AND METHODS

2.1. Materials

Materials: Root tissue samples were collected from Vetiver plants grown at Bien Hoa airbase, Dong Nai province.

Microbial strains: A laboratory strain of *Bacillus subtilis* VS1 used for reference control was provided by the Department of Microbiology, Faculty of Biology, University of Science, Vietnam National University, Hanoi.

Media used for bacterial growth and detecting biological activities:

Luria-Bertani (LB) medium contains 10 g peptone, 5 g yeast extract, 5 g NaCl, 20 g agar, and distilled H_2O to 1000 mL.

LB-Tryptophan medium includes 2 % peptone, 0.15 % K_2HPO_4, 1.5 % $MgSO_4.7H_2O$, 1.5 % glycerol, 0.05 % tryptophan, and distilled H_2O to 1000 mL.

Phytase screening medium (PSM) comprises 1 % glucose, 0.4 % sodium phytate, 0.2 % $CaCl_2$, 0.5 % $(NH_4)_2SO_4$, 0.05 % KCl, 0.05 % $MgSO_4.7H_2O$, 0.001 % $FeSO_4$, 2 % agar, and distilled H_2O to 1000 mL.

Pikovaskya medium contains 1 % glucose, 0.5 % $Ca_3(PO_4)_2$, 0.05 % $(NH_4)_2SO_4$, 0.01 % $MgSO_4.7H_2O$, 0.02 % KCl, 0.05 % yeast extract, 0.0002 % $MnSO_2.H_2O$, 2 % agar, and distilled H_2O to 1000 mL.

2.2. Methods

Collecting Vetiver root samples: Root samples were taken at the root system of the Vetiver grass grown at Bien Hoa airbase, Dong Nai province. Firstly, the soil surface of the sampling locations was cleaned by removing all gravel and rocks. Subsequently, pickaxes, shovels, and scissors were used to dig and collect root samples (50 g/sample) at the selected locations. Root samples were rinsed with tap water to eliminate soil and sand. In the following step, the samples were washed sequentially with distilled water, hexane, and acetone to eliminate contaminated soil and toxic chemicals. The samples were air-dried and divided into different zip-bags with specific sample labels. The samples were stored at 4 °C and transported to the laboratory for further analysis.

Isolation of endophytic bacteria: The root samples were washed under running water for 3 min and then cut into small pieces of 5 cm. The surface of root samples was sterilized with 96 % ethanol (3 min), 3 % hydrogen peroxide (3 min), and then 1 % sodium hypochlorite (3 min). Finally, the samples were rinsed with sterile distilled water four times. To examine the effectiveness of the surface sterilization, 100 µL of the water of the last wash was spread on an LB plate, and the plate was incubated at 37 °C for 24 h. If there is no bacterial growth, surface disinfection is successful. The sterilized root samples were ground well using a mortar and a pestle with 1 mL of sterile distilled water. A volume of 100 µL of the liquid was spread on an LB plate, and the plate was kept at 30 - 37 °C to collect single bacterial colonies.

Genomic DNA extraction from bacterial strains: The protocol for genomic DNA extraction was conducted as previously reported [16] with some minor adjustments. Bacterial strains were grown in the LB liquid medium for 24 h at 30 °C using an orbital shaking incubator. A volume of 2 mL of each bacterial culture (OD_{600} = 1.5 - 2.0) was centrifuged at 12,000 rpm for 1 min to collect the cells. The cell pellet was resuspended in 70 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8), and the tube was vortexed vigorously for 15 s. The sample was added with 30 µL lysozyme (10 mg/mL), and incubated at 25 °C for 10 min. In the next step, 600 µL of GX extraction buffer and 3 µL proteinase K (20 mg/mL) were added to the sample. The tube was gently mixed for 15 s and incubated at 60 ºC for 30 min. A volume of 300 µL sodium acetate 3M (pH 5.2) was added, and the tube was centrifuged at 4 ºC, 12000 rpm for 20 min. The supernatant phase was transferred to a new 1.5 mL Eppendorf tube, and an equal volume of cold isopropanol was added to precipitate DNA. The tube was then centrifuged at 12000 rpm, 4 ºC for 10 min. The supernatant was discarded, and 700 µL of 70 % ethanol was added to wash the DNA pellet. The DNA pellet was collected by centrifugation at 4 ºC, 12000 rpm for 5 min. The DNA sample was air-dried and dissolved in 50 µL of the TE buffer. RNA was digested by 3 µL RNase A (10 mg/mL) at 60 ºC for 30 min. The DNA sample was stored at 4 °C for further experiments.

DNA sequencing and identification of bacterial strains: 16S rRNA gene was amplified by PCR using the universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT). The thermal cycle for the PCR amplification includes 95 °C (3 min); 35 cycles of 94 °C (45 s), 53 °C (45 s), 72 °C (1 min); and 72 °C (7 min). PCR products were checked on an agarose gel by electrophoresis and purified by MEGAquick-spin™ Plus Total Fragment DNA Purification Kit (iNtRON Biotechnology, South Korea). The purified DNA samples were sequenced from the forward direction by the 1st Base company (Singapore). DNA sequences were checked for quality using BioEdit 7.2 software and comparative analysis with the GenBank database using the BLAST tool.

Assay for the production of IAA: A bacterial strain was freshly grown in the LB medium at 37 °C for 24 h. Subsequently, 0.4 mL of the culture was added to a flask containing 40 mL of the LB-Tryptophan medium. Negative control flasks were not inoculated with bacterial strains. Flasks were shaken in an incubator at 37 °C, 150 rpm for 72 h in darkness. A volume of 1.5 mL of the culture was pipetted into an Eppendorf tube, and the bacterial cells were removed by centrifugation at 6000 rpm for 10 min. The supernatant of 1 mL was transferred to a new Eppendorf tube, and 4 mL of the Salkowski reagent (0.5 M $FeCl_3$ dissolved in 35 % H_2SO_4 at the 1 : 50 ratio) was added. The sample was mixed well and kept at room temperature for 25 min. The sample was quantified for IAA concentration at 530 nm, as previously described [17, 18].

Assay for phytase activity: 50 µL of bacterial culture was added to a well in a PSM agar plate. After 24 h of incubation at 30 ^{o}C, phytate degradation was confirmed by a clear halo around the colony. For phytase activity quantification, a bacterial strain was grown in the PSM medium at 200 rpm, 37 °C for 72 h. The culture was centrifuged at 6000 rpm, 20 °C for 30 min. The supernatant as a crude enzyme solution was transferred to a new tube. The reaction mixture contained 125 µL of the crude enzyme solution, 125 µL of 15 mM sodium phytate (Sigma-Aldrich, USA), and 125 µL of 200 mM sodium acetate buffer (pH 5.5). The mixture was then incubated at 55 °C for 30 min, and the reaction was stopped by adding 0.5 mL 15 % TCA. The development of yellow color began when 125 µL of the mixture was mixed with 1 mL of AAM solution (10 mM ammonium molybdate, 100 % acetone, 5 N sulfuric acid, 1:2:1). After 2 min of incubation at room temperature, 100 µL of 1 M citric acid was added. The sample was measured at 355 nm for the phytase activity quantification [19].

Detection of insoluble phosphate degradation [20]: Bacterial strains were grown on the Pikovaskya medium at 37 °C for 72 h. The ability of phosphate degradation was detected via clear halos around the bacterial colonies.

Assay for the tomato growth promotion by the selected bacterial strains: tomato seeds were inoculated to the nursery soil for 15 days. Afterward, 30 plants with the same length of stem, number of leaves, and root length were selected for the next experiment. The root tips of all the plants were injured by cutting. For bacterial inoculation, 10 plants were soaked in a bacterial culture (10^6 CFU/mL) containing one of two selected strains for 20 min. For the control, 10 plants were grown without bacterial inoculation. Plant height was measured at 7, 14, 21, and 28 days. The experiment was repeated twice.

Statistical analysis: Data were presented as means ± standard deviations. Statistical analyses were performed with GraphPad Prism 8.0 using one-way ANOVA and Tukey's test. A statistical difference was considered when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Successful isolation of culturable endophytic bacteria from Vetiver root samples

After surface sterilization of the Vetiver root samples, endophytic bacteria from the root tissues were isolated. Bacterial colonies appearing on the agar plates were separated and purified to obtain pure single strains (Figure 1). Sixteen bacterial isolates with diversified colony morphologies and colors were selected. These endophytic strains form mainly round colonies. Results from Gram staining showed that there are 10 Gram-negative and 6 Gram-positive bacterial strains (Figure 2). Further, cell morphological analysis under microscopy revealed that all 16 strains are rod-shaped bacteria or bacilli (Table 1).

The isolated endophytic bacteria were identified based on morphological characteristics, Gram staining, and partial sequencing of the 16S rRNA gene. Genomic DNA samples were prepared from these strains for PCR amplification of the 16S rRNA gene using universal primer pair 27F/1492R. Analysis of PCR products on an agarose gel showed a single DNA band of approximately 1500 bp for all the strains (Figure 3A). Purified PCR products were sequenced from the forward direction, and the DNA sequences were compared with the GenBank database using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All the 16S rRNA sequences of the endophytic bacterial strains were deposited in GenBank under accession numbers (OR921912- OR921927). Based on the results of 16S rRNA sequence analysis, 16 endophytic strains were preliminarily classified into 9 different species, including *Klebsiella variicola*, *Priestia megaterium* (previously known as *Bacillus megaterium*), *Citrobacter freundii*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Enterobacter hormaechei*, *Bacillus cereus*, *Enterobacter asburiae*, and *Serratia marcescens* (Figure 3B). However, a major limitation of the 16S rDNA sequence is that it is unreliable for discriminating among closely related bacterial species of a genus. To solve this problem, sequencing of other bacterial target genes, such as *rpoB* (β-subunit of RNA polymerase), *tuf* (elongation factor Tu), *gyrA* or *gyrB* (gyrase A or B), or *sodA* (manganese-dependent superoxide dismutase) may provide better separation of closely related species [21]. Therefore, some endophytic strains of the same genus from this study still need to be further confirmed for accurate species classification.

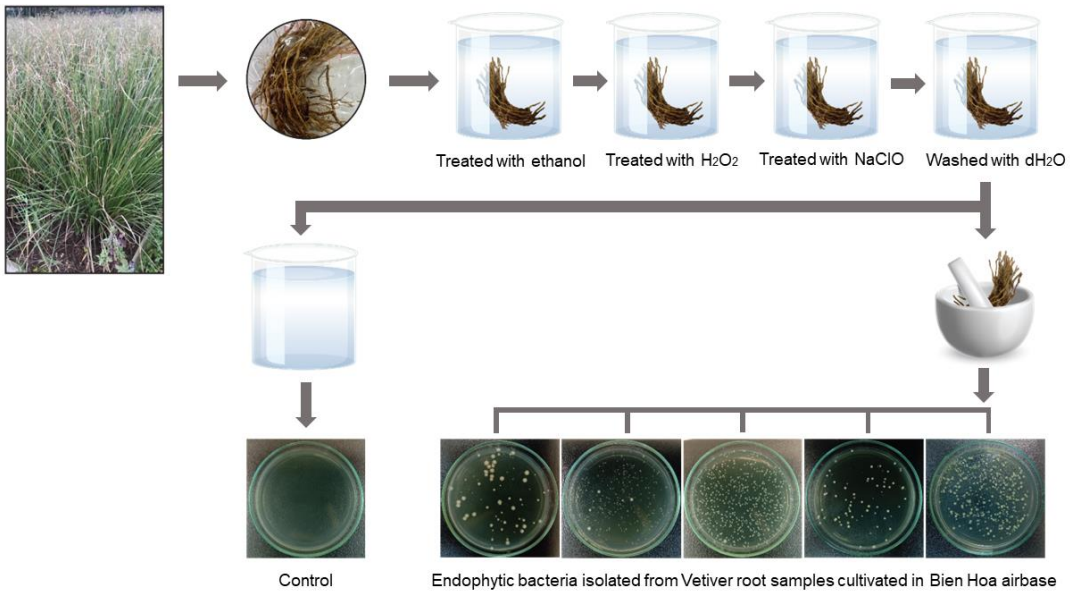


Figure 1. The schematic strategy for isolating endophytic bacteria from Vetiver grass roots.

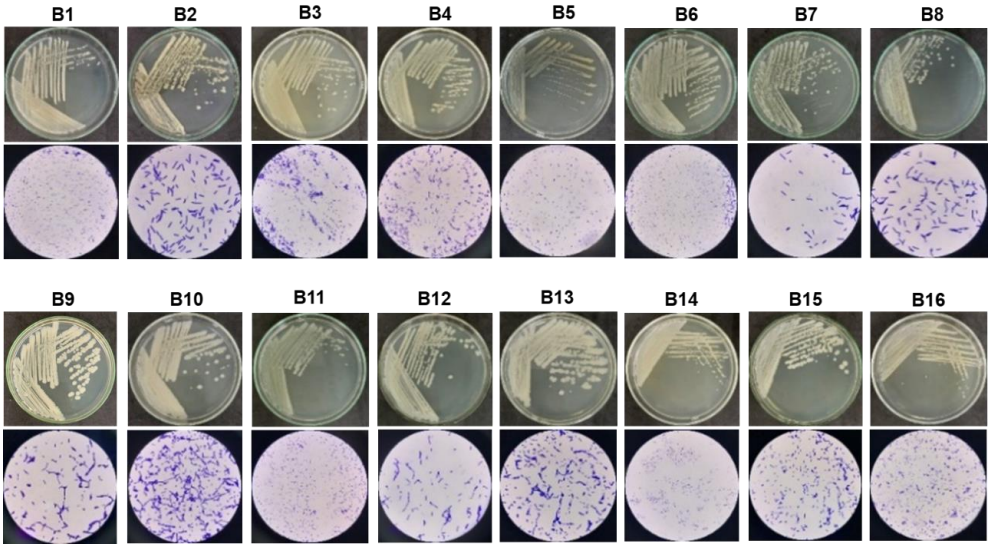


Figure 2. Phenotypic characteristics of sixteen endophytic bacterial strains. The strains were cultivated on the LB agar medium to examine colony morphology. Cell morphologies of these bacteria were observed under microscopy.

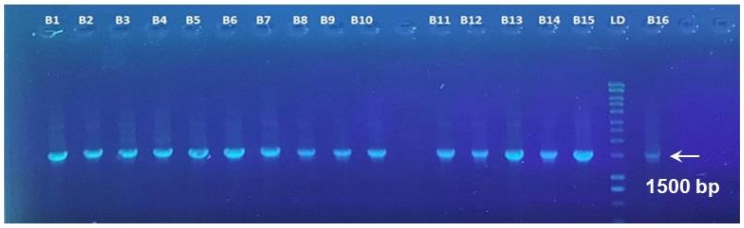
Table 1. Phenotypic characteristics and Gram staining of the isolated endophytic bacteria.

Strain	Colony morphology				Cell morphology	Gram staining
	Surface of colony	Opacity of colony	Margin of colony	Color of colony		
B1	Smooth	Transparent	Entire	Opalescent	Rod-shaped	-
B2	Viscous	Opaque	Entire	Opalescent	Rod-shaped	+
B3	Smooth	Transparent	Entire	White	Rod-shaped	-
B4	Smooth	Transparent	Undulate	Opalescent	Rod-shaped	-
B5	Smooth	Opaque	Entire	Blue white	Rod-shaped	-
B6	Smooth	Transparent	Entire	Clear white	Rod-shaped	-
B7	Viscous	Opaque	Entire	Opalescent	Rod-shaped	-
B8	Viscous	Opaque	Entire	Opalescent	Rod-shaped	+
B9	Viscous	Opaque	Undulate	Opalescent	Rod-shaped	+
B10	Viscous	Opaque	Undulate	Opalescent	Rod-shaped	-
B11	Viscous	Transparent	Entire	Clear white	Rod-shaped	-
B12	Viscous	Opaque	Entire	Opalescent	Rod-shaped	+
B13	Viscous	Opaque	Undulate	Opalescent	Rod-shaped	+
B14	Smooth	Transparent	Undulate	Opalescent	Rod-shaped	-
B15	Viscous	Opaque	Undulate	Opalescent	Rod-shaped	+
B16	Smooth	Transparent	Undulate	Opalescent	Rod-shaped	-

All the endophytic bacterial strains reported in this study were already described as endophytes in different plants, such as *Klebsiella variicola* from *Saccharum officinarum* (sugarcane) [22], *Priestia megaterium* from *Bolboschoenus planiculmis* [23], *Citrobacter freundii* from *Zea mays* (maize) [24], *Enterobacter cloacae* from *Ocimum sanctum* [25],

Pseudomonas aeruginosa from *Phragmites australis* [26], *Enterobacter hormaechei* from *Stevia rebaudiana* [27], *Bacillus cereus* from *Clitoria ternatea* [28], *Enterobacter asburiae* from *Phoenix dactylifera* [29], and *Serratia marcescens* from *Oryza sativa* (rice) [30]. However, this is the first report on culturable endophytes isolated from the root tissues of Vetiver grass cultivated in dioxin-contaminated soil at Bien Hoa airbase.

A



B

Bacterial strain (Accession number)	Nucleotide identity (%) to GenBank database	GenBank database (Accession numbers)	Suggested bacterial species
B1 (OR921912)	100	MT509531	*Klebsiella variicola*
B2 (OR921913)	100	MH559568	*Priestia megaterium*
B3 (OR921914)	100	MN420979	*Citrobacter freundii*
B4 (OR921915)	99.87	MZ676567	*Enterobacter cloacae*
B5 (OR921916)	100	CP133753	*Pseudomonas aeruginosa*
B6 (OR921917)	100	MT613371	*Enterobacter cloacae*
B7 (OR921918)	100	CP042551	*Enterobacter hormaechei*
B8 (OR921919)	100	OR793900	*Priestia megaterium*
B9 (OR921920)	100	OR793125	*Bacillus cereus*
B10 (OR921921)	99.76	MT111594	*Enterobacter cloacae*
B11 (OR921922)	100	MT613375	*Enterobacter asburiae*
B12 (OR921923)	100	CP138336	*Bacillus cereus*
B13 (OR921924)	100	CP138336	*Bacillus cereus*
B14 (OR921925)	100	MN069331	*Serratia marcescens*
B15 (OR921926)	100	CP138336	*Bacillus cereus*
B16 (OR921927)	100	MG461554	*Serratia marcescens*

Figure 3. Molecular identification of the endophytic bacterial strains. (A) The 16S rRNA gene was amplified from genomic DNA of 16 strains by PCR. Purified PCR products of the 16S rRNA gene were partially sequenced from the forward direction. (B) Comparative analysis of the 16S rRNA sequences using the GenBank database and the BLAST tool.

3.2. The endophytic bacteria isolated from Vetiver grass produce high amounts of indole acetic acid

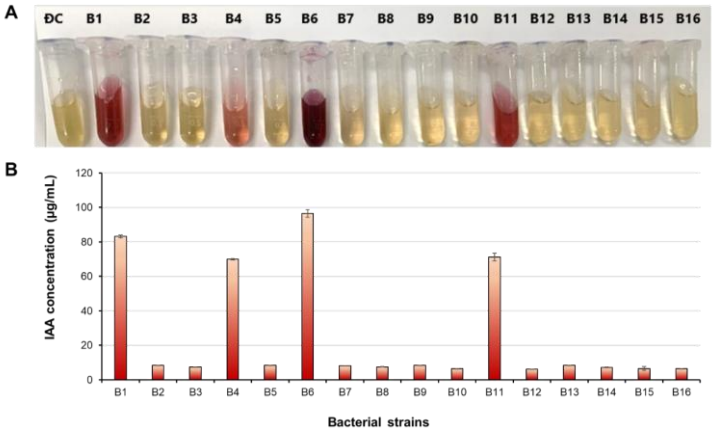


Figure 4. The IAA biosynthesis in the endophytic bacterial strains. (A) Detection of IAA by the color changes of the reactions. (B) Quantification of IAA amounts produced by the bacterial strains.

Indole acetic acid (IAA) is a phytohormone of the auxin class, which plays a vital role in promoting the growth and development processes in plants, such as cell division, elongation, and differentiation [31]. This compound is also produced by numerous microorganisms, including bacterial endophytes [12, 13, 32]. Assays for the IAA production showed that all the endophytic bacterial strains from this study exhibited the IAA production ability with the amounts of 6.2 ± 0.02 to 96.5 ± 2.24 µg/mL (Figure 4).

Kim et al. (2017) indicated that *K. variicola* AY13 could synthesize IAA at a concentration of 84.27 ± 3.55 µg/mL [33]. An endophytic bacterium *E. cloacae* MG00145, isolated from the stem of *Ocimum sanctum*, produced IAA at a concentration of 17.807 µg/mL [25]. Our study showed that 4 endophytic bacterial strains isolated from Vetiver grass had high amounts of IAA in the liquid cultures after 72 h of the cultivation (Figure 4). These strains include *K. variicola* B1 (83.2 ± 0.89 µg/mL), *E. cloacae* B4 (69.9 ± 0.45 µg/mL), *E. cloacae* B6 (96.5 ± 2.24 µg/mL), and *E. asburiae* B11 (71.2 ± 2.23 µg/mL).

3.3. The endophytic bacterial strains exhibit the ability of phosphate solubilization

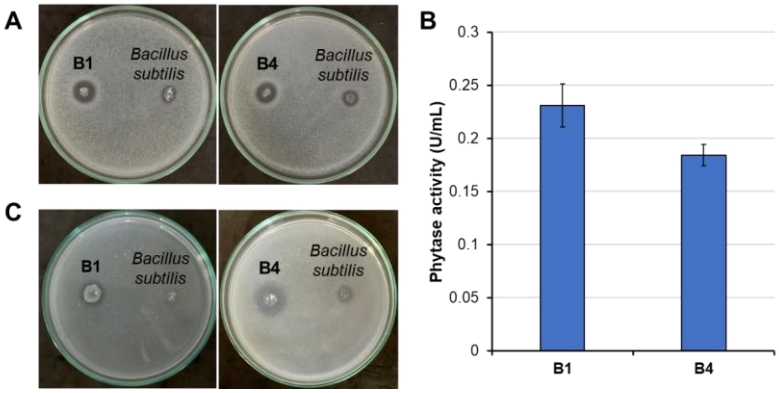


Figure 5. The ability for enzyme production of two selected endophytic bacteria. (A) Phytate degradation by phytase activity of B1 and B4. *Bacillus subtilis* was used as a reference. (B) Quantification of phytase activity. (C) Phosphate degradation by B1 and B4.

Phosphate solubilization in plant rhizosphere by bacteria is a key driving force enabling higher P use efficiency and crop productivity [34]. Microbial degradation processes of phytate and insoluble phosphate in soil are usually involved in phytases and phosphatases [19, 34, 35]. Among the endophytic bacterial strains, 7 strains (B1, B2, B3, B4, B5, B6, and B8) were able to degrade phytate, and only 3 strains (B1, B4, B5) showed phosphate degradation. Notably, *K. variicola* B1 and *E. cloacae* B4 could degrade both phytate and phosphate (Figure 5).

Phytate is the major form of organic phosphorus in soil. It is not a source of phosphorus readily available to plants because it either forms a complex with cations or adsorbs to various soil components. Phosphate-solubilizing microorganisms are ubiquitous in soils and are vital in supplying P to plants [35]. Some bacterial genera, including *Pseudomonas*, *Enterobacter*, and *Pantoea*, were proven to produce phosphatases that lead to high performance of inorganic phosphate release from insoluble phosphate [36]. Therefore, bacterial strains that are capable of degrading both phosphate and phytate are promising candidates for promoting plant growth.

3.4. *K. variicola* B1 and *E. cloacae* B4 promote the growth of tomato plants

Endophytic strain *K. variicola* DX120E, isolated from roots of sugarcane, was reported to be able to colonize sugarcane roots and shoots and promote plant host growth [22]. Kim et al.

(2017) indicated that *K. variicola* AY13 could synthesize IAA at a concentration of 84.27 ± 3.55 µg/mL, which promoted soybean growth and root development [33].

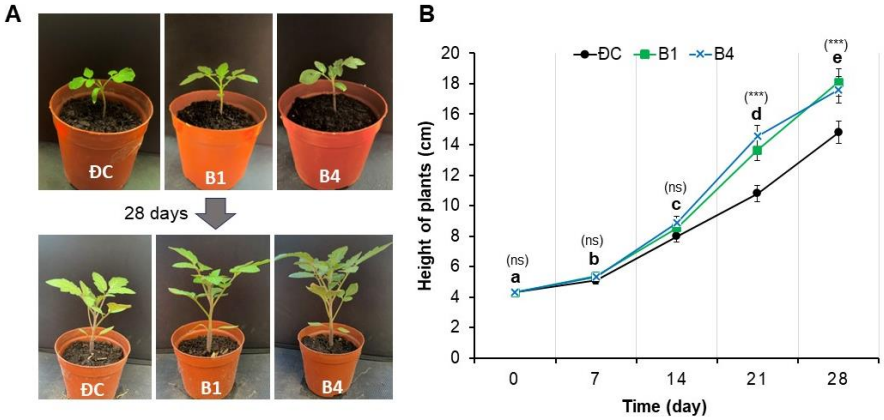


Figure 6. In vitro assays for promoting the growth of tomato plants by two endophytic bacterial strains (B1, B4). (A) The plants are at 0 day and 28 days of cultivation. (B) Quantification of the plant height through periods of cultivation. ĐC: control plant growth, B1: The growth of tomato plants inoculated with *K. variicola* B1, B4: The growth of tomato plants inoculated with *E. cloacae* B4. Data were expressed as means ± standard deviations. One-way ANOVA analysis with Tukey's test was used for multiple comparisons. The lowercase letters indicate significant differences when the mean values of plants at different time points were compared. Asterisks (***) indicate a statistically significant difference ($p < 0.001$) between the means of tested plants and the means of control plants, and (ns) shows a non-significant difference.

Endophytic bacterial strain *E. cloacae* MG00145, isolated from the stem of *Ocimum sanctum,* produced IAA at a concentration of 17.807 µg/mL. Plant assays with the inoculation of strain MG00145 proved that this strain significantly enhanced the growth of four crops [25]. In this study, strains *K. variicola* B1 and *E. cloacae* B4 could produce high amounts of IAA (Figure 4). They were also able to degrade phytate and insoluble phosphate (Figure 5). Therefore, these strains were selected to evaluate their ability to stimulate the growth of tomato plants. After 28 days of bacterial inoculation, the plants inoculated with B1 or B4 increased the plant height to 19 - 22 % compared to the uninoculated control plants (Figure 6). Conclusively, strains B1 and B4 could promote the growth of tomato plants in vitro. These strains may also contribute to the growth simulation of Vetiver grass cultivated in dioxin-contaminated soil at Bien Hoa airbase.

4. CONCLUSIONS

In this study, we successfully isolated 16 endophytic bacterial strains from the root tissues of Vetiver grass cultivated in dioxin-contaminated soil at Bien Hoa airbase. Based on morphological characteristics and DNA sequencing of the 16S rRNA gene, these bacterial endophytes were preliminarily identified to belong to *Klebsiella variicola* (1 strain), *Priestia megaterium* (2 strains), *Citrobacter freundii* (1 strain), *Enterobacter cloacae* (3 strains), *Pseudomonas aeruginosa* (1 strain), *Enterobacter hormaechei* (1 strain), *Bacillus cereus* (4 strains), *Enterobacter asburiae* (1 strain), and *Serratia marcescens* (2 strains). Among them, 4 strains, including *K. variicola* B1, *E. cloacae* B4, *E. cloacae* B6, and *E. asburiae* B11, produce high amounts of the IAA biostimulant. Notably, strains B1 and B4 also possess the ability to solubilize insoluble phosphate. In vitro assays indicated that strains B1 and B4 can promote the

growth of tomato plants. This study opens up the potential of using the bacterial endophytes from Vetiver grass to produce biofertilizers to support plant growth.

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CRediT authorship contribution statement. Vu Thi Lan Anh: Methodology, Formal analysis, Data Visualization, Writing original draft. Dang Thi Ha Thu and Nguyen Thi Nhan: Formal analysis. Ngo Thi Thuy Huong, Nguyen Quoc Dinh: Methodology, Writing-Review and Editing. Tran Van Tuan: Conceptualization, Supervision, Writing-Review and Editing, Funding acquisition.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Das P., Datta R., Makris K. C., Sarkar D. - Vetiver grass is capable of removing TNT from soil in the presence of urea. Environmental Pollution. **158** (2010) 1980-1983. https://doi.org/10.1016/j.envpol.2009.12.011.
2. Banerjee R., Goswami P., Pathak K., Mukherjee A. - Vetiver grass: An environment clean-up tool for heavy metal contaminated iron ore mine-soil. Ecological Engineering. **90** (2016) 25-34. https://doi.org/10.1016/j.ecoleng.2016.01.027.
3. Chen X. W., Wong J. T. F., Wang J.-J., Wong M. H. - Vetiver grass-microbe interactions for soil remediation. Critical Reviews in Environmental Science and Technology. **51** (2020) 897-938. https://doi.org/10.1080/10643389.2020.1738193.
4. Chen Y., Shen Z., Li X. - The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. Applied Geochemistry. **19** (2004) 1553-1565. https://doi.org/10.1016/j.apgeochem.2004.02.003.
5. Grover M., Behl T., Virmani T., Bhatia S., Al-Harrasi A., Aleya L. - *Chrysopogon zizanioides* - a review on its pharmacognosy, chemical composition and pharmacological activities. Environmental Science and Pollution Research. **28** (2021) 44667-44692. https://doi.org/10.1007/s11356-021-15145-1.
6. Huyen D. T., Igarashi T., Shiraiwa T. - Vertical distribution of dioxins in soil of Bien Hoa airbase, Vietnam. SpringerPlus. **4** (2015) 300. https://doi.org/10.1186/s40064-015-1064-x.
7. Nguyen Q. D., Ngo H. T. T., Nguyen T. T. T. - Assessment of the potential of Vetiver grass in mitigation of dioxin and arsenic in contaminated soils at Bien Hoa airbase. VNU Journal of Science: Earth and Environmental Sciences. **34** (2018) 45-54. https://doi.org/10.25073/2588-1094/vnuees.4262.
8. Munakata Y., Gavira C., Genestier J., Bourgaud F., Hehn A., Slezack-Deschaumes S. - Composition and functional comparison of Vetiver root endophytic microbiota originating from different geographic locations that show antagonistic activity towards *Fusarium graminearum.* Microbiological Research. **243** (2021) 126650. https://doi.org/10.1016/j.micres.2020.126650.
9. Ho Y. N., Hsieh J. L., Huang C. C. - Construction of a plant–microbe phytoremediation system: Combination of Vetiver grass with a functional endophytic bacterium, *Achromobacter xylosoxidans* F3B, for aromatic pollutants removal, Bioresource Technology. **145** (2013) 43-47. https://doi.org/10.1016/j.biortech.2013.02.051.

10. Ahluwalia O., Singh P. C., Bhatia R. - A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. Resources, Environment and Sustainability. **5** (2021) 100032. https://doi.org/10.1016/j.resenv.2021.100032.
11. Afzal M., Khan Q. M., Sessitsch A. - Endophytic bacteria: Prospects and applications for the phytoremediation of organic pollutants. Chemosphere. **117** (2014) 232-242. https://doi.org/10.1016/j.chemosphere.2014.06.078.
12. Fadiji A. E., Babalola O. O. - Exploring the potentialities of beneficial endophytes for improved plant growth. Saudi Journal of Biological Sciences. **27** (2020) 3622-3633. https://doi.org/10.1016/j.sjbs.2020.08.002.
13. Park S., Kim A. L., Hong Y.-K., Shin J.-H., Joo S.-H. - A highly efficient auxin-producing bacterial strain and its effect on plant growth. Journal of Genetic Engineering and Biotechnology. **19** (2021) 179. https://doi.org/10.1186/s43141-021-00252-w.
14. Liu H., Carvalhais L. C., Crawford M., Singh E., Dennis P. G., Pieterse C. M. J., Schenk P. M. - Inner plant values: diversity, colonization and benefits from endophytic bacteria. Frontiers in Microbiology. **8** (2017) 2552. https://doi.org/10.3389/fmicb.2017.02552.
15. Eke P., Kumar A., Sahu K. P., Wakam L. N., Sheoran N., Ashajyothi M., Patel A., Fekam F. B. - Endophytic bacteria of desert cactus (*Euphorbia trigonas* Mill) confer drought tolerance and induce growth promotion in tomato (*Solanum lycopersicum* L.). Microbiological Research. **228** (2019) 126302. https://doi.org/10.1016/j.micres. 2019.126302.
16. Tran V. T., Do T. B. X. L., Nguyen T. K., Vu X. T., Dao B. N., Nguyen H. H. - A simple, efficient and universal method for the extraction of genomic DNA from bacteria, yeasts, molds and microalgae suitable for PCR-based applications. Vietnam Journal of Science, Technology and Engineering. **59** (2017) 66-74. https://doi.org/10.31276/VJSTE.59(4).66.
17. Chandra S., Askari K., Kumari M. - Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. Journal of Genetic Engineering and Biotechnology. **16** (2018) 581-586. https://doi.org/10.1016/j.jgeb.2018.09.001.
18. Glickmann E., Dessaux Y. - A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria, Applied and Environmental Microb. **61** (1995) 793-796. https://doi.org/10.1128/aem.61.2.793796.1995.
19. Demirkan E., Baygin E., Usta A. - Screening of phytate hydrolysis *Bacillus* sp. isolated from soil and optimization of the certain nutritional and physical parameters on the production of phytase. Turkish Journal of Biochemistry. **39** (2014) 206-214. https://doi.org/10.5505/tjb.2014.26817.
20. Nautiyal C. S. - An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS microbiology Letters. **170** (1999) 265-270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x.
21. Reller L. B., Weinstein M. P., Petti C. A. - Detection and identification of microorganisms by gene amplification and sequencing. Clinical Infectious Diseases. **44** (2007) 1108-1114. https://doi.org/10.1086/512818.
22. Wei C. Y., Lin L., Luo L. J., Xing Y. X., Hu C. J., Yang L. T., Li Y. R., An Q. - Endophytic nitrogen-fixing *Klebsiella variicola* strain DX120E promotes sugarcane growth. Biology and Fertility of Soils. **50** (2013) 657-666. https://doi.org/10.1007/s00374-013-0878-3.

23. Hwang H. H., Chien P. R., Huang F. C., Yeh P. H., Hung S. H. W., Deng W. L., Huang C. C. - A plant endophytic bacterium *Priestia megaterium* strain BP-R2 isolated from the halophyte *Bolboschoenus planiculmis* enhances plant growth under salt and drought stresses, Microorganisms **10** (2022) 2047. https://doi.org/10.3390/microorganisms 10102047.
24. Fadiji A. E., Ayangbenro A. S., Babalola O. O., Klepac-Ceraj V. - Draft genome sequence of *Citrobacter freundii* AYS58, a potential plant growth-promoting endophyte. Microbiology Resource Announcements. **12** (2023) e0014223. https://doi.org/10.1128/ mra.00142-23.
25. Panigrahi S., Mohanty S., Rath C. C. - Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South African Journal of Botany. **134** (2020) 17-26. https://doi.org/10.1016/j.sajb.2019.09.017.
26. Wu T., Xu J., Xie W., Yao Z., Yang H., Sun C., Li X. - *Pseudomonas aeruginosa* L10: A hydrocarbon-degrading, biosurfactant-producing, and plant-growth-promoting endophytic bacterium isolated from a reed (*Phragmites australis*). Frontiers in Microbiology. **9** (2018) 1087. https://doi.org/10.3389/fmicb.2018.01087.
27. Oviedo-Pereira D. G., López-Meyer M., Evangelista-Lozano S., Sarmiento-López L. G., Sepúlveda-Jiménez G., Rodríguez-Monroy M. - Enhanced specialized metabolite, trichome density, and biosynthetic gene expression in *Stevia rebaudiana* (Bertoni) Bertoni plants inoculated with endophytic bacteria *Enterobacter hormaechei*. PeerJ. **10** (2022) e13675. 10.7717/peerj.13675.
28. Khaksar G., Treesubsuntorn C., Thiravetyan P. - Effect of endophytic *Bacillus cereus* ERBP inoculation into non-native host: Potentials and challenges for airborne formaldehyde removal. Plant Physiology and Biochemistry. **107** (2016) 326-336. https://doi.org/10.1016/j.plaphy.2016.06.020.
29. Yaish M. W. - Draft genome sequence of endophytic bacterium *Enterobacter asburiae* PDA134, isolated from date palm (*Phoenix dactylifera* L.) roots. Genome Announcements. **4** (2016). https://doi.org/10.1128/genomea.00848-16.
30. Gyaneshwar P., James E. K., Mathan N., Reddy P. M., Reinhold-Hurek B., Ladha J. K. - Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. Journal of Bacteriology. **183** (2001) 2634-2645. https://doi.org/10.1128/jb.183.8.2634-2645.2001.
31. Teale W. D., Paponov I. A., Palme K. - Auxin in action: signalling, transport and the control of plant growth and development. Nature Reviews Molecular Cell Biology. **7** (2006) 847-859. https://doi.org/10.1038/nrm2020.
32. Wagi S., Ahmed A. - *Bacillus* spp.: potent microfactories of bacterial IAA. PeerJ. **7** (2019) e7258. 10.7717/peerj.7258.
33. Kim A.-Y., Shahzad R., Kang S.-M., Seo C.-W., Park Y.-G., Park H.-J., Lee I.-J. - IAA-producing *Klebsiella variicola* AY13 reprograms soybean growth during flooding stress. Journal of Crop Science and Biotechnology. **20** (2017) 235-242. https://doi.org/10.1007/s12892-017-0041-0.
34. Elhaissoufi W., Ghoulam C., Barakat A., Zeroual Y., Bargaz A. - Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. Journal of Advanced Research. **38** (2022) 13-28. https://doi.org/10.1016/j.jare.2021.08.014.

35. Singh B., Satyanarayana T. - Microbial phytases in phosphorus acquisition and plant growth promotion. Physiology and Molecular Biology of Plants. **17** (2011) 93-103. https://doi.org/10.1007/s12298-011-0062-x.
36. Jorquera M. A., Hernández M. T., Rengel Z., Marschner P., de la Luz Mora M. - Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biology Fertility of Soils. **44** (2008) 1025-1034. https://doi.org/10.1007/s00374-008-0288-0.