Morphological and molecular characterization of plant growth promoting salt-tolerant bacteria associated with halophytes in the Southeast seaside of Vietnam

Ha T. N. Vo*, Vy T. Do, Ti Ta Danh, Quan H. Nong, & Huyen K. Pham
Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam

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***Corresponding author**

Vo Thi Ngoc Ha
Email: ha.vothingoc@hcmuaf.edu.vn

ABSTRACT

Halophytes are found in high-salt environments naturally, and their roots may be associated with promising microbial candidates for promoting crop growth and salt tolerance. In this study, halotolerant bacteria were isolated from soil and root samples of *Rhizophora apiculate* (*R. apiculate*), *Avicennia officinalis* (*A. officinalis*), *Thespesia populnea* (*T. populnea*), *Acanthus ilicifolius* (*A. ilicifolius*) and *Trichophorum cespitosum* (*T. cespitosum*), five native halophytes of southeast seaside of Vietnam. Isolates were tested for maximum salt tolerant and screened for the ability of phosphate solubilization and indole acetic acid (IAA) production. Colony morphology, pigmentation, and Gram staining of each IAA production halotolerant isolate were determined. The bacterial isolates showed the highest salt tolerance and IAA production were identified by sequencing the 16S rRNA gene. A total of 54 isolates which were able to grow in the presence of up to NaCl 3M were isolated. Twenty-three halotolerant bacterial isolates had the capacity of IAA production, 60.9% from which were Gram positive with a cocci shape, colony in opaque/transparent yellow or opaque/off white, 1 - 2 mm or 2 - 3 mm in diameter with the convex surface. Three isolates VTDD1, VTTD2, and KGOR1 were able to solubilize insoluble phosphorus. The highest IAA production was observed in VTDR1 (93.77 µg/mL) followed by VTMR1 (75.23 µg/mL) and VTDR2 (60.00 µg/mL), while the smallest IAA production was observed in CGOD1 (0.50 µg/mL). The isolates VTDR1 and VTDR2 were identified as *Salinicola tamaricis* (99.58% and 99.67% identity respectively), while VTMR1 was found to be *Salinicola peritrichatus* (98.37% identity).

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1. Introduction

Soil salinization is a significant problem that affects and degrades land. It is widespread in desert or semi-arid places and has a direct impact on the sustainability of agricultural cultivation around the world, even posing a threat to global food security (Aragüés et al., 2015). It is one of the agricultural sector's most pressing problems because of its direct impact on the productivity and quality of agricultural products (Wild, 2003). Various approaches have recently been used to solve the problem of soil salinity, one of which is using plant growth-promoting rhizobacteria (PGPR) associated with halophytes.

Halophytes are naturally salt-tolerant plants that can stand up to concentrations of 1M NaCl (Kumari et al., 2015). Halophytic plants have

evolved various strategies to live in different salinity levels, one of which is ability to exploit the benefits provided by microbial systems around their roots, including endophytes and rhizosphere microorganisms (Sgroy et al., 2009; Ruppel et al., 2013).

There are many salt-tolerant halophyte rhizosphere and endophytic bacteria were investigated and reported to have the potential to contribute significantly to the ability of plants to adapt to adverse conditions (Numan et al., 2018). Salt tolerant bacteria that had the ability to grow at varied NaCl concentrations ranges from 50 to 600 mM (Zerrouk et al. 2019), from 1M to 4M (Kearl et al., 2019) or from 2% to 10% (Sharma et al. 2021). Among the salt-tolerant microorganisms associated with halophytes, PGPR has been effective at improving plant stress tolerance (Etesami & Beattie, 2018; Etesami & Maheshwari, 2018), especially phosphate solubilizing and IAA production bacteria. In the saline soil halotolerant bacteria reduce plant uptake of sodium ions from the soil by forming the biofilms in the rhizosphere that trap water and nutrients (Nadeem et al., 2014). Some other halophilic bacteria could stimulate plant growth based on binding of salt ions by the bacteria or production of volatile compounds or other signals that stimulate expression of genes to enhance growth via increased photosynthesis or other changes in the host plant (Meena & Meena., 2017; Numan et al., 2018). Phosphate (P) solubilizing bacteria convert unavailable P into available P, consequently improving the P supply available to the plant. Applying of phosphate solubilizing bacteria also decreases the pH of the soil and forms a P-offering microarea around the plant rhizosphere, and strengthening the activity of other beneficial microorganisms, promote the absorption of nutritive element ions of plant in salinity soil (Chen & Liu, 2019). In extremely condition plants are tried to use exogenous IAA hormone, that produced by rhizosphere organisms to improve budding and root hair formation. Root hair growth is stimulated by the presence of rhizosphere bacteria that produce IAA (Larekeng et al., 2020). These groups of bacteria were identified and used as inoculants to stimulate growth of non-host plants under saline conditions, and reduce pressure on arable lands. In the present study, bacteria were isolated from saline soils and roots of halophytes, and then screened for salt tolerance, phosphate solubilization, and IAA production.

2. Materials and Method

2.1. Materials

Total 30 soil and root tissues samples of halophytes *Rhizophora apiculate* (*R. apiculate*), *Avicennia officinalis* (*A. officinalis*), *Thespesia populnea* (*T. populnea*), *Acanthus ilicifolius* (*A. ilicifolius*) and *Trichophorum cespitosum* (*T. cespitosum*) and three soil samples of bare areas.

Chemicals: Yeast extract (India), meat extract (India), NaCl (99%, India), K_2HPO_4 (99.5%, China), L-triptophane ($\geq$ 99.0%, Germany), KCl (99%, Germany), Na_2HPO_4 ($\geq$ 99.0%, China), KH_2HPO_4 (99%, China), Tryptone (India), IAA (IAA $\geq$ 99.0%, China), $FeCl_3$.6H2O (98%, China), Agar powder (100%, Vietnam) $MnSO_4.H_2O$ (99%, China), $FeSO_4.7H_2O$ (99%, China) and $Ca_3(PO_4)_2$ (99%, China).

Equipment: Biosafety level 2AC2-6E8 incubator (2AC2-6E8, Esco, Singapore), MC40L autoclave (MC40L, ALP, Japan), Shaker HS260 (HS 260 control (0003066700), IKA, Germany), Spectrophotometry (NanoVue plus, Bichochrom, US), Freezer (MPR-414F-PE, Panasonic (PHC Corporation, Japan), single-channel micropipette 10÷1000 µL (Nichipet EXII, Japan), pH meter LAB845 (SI Analytics, Germany), ultrasound waves (S300H, Elma, Germany).

2.2. Collection of samples

Samples were collected as described by Kearl et al. (2019) from predominant halophyte species, such as *R. apiculate*, *A. officinalis*, *T. populnea*, *A. ilicifolius* and *T. cespitosum* around the seaside of Vung Tau (coordinate: 10°26'51.2"N 107°08'50.8"E), Can Gio (coordinate: 10°23'22.2"N 106°55'16.3"E) and Kien Giang (coordinate: 10º06'15.9"N 104°52'19.8"E). Five pooled soil and five pooled root tissue samples from each coordinate were collected. Each pooled sample was collected from 5 to 10 individual plants of each halophyte species. Samples were stored at 4ºC until be analyzed. Soil was also collected from bare areas where no plants were growing for comparison. Soil electrical conductivity (EC) as dS/m was measured using a Beckman RC-16C conductivity bridge. Soil samples were mixed with deionized water, the saturated mix was allowed to sit overnight for the soil to settle, and the pH of the liquid was measured with a standard pH meter.

2.3. Isolation and Characterization of salt tolerant Bacteria

Rhizosphere soil samples were left at room temperature for 24 h, then 0.5 g sample was vortexed in 1 mL sterile 1 X PBS buffer (phosphate buffered saline containing of NaCl 8.5 g/L, KCl 200 mg/L, Na_2HPO_4 1.44 g/L, KH_2PO_4 245 mg/L, pH 7.4) and plated on Luria broth (LB) agar plates containing of 1 M NaCl. To isolate endophytic bacteria, root samples were surface sterilized (by washing twice in sterile distilled water, once for 10 min in 70% ethanol, and twice in sterile 1 X PBS buffer) and ground in PBS buffer. Single colonies were continuously subcultured on Luria agar (LB) medium containing 1M NaCl to obtain pure colonies. Cultures were re-streaked on LB media containing increasing amounts of NaCl (2M, 3M, 4M) to determine the maximum salt tolerance of each isolate (Sharma et al., 2021). Colony morphology, pigmentation, and Gram staining of each isolate were also determined. Stock cultures of each isolate were stored at -20°C in 50% glycerol.

2.4. Phosphate solubilization

Phosphorus solubilizing activity of the isolates was determined qualitatively according to the method described by Nautiyal (1999) on Pikovskaya's agar medium. $Ca_3(PO_4)_2$ (0.5%) was used as the inorganic form of phosphate. The bacteria were spotted on the center of the plates, and the plates were then incubated at 28°C for 7 days. The experiment for each isolate were repeated for a total of three replicates. Transparent halo zone around the bacterial colony indicates the phosphate solubilizing activity of the bacterial isolates.

2.5. Characterization of indole acetic acid production

To determine IAA producing capability, and amounts of IAA produced by each salt tolerance isolate, a colorimetric technique was performed with Salkowski reagent using the Salkowski's method (Ehmann, 1977). The isolates were grown in L-triptophan Luria broth and incubated at 28°C for 4 days. The bacterial suspension was centrifuged at 4000 rpm for 20 min, then 2 mL supernatant was collected and mixed with 8 mL of Salkowski's reagent (2% $FeCl_3$ in 36% H_2SO_4 solution) to determine IAA producing capability. If the mixture color changed to pink, the mixture was kept in the dark for 30 min to determine the amount of IAA produced (Sarwar & Kremer, 1995) by recording the optical density (OD) at 530 nm using spectrophotometer. The experiment for each isolate were repeated for a total of three replicates. Sterile LB medium (with and without L-tryptophan) were used as controls.

2.6. Bacterial Identification

The bacterial isolates with high salt tolerance and IAA production were selected for identification. Genomic DNA was obtained from individual isolates using a DNA isolation kit GeneJET Plant Genomic DNA Purification - Thermo (America). The 16S ribosomal RNA (rRNA) gene was amplified by PCR with MyTaq™ DNA Polymerase - Bioline using the 63F and 1492R primers (Turner et al., 1999). The PCR products were submitted for sequencing at the Biotechnology Department of University of Science, Vietnam National University Ho Chi Minh City. The obtained sequences were were analyzed and determined by the BLAST program on the NCBI (https://www.ncbi.nlm.nih.gov).

3. Results

3.1. Isolation and characterization of rhizospheric and endophytic salt tolerance bacteria

Total 30 pooled soil and root tissue samples were collected around the dominant halophyte species at seaside of Vung Tau, Kien Giang, Can Gio areas including *R. apiculate*, *A. officinalis*, *T. populnea*, *A. ilicifolius* and *T. cespitosum* (Figure 1).

Soil salinity around the plants ranged from 4.10 to 12.75 dS/m (Table 1), according to Kotuby-Amacher et al. (2000) and Richards (1954), soils are moderately saline and strongly saline. In the bare-no plant, salinity was varied between 13.90 and 16.40 dS/m depending on the areas. The pH of all soil samples was ranged from 4.00 to 7.90 (Table 1).

Fifty-four isolates of bacteria were isolated from the rhizosphere soil and root samples on LB agar plates containing 1 M NaCl. Among which, 27 isolates were endophytic bacteria and 27 isolates were rhizosphere bacteria. The highest salt

Figure 1. Photos of the halophyte species: a) *R. apiculate*; b) *A. officinalis*; c) *T. populnea*; d) *T. cespitosum*; e) *A. ilicifolius*.

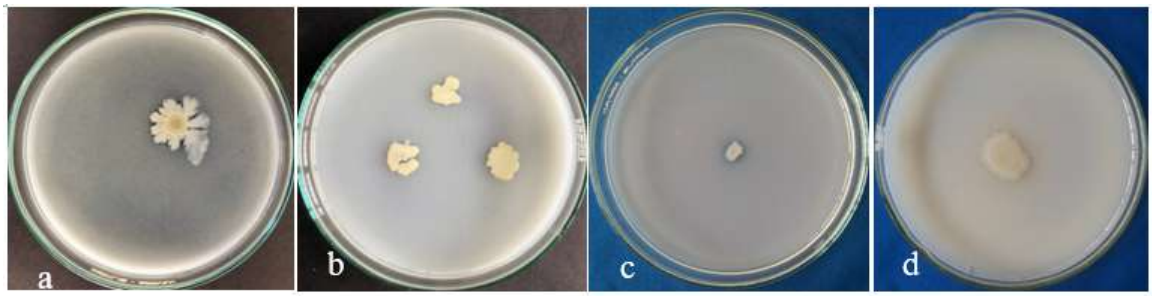

Figure 2. The phosphate solubilizing ability of three selected isolates a) VTDD1; b) VTTD2; c) KGOR1, and d) KGOD1 the isolate without solubilizing phosphate.

Table 1. Physicochemical analysis of soil samples

Plant species	Vung Tau		Can Gio		Kien Giang	
	EC dS/m	pH	EC dS/m	pH	EC dS/m	pH
Rhizophora apiculate	6.27	5.00	7.27	5.50	11.56	7.00
Avicennia officinalis	4.10	4.52	5.10	5.12	6.70	7.50
Thespesia populnea	8.50	5.00	9.62	5.83	4.90	5.45
Trichophorum cespitosum	4.50	4.00	4.87	4.30	4.68	5.40
Acanthus ilicifolius	5.62	5.95	7.52	5.90	12.75	6.30
Bare-no plants	13.90	5.35	14.93	5.05	16.40	7.90

concentration tolerance for growth up to 3 M was discovered in a variety of isolates. There were 18 isolates (33.3%) growing in the presence of NaCl 3 M, 32 isolates (59.3%) growing in the presence of NaCl 2M, and 4 isolates (7.4%) growing in the presence of NaCl 1 M. High salt tolerant rhizospheric isolates were found in associated with *R. apiculate* and *A. ilicifolius*, while the high salt tolerant endophytic isolates was found in *T. cespitosum*. The isolates grew equally well on minimal media agar plates at the same salt concentrations. The Gram staining, cell morphology, pigmentation and colony morphology were recorded for some isolates (Table 2). In LB plates containing 1M NaCl, none of rhizosphere bacteria were observed from soil sample of bare-no plants.

Most of the isolates are Gram positive, cell of isolates had a cocci shape (60.9% of bacterial isolates), and colony in opaque/transparent yellow or opaque/off white, 1-2 mm or 2-3 mm in diameter and a convex surface. Some others were found to have rod or short rod shape of cell, sometimes with opaque white or yellowish white colony with flat surface, core in centre or coreless.

3.2. Characterization of salt-tolerant bacterial isolates for ability of phosphate solubilization

The method for determination of phosphate solubilizing ability is based on the formation of visible halo/zone on Pikovskaya agar plates. In the present study, the phosphate solubilization was investigated in three salt-tolerant bacterial isolates VTDD1, VTTD2 and KGOR1. However, the visible halo zones on agar plates created by these isolates were very weak (Figure 2).

Table 2. Morphological characteristic of Salt tolerant and indole acetic acid production bacteria

Rhizosphere bacterial isolates				
Bacterial isolate	Plant species	Gram staining and cell morphology/ Pigment and morphology of the colony	Salt concentration tolerance	IAA, µg/mL
VTTD2	*T. populnea*	Gram + cocci/ opaque yellow 1-2 mm in diameter, convex surface, core in centre	3M	18.33
VTTD3	*T. populnea*	Gram + rod/ transparent yellow 2-3 mm in diameter, convex surface, core in centre	2M	48.20
KGTR1	*T. populnea*	Gram + cocci/ opaque yellow 1-2 mm in diameter, convex surface, core in centre	2M	41.10
CGMD3	*A. officinalis*	Gram - cocci/ off white 2-3 mm in diameter, the surface is smooth, shiny, and wet, coreless	3M	49.10
KGMR2	*A. officinalis*	Gram + cocci/ opaque white 3-6 mm in diameter, irregular, flattened surface, coreless	2M	12.00
CGDD1	*R. apiculate*	Gram + cocci/ off white 2-3 mm in diameter, the surface is smooth, shiny, and wet, coreless	3M	21.80
KGDR1	*R. apiculate*	Gram + cocci/ opaque white 1–2 mm in diameter, flat surface, core in centre	3M	47.97
KGOR2	*A. ilicifolius*	Gram + short rod/ yellowish white 1-2 mm in diameter, convex surface, and circular division, coreless	3M	19.10
CGOD1	*A. ilicifolius*	Gram + cocci/ pale orange 2-3 mm in diameter, slimy and fluid with a core	3M	0.50
CGOD2	*A. ilicifolius*	Gram + cocci/ orange 1-2 mm in diameter, slimy and fluid with a core	2M	59.30
CGCD1	*T. cespitosum*	Gram - cocci/ orange 1-2 mm in diameter, smooth, glossy, and wet surface, coreless	2M	30.30
CGCD2	*T. cespitosum*	Gram + cocci/ opaque white 2-3 mm in diameter, smooth, glossy and wet surface, coreless	2M	18.40
Endophytic bacterial isolates				
VTMR2	*A. officinalis*	Gram - short rod/ pale yellow 0,5-1 mm in diameter, clarity, convex surface	3M	32.00
VTMR1	*A. officinalis*	Gram - cocci/ translucent yellow, 1-2 mm in diameter, convex surface, core in centre	2M	75.23

Table 2. Morphological characteristic of Salt tolerant and indole acetic acid production bacteria (continued)

Endophytic bacterial isolates				
CGMR1	*A. officinalis*	Gram + cocci/ pale yellow 1 - 2 mm in diameter, the surface is smooth, shiny, and wet, coreless	2M	37.10
VTDR1	*R. apiculate*	Gram + cocci/ transparent yellow 1 - 2 mm in diameter, convex surface, core in centre	2M	93.77
VTDR2	*R. apiculate*	Gram + short rod/ yellowish white 1 - 2 mm in diameter, convex surface, circular division, coreless	3M	60.00
KGDD1	*R. apiculate*	Gram + rod/ opaque white 1 - 2 mm in diameter, flat surface, core in centre	2M	15.50
KGMD1	*R. apiculate*	Gram + cocci/ opaque white 2 - 4 mm in diameter, convex surface, pink core	2M	19.77
KGCD1	*T. cespitosum*	Gram + rod/ transparent yellow 2 - 3 mm in diameter, convex surface, core in centre	3M	14.90
KGCD2	*T. cespitosum*	Gram + cocci/ opaque yellow 2 - 3 mm in diameter, coreless	3M	39.37
VTCR1	*T. cespitosum*	Gram + cocci/ opaque yellow 2 - 3 mm in diameter, coreless	3M	15.23
CGCR1	*T. cespitosum*	Gram + cocci/ off white 2 - 3 mm in diameter, dry and rough surface, coreless	2M	16.70

3.3. Characterization of salt-tolerant bacterial isolates for ability of indole acetic acid production

The IAA production of the bacterial isolates was determined based on the change in color of the culture supernatant after the addition of the Salkowski reagent. Twenty-three bacterial isolates from fifty-four salt tolerant isolates were observed able to produce IAA (Figure 3). The color density was varied depending on the level of IAA produced by the tested isolates, as in Figure 3h and Figure 3g are bright-less pink, in Figure 3b - 3f are pink and brighter pink, in comparison to positive control (Figure 3a) containing 50 µg/mL IAA, and negative control (Figure 3i). The interaction between IAA, produced by bacterial isolates and Fe elements in Salkowski reagent forms a complex compound $[Fe_2(OH)_2(IA)_4]$, that makes the pink as result of positive reaction. The brighter pink color indicates that the higher content of IAA produced by bacterial isolate (Susilowati et al., 2018).

Indole acetic acid concentration values were obtained by preparing a standard curve from IAA solution by suspending IAA in water and mixed by ultrasound waves at a concentration of 1 mg/mL and diluting to a concentration of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 and 0 µg/mL. Spectrophotometric test results show the correlation between IAA standard solution (x) and absorbance (y) by obtaining a regression equation $Y = 0.0006x + 0.0272$ with correlation coefficient $R^2 = 0.9833$, which was met the requirement of IAA curve standard (Brick et al., 1991). The ability of IAA production of endophytic and rhizosphere bacterial isolates from halophyte plants is presented in Table 2. The highest IAA concentration is produced by the VTDR1 isolate at 93.77 µg/mL, followed by the VTMR1 isolate at 75.23 µg/mL, and the VTDR2 isolate at 60.00 µg/mL. The lowest IAA concentration is produced by the CGOD1 isolate at 0.50 µg/mL. IAA is a key hormone for various aspects of plant growth that can regulate many physiological processes, such as cell division, differentiation, and protein

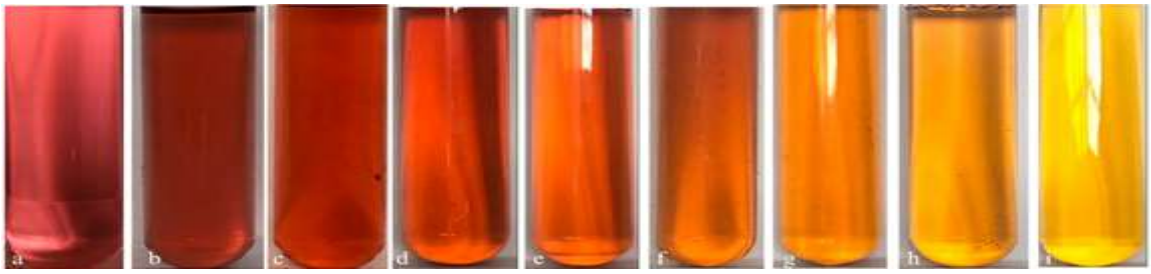

Figure 3. IAA test results of bacterial isolates based on differences in color obtained: a) IAA 50µg/mL; b)VTDR1; c)VTMR1; d)VTDR2; e)VTMR2; f)VTTD3; g)VTTD2; h)VTCR1; i) Salkowski.

synthesis. IAA produced by PGPR are required for improving root hair budding, formation, and growth, helping plants to cope with salt stress (Egamberdieva & Kucharova, 2009; Patil, 2011). IAA production varies greatly between species and strains, the host plant, different areas, and is also affected by environmental conditions, growth rates, and availability of substrates such as amino acids.

3.4. Bacterial identification

Three bacterial isolates (VTDR1, VTMR1 and VTDR2) with the highest IAA production and salt tolerance were selected for identification. The 16S rRNA gene from these isolates was successfully amplified. The PCR is about 1400 bp to 1500 bp (Figure 4) in length was sequenced. The 16S rRNA sequences of the bacterial isolates VTDR1 and VTDR2 show high identity (99.58% and 99.67%, respectively) to the *Salinicola tamaricis* sequence, whereas the VTMR1 show high identity (98.37%) in 16S rRNA sequence to *Salinicola peritrichatus*.

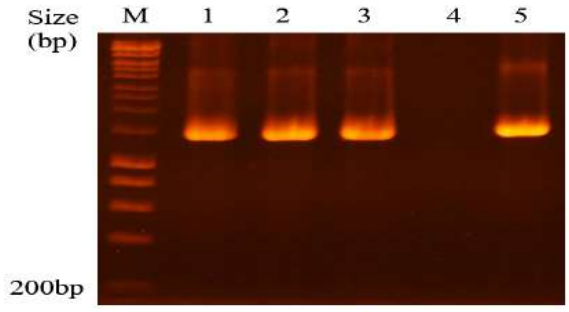

Figure 4. PCR amplified product of 16S rDNA of bacterial isolates. (Lad M: molecular size marker 1 kb; lane 1: isolate VTDR1; lane 2: VTDR2; lane 3: VTMR1; lane 4: control without DNA; lane5: DNA of *Bacillus subtilis*).

4. Discussion

Salinity stress which causes the decrease in yield of important crops as rice is increasing year by year in many countries due to global climate change. Every year, saline soil reduces agricultural cultivable area by 1 - 2 percent, leads to reducing food production. A strategy that has received much attention in recent years is using salt-tolerant microorganisms as plant growth-promoting rhizobacteria (PGPR) in combination with fertilizers to increase the salt tolerance of plants grown in flooded areas (Etesami & Beattie, 2018).

In the present study, the salt tolerant bacteria were isolated from saline soils and roots of the dominant halophytes in Southeast seaside of Vietnam as *Rhizophora apiculate*, *Avicennia officialist*, *Thespesia populnea*, *Acanthus ilicifolius* L., and *Trichophorum cespitosum*. A total of fifty-four isolates were found salt tolerant at different NaCl concentrations. About 33.3% of them have shown high salt tolerance at 3M NaCl. In a similar study, Kearl et al. (2019) isolated, screened, and characterized salt tolerant rhizosphere bacteria from the native halophyte of Ulta (*Salicornia rubra*, *Sarcocornia utahensis*, and *Allenrolfea occidentalis*). Forty-one isolates of three bacteria genus *Halomonas*, *Bacillus*, and *Kushneria* were demonstrated as salt tolerant bacteria. Some of which were able to grow in the presence of NaCl up to 4 M and stimulated plant growth of alfalfa in the presence of NaCl 1%. The diazotrophic salt tolerant bacterial strains of *Klebsiella*, *Agrobacterium*, *Pseudomonas*, and *Ochrobactrum* isolated from the roots of a halophytic plant (*Arthrocnemum indicum*) showed salinity tolerance ranging from 4% to 8%, and improved the productivity of peanut in saline conditions by increasing in total nitrogen (N) con-

tent up to 76% (Sharma et al., 2016). Zerrouk et al. (2019) found that *Pseudomonas plecoglossicida* strain Pp20 had the ability to grow at varied NaCl concentrations ranging from 50 to 600 mM. The study also revealed a positive impact on stem weight, seminal roots, lateral roots, and root length of maize in greenhouse experiment.

Different bacterial species stimulate the growth of a variety of plant species depending on the plant host and bacterial genus (Yuan et al., 2016). For example, the endophytic strain of *Bacilllus amyloliquefaciens* protects against salinity stress in rice by producing abscisic acid in response to increasing ability of glutamic acid and proline production of to increase resistance to salinity (Shahzad et al., 2017). In another study, Sarkar et al. (2018b) demonstrated that halotolerant *Enterobacter* sp. strain P53 can stimulate rice growth under salt stress due to the capacity of IAA production, HCN, siderophore and antioxidant activity. Mukhtar et al. (2020) found that, under salinity stress conditions halophilic PGP bacteria genus *Bacillus*, *Halobacillus*, and *Pseudomonas* associated with *Salsola stocksii* and *Atriplex Amnicola* improved plant growth and grain yield of maize by solubilization of phosphorus, nitrogen fixation and production of growth-promoting hormones indole acetic acid, siderophores and HCN.

In the present study, the bacterial isolates were further screened for the ability of phosphate solubilization and IAA production. There are only 3 bacterial isolates with the capacity to solubilize phosphate but the visible zones were weak. In the recent study, Sharma et al. (2021) reported that, the phosphate solubilization was demonstrated by three salt-tolerant bacterial isolates from saline agricultural fields of Haryana, India HB4N3, HB6P2 and HB6J2. However, the diameter of visible zones on the plates was impossible to measure, which was the same as the present study. We would suggest that, there are many factors that could affect the solubilization of insoluble phosphorus of halotolerant bacteria on Pikovskaya agar plates. Therefore, the method needs to be improved for optimization of the phosphorus solubilization capacity of salt tolerant bacteria.

There are twenty-three bacterial isolates in this study with capacity to produce IAA at different concentrations. The concentration of produced IAA was high in comparison with the others results. In previous results, IAA production of Bacillus sp. from saline agricultural fields of Haryana, India were ranges from 8.91 to 15.89 ppm (Sharma et al. 2021). *Bacillus subtillis* from cultivated soil sample in Egypt had capacity of IAA production with variable degrees ranged between 13.0-25.5 mg/L (Naeima, 2018). According to Mirza et al. (2001), IAA production by microorganisms depend on the culture conditions and substrate stage growth conditions and may vary between different species and strains of the same species. In present study, the endophytic bacteria could produce IAA at a higher concentration than rhizosphere bacteria from the same halophyte species. IAA synthesis by bacteria can be promoted by the presence of L-tryptophan (Mohite, 2013). L-tryptophan is an amino acid with an indole group that can function as a physiological precursor of IAA in plants and microorganisms because it contains active compounds that can enhance IAA biosynthesis, active microbial growth. IAA biosynthesis of microbes from plant roots, especially in the rhizosphere, which has a metabolism pathway through the synthesis of L-tryptophan derived from root exudates (Dewi et al., 2016).

The isolates VTDR1, VTMR1 and VTDR2 with high salt tolerance and IAA production ability were sequenced for amplified 16S rRNA gene and identified as Salinicola tamaricis (VTDR1, VTDR2) and Salinicola peritrichatus (VTMR1). In previous findings, many salt tolerance plant growth promoting rhizobacteria were identified as Klebsiella, Pseudomonas, Rhizobium, Azospirillum, Enterobacter, Serratia, Alcaligenes, Arthrobacter, Azotobacter, Burkholderia and Bacillus (Sarkar et al., 2018a; Ipek et al., 2019; Sayyed et al., 2019). Salinicola sp. might be a new bacterial species in PGPB group, that could be beneficial in improving crop yields in salt-affected agricultural fields.

5. Conclusions

Fifty-four isolates of rhizosphere and endophytic bacteria were able to grow in the presence of up to 3M NaCl were isolated from soil and root samples of five native halophytes of *Rhizophora apiculate*, *Avicennia officinalis*, *Thespesia populnea*, *Acanthus ilicifolius* and *Trichophorum cespitosum* in the southeast seaside of Vietnam. These bacterial isolates varied in pigmentation and colony morphology. Among which,

twenty-three halotolerant bacterial isolates produced IAA ranged from 0.50 µg/mL to 93.77 µg/mL, and three isolates had the ability of phosphate solubilization. The VTDR1 and VTDR2 isolates, which could grow in the presence of 3M NaCl, produced IAA at 93.77 µg/mL and 60.00 µg/mL, respectively, were identified as *Salinicola tamaricis* (99.58% and 99.67% identity, respectively). The VTMR1 isolate grew in 3M NaCl and produced IAA at 75.23 µg/mL was found to be *Salinicola peritrichatus* (98.37% identity).

Conflict of interest declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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