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Isolation of salt-tolerant *Pseudomonas* strains with potential for alleviation of salt stress in peanut plant (*Arachis hypogaea* L.)

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Isolation of salt-tolerant *Pseudomonas* strains with potential for alleviation of salt stress in peanut plant (*Arachis hypogaea L.*)

Abstract: Plant growth-promoting rhizobacteria (PGPR) is a promising solution to improve plant growth under salt stress. Among PGPR, *Pseudomonas* is a genus of bacteria that possesses a variety of mechanisms in promoting plant growth and inducing resistance to biological as well as non-biological stress. This study aimed to isolate the genus *Pseudomonas* from the salty-contaminated rhizosphere of plant root collecting at Nam Dinh, and also investigate their functions in promoting the growth of peanut seedlings under salty conditions. Nine *Pseudomonas* bacteria were isolated, but only seven of them were identified by *Pseudomonas*-specific primers. Two of those seven isolates, ND06 and ND09, were chosen based on their characteristics in promoting plant growth such as the production of indole-3-acetic acid (IAA), phosphate solubilization, and nitrogen fixation. In addition, both two strains also carried the coding gene for 1-aminocyclopropane-1-carboxylate (ACC) deaminase which plays an important role in supporting plants to withstand various stress conditions. Especially, the ND09 strain improved the growth parameters of peanut seedlings under normal and salty stress conditions; while the ND06 only presented the plant growth enhancement under salty stress but not in normal conditions. These results suggest the ND09 strain may be used as a biological agent for eco-friendly agricultural practices in the future.

Key words: peanut plant; PGPR; *Pseudomonas*; salt stress resistance

Izolacija na sol tolerantnih sevov bakterij iz rodu *Pseudomonas* s potencialom zmanševanja solnega stresa pri arašidu (*Arachis hypogaea* L.)

Izvleček: Uporaba rast vzpodbujajočih rizobakterij (PGPR) je obetajoča rešitev za izboljšanje rasti rastlin v razmerah solnega stresa. Med PGPR imajo bakterije iz rodu *Pseudomonas* mehanizme, ki vzpodbujajo rast rastlin in povečujejo njihovo odpornost v razmerah biotičnega in abiotičnega stresa. Namen raziskave je bil izolirati bakterije iz rodu *Pseudomonas* iz rizosfere rastlin nabranih v zasoljenih tleh na območju Nam Dinh in preučiti njihove fukcije pri vzpodbujanju rasti sejank arašidov, rastočih v slanih tleh. Izoliranih je bilo devet vrst bakterij iz rodu *Pseudomonas*, vendar je bilo samo sedem od teh potrjenih s specifičnimi primerji za rod *Pseudomonas*. Dva od teh sedmih izolatov, ND06 in ND09, sta bila izbrana na osnovi njunih lastnosti vzpodbujanja rasti rastlin s tvorbo indol-3-ocetne kisline (IAA), raztaplanja fosfatov in fiksacije dušika. Dodatno sta oba seva vsebovala gen za kodiranje 1-aminociklopropan-1-karboksilaze (ACC), deaminaze, ki ima pomembnmo vlogo pri podpori rastlinam za prenašanje različnih stresnih razmer. Še posebej je sev ND09 izboljšal rastne parametre sejank arašidov v normalnih razmerah in ob solnem stresu. Med tem je sev ND06 izboljšal rast rastlin samo v razmerah solnega stresa, ne pa v normalnih razmerah. Razultati nakazujejo, da bi v prihodnosti sev ND09 lahko uporabili kot biotični aganes pri okolju prijaznem kmetovanju.

Ključne besede: arašid; PGPR; *Pseudomonas*; odpornost na solni stres

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1 INTRODUCTION

Peanuts (*Arachis hypogaea* L.), a plant of high economic value in agriculture, is considered the most widely produced and consumed oilseed plant all over the world and also in Vietnam. However, peanuts are quite sensitive to salt stress, which causes inhibitory effects to plant development and subsequently a decrease in peanuts production (Goswami et al., 2014; Sharma et al., 2016; Zörb et al., 2019). Recently, many methods have been applied to reduce soil salinity and acidity (Upadhyay and Singh, 2015; El-Nahrawy and Yassin, 2020). Among those, the phytoremediation and bioremediation methods are promising alternative approaches to retrieving salt-affected soils (Singh et al., 2015).

Plant growth-promoting rhizobacteria (PGPR) are bacteria that support growth and control pathogens in plants. Especially, the PGPR inoculation presented the alleviation of salt stress in the development of various plants such as tomato, pepper, canola, bean, *Arabidopsis*, and lettuce (Kang et al., 2009; Chu et al., 2019). The mechanism of plant growth stimulation of PGPR (such as *Rhizobium*, *Azospirillum*, *Pseudomonas*, *Flavobacterium*, *Arthrobacter*, and *Bacillus*) under saline conditions involves the biosynthesis of growth regulators such as indole-3-acetic acid (IAA); the enhancement of nutrients absorption for plants through the process of phosphate solubilization, nitrogen fixation; help plants maintain ionic balance; biosynthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase; inducing systemic tolerance (IST) (Goswami et al., 2014; Chu et al., 2019). Among those, fluorescent *Pseudomonas* sp. is the most studied and exploited bacteria because of some advanced abilities such as an excellent root-colonizing capability and plant growth-promoting activity (Egamberdieva, 2011); is also salt tolerant and able to alleviate salt stress in plants (Shafi et al., 2017). For example, Egamberdieva (2011) reported a significant increase in shoot length (up to 50 %) of beans in salt stress (at 5.0, 7.5, and 10.0 dS m^{-1}) when inoculated beans with *P. extremorientalis* TSAU20 and *P. chlororaphis* TSAU13. Hence, the selection of native PGPRs with characteristics related to bacterial suitability in the potential environment should be considered.

In this study, we aimed to isolate the genus *Pseudomonas* from the salt-contaminated rhizosphere of plants in Nam Dinh and to assess their abilities in stimulating peanuts' growth under conditions of salty stress. The results suggest a promising PGPR to further exploit as a bioinoculant in the future.

2 MATERIAL AND METHODS

2.1 ISOLATION AND SCREENING OF *PSEUDOMONAS* FOR SALT TOLERANCE FROM SALT-CONTAMINANT SOIL

Bacteria were isolated from 18 samples of the rhizosphere of corn (*Zea mays* L.), rice (*Oryza sativa L.),* and peanuts *(Arachis hypogaea*) obtained from salt-contaminated land in Quat Lam, Giao Thuy, Nam Dinh, Vietnam. The 10-fold serial dilutions of the samples were plated on sterile LB agar plates supplemented with 10 % NaCl. After 48 hours of incubation (30 ± 1) °C, the fluorescent colonies under a 366 nm wavelength UV lamp were selected, purified, sub-cultured, and preserved by deep freezing techniques at -80 °C.

Isolated strains were then reconfirmed by using PCR techniques to detect 16S rDNA sequences specifically for *Pseudomonas*. The bacteria proliferated on the Tryptone Soya Broth (TSB) agar medium around 18 hours in (30 ± 1) °C. Use the PureLink™ Genomic DNA Mini Kit (Thermo Fisher) to extract the bacterial gDNA according to the manufacturer's instructions. PCR reaction (25 µl) consists of 2.5 µl gDNA bacteria; 2.5 µl PCR reactive buffer solution (10X); 1 µl per primer (10 nM) and 1 U Phusion High-Fidelity DNA Polymerase (Thermo Fisher), and deionized water was added to get the desired volume. Forward primer (Psmn289: 5'–GGTCTGAGAGGATGATGATCAGT–3') and reverse primer (Psmn1258: 5'–TTAGCTCCACCTCGCGGC–3') were used (Widmer et al., 1998). The PCR program included 5 minutes at 95 °C; 25 cycles (15 seconds at 94 °C, 30 seconds at 55 °C, and 1 minute at 72 °C); 10 minutes at 72 °C. PCR product (about 960 bp) was then detected by electrophoresis on the 1 % agarose gel.

2.2 CHARACTERIZE THE SALT-TOLERANT BACTERIA STRAINS FOR PLANT-GROWTH PROMOTING PROPERTY

2.2.1 Salt tolerance

Strains of bacteria cultured on the TSB media supplemented with different NaCl concentrations ranging from 10 to 24 %. The culture was incubated on a shaker at 150 rpm at (30 ± 1) °C. The results were recorded after 1-4 days of incubation.

2.2.2 Phosphate solubilization

Strains of bacteria were grown on Pikovskaya

(PVK) media agar plates (Pikovskaya, 1948). The plates were incubated at 30 °C for 7 days. Each treatment was done in triplicates. The bacterial colonies with clear halos in the PVK agar plate indicated solubilizing activity of the phosphate. These were sub-cultured on PVK media (Biobasic, Canada).

The phosphate solubilization index (PSI) of bacteria grown on plates was measured as the following formula:

Phosphate solubilizing index (PSI) = [(colony diameter + clearing zone)/ colony diameter]

2.2.3 IAA production

The IAA content produced by isolates was determined by the color reaction with the improved Salkowski reagent (Glickmann and Dessaux, 1995). Bacteria were grown in TSB media containing 5 % NaCl, with an additional 0.1 g l^{-1} tryptophan. After 5 days of incubation at 150 rpm, (30 ± 1) °C, 1 ml of bacteria was collected and centrifuged to remove biomass. The bacterial supernatant was then added Salkowski reagent (1:2 ratio). The reaction was kept for 1 hour at room temperature. The positive reaction with the color from pink to red has measured the absorption at a wavelength of 530 nm to determine the IAA content based on the IAA standard line.

2.2.4 Nitrogen fixation

The bacterial isolates were cultured on a nitrogen-free mineral media containing 3 % NaCl (Wright and Weaver, 1981). Bacteria with the ability in forming colonies, and change the color of the media after 5 days of culture were identified as nitrogen-fixed bacteria.

2.2.5 Biofilm formation

The experiment was carried out in 96-well polystyrene microtiter plates (Biobasic, Canada), using the method described by O'Toole and Kolter (1998). The isolates were grown overnight in LB and LB + 0.3 M NaCl (Costa-Gutierrez et al., 2020a). Then overnight culture was diluted to an OD600 = 0.1 before placing in the wells. The plate incubation was done at 30 °C without agitation. After the indicated times, the biofilm formation was observed by staining with crystal violet (0.4 %) and then using 30 % glacial acetic acid solution to solubilize the dye before qualifying the biofilm formation by measuring absorbance at 540 nm.

2.2.6 Identification of the ACC deaminase encoding genes

PCR reaction (25 µl) consists of 2.5 µl bacterial g DNA; 2.5 µl PCR reactive buffer solution (10X), 1 µl per primer (10 nM), and 1 U Phusion High-Fidelity DNA Polymerase (Thermo Fisher) and deionized water was added to get the desired volume. Forward primer (5'–ATGAACCTGCTGCAACGATTC–3') and reverse primer (5'–TCAGCCGTCGGAAGAT–3') were applied (Saravanakumar and Samiyappan, 2007). The PCR program included 5 minutes at 95 °C; 25 cycles (15 seconds at 95 °C, 15 seconds at 58 °C, and 75 seconds at 72 °C); 5 minutes at 72 °C. PCR product (about 750 bp) was then detected by electrophoresis on the 1 % agarose gel.

2.3 EVALUATE THE PEANUT GROWTH PROMOTION OF BACTERIA UNDER SALTY STRESS AND IN VITRO CONDITIONS

Peanut seeds (*Arachis hypogaea* LDH12) were disinfected and germinated on cotton wool that was impregnated with ½ MS media. After 7-day incubation, the seedlings were transferred to ½ MS media with or without bacteria; and ½ MSmedia supplemented with 100 mM NaCl with or without bacteria (Sharma et al., 2016). The density of bacteria added to the media was 10^6 CFU ml^{-1}. The seedling was grown in long-day conditions (day/night ratio was 16 hours/8 hours); room temperature ranged from 23 to 27 °C. The fresh biomass of the seedling was measured after 4 weeks of growth.

2.4 EVALUATE THE PEANUT GROWTH PROMOTION OF BACTERIA UNDER SALTY STRESS AND GREENHOUSE CONDITION

Peanut seeds (*Arachis hypogaea* LDH12) are disinfected and germinated on cotton wool impregnated with ½ MS mineral media with or without bacterial supplements at a density of 10^6 CFU ml^{-1}. After the seeds germinate, peanut seedlings were transferred to pots containing soil not treated with salt or the soil is mixed with NaCl 75 mM (Goswami et al., 2014). The seedling was watered twice a week. In bacterial treatment experiments, bacterial suspension was added to the water reaching a density of 10^6 CFU ml^{-1}, and watered every 2 weeks. With the salt treatment test, 2 weeks will be additionally watered with a 50 mM NaCl solution (Goswami et al., 2014). After 40 days of sowing seeds, fresh biomass of seedlings was recorded. Temperature conditions (day: 34-38 °C; night: 29-32 °C) and relative humidity of 48-62 % in the nursery

were recorded during the experiment.

2.5 DATA ANALYSIS

All experiments were repeated three times the results were presented as mean values with ± SD. Tukey's honestly significant difference (HSD) method in SPSS (version 17) was applied to compare the means in all experiments.

3 RESULTS AND DISCUSSION

3.1 ISOLATION AND IDENTIFICATION OF BACTERIA

From 18 root rhizosphere soil samples, we isolated 10 bacteria strains including 6 strains (ND01, ND02, ND03, ND04, ND05, and ND06) from corn rhizosphere; 2 strains (ND07 and ND08) from rice rhizosphere, and 2 strains (ND09 and ND10) from peanut rhizosphere. In fact, *Pseudomonas* is a genus of bacteria that is very common in the soil and root rhizosphere of plants, but due to the diversity of specie composition along with the relatively low selective efficiency of the LB media limits the ability to isolate target bacteria, especially for those samples with mold growth on agar plates. To confirm that selected strains belong to the genus of *Pseudomonas*, the PCR reaction was used to amplify a 16S rDNA sequence specific for the *Pseudomonas* (Kim et al., 2013; Yadav et al., 2014). The experiment results showed that only 7 isolates (ND01, ND03, ND04, ND06, ND07, ND09, and ND10) gave a band on the electrophoresis. These bacteria strains were evaluated for growth-promoting characteristics.

3.2 SCREENING THE BACTERIAL ISOLATES FOR SALT TOLERANCE AND PGPR TRAITS

All 7 isolates were used to evaluate their possibilities of growing in high salt conditions. The isolates were cultured in the media containing a gradual increase in NaCl concentration from 10 % to 24 % (the gap between concentrations is 2 %). The results showed that isolated strains in Nam Dinh are likely to survive in media containing a quite high salt concentration, especially the two strains ND06 and ND09, which could grow in media supplemented with up to 18 % and 22 % NaCl, respectively (Table 1).

The experimental results also presented in Table 1 showed that all isolates were capable of producing IAA. However, the amount of IAA produced by bacterial strains after 7 days of culture was relatively low in the range of 2.021 – 3.549 μg ml^{-1} (Malik and Sindhu, 2011). According to Egamberdieva (2015), three main factors affecting the IAA production of rhizobacteria were the bacterial strains; culture time, bacterial growth stage; and precursor to IAA synthesis. Many studies have shown that different strains of bacteria have different IAA production. Several strains of bacteria that are prominent for IAA production, such as *Pseudomonas aureantiaca* TSAU22 (Sheehy et al., 1991), *Pseudomonas extremorientalis* TSAU6 and *Pseudomonas extremorientalis* TSAU20 (Egamberdieva, 2011), significantly increased root growth by up to 25 % under normal conditions and up to 52 % under 100 mM NaCl condition compared with control plants (Botelho and Mendonça-hagler, 2006; Egamberdieva, 2009).

One of the important traits of PGPR is nitrogen fixation. Hence, the isolates were also investigated the nitrogen fixation ability on media without nitrogen sources. The results were shown in Table 1, the majority of iso-

Table 1: Characterization of plant growth-promoting bacteria isolated under salty stress conditions

Bacterial strain	Highest NaCl concentration (%)	IAA concentration (μg ml^{-1})	Phosphate Solubility Index	Nitrogen fixation
ND01	12	3.521 ± 0.113^{a}	1.557 ± 0.211^{b}	-
ND03	12	3.327 ± 0.106^{ab}	2.013 ± 0.131^{a}	+
ND04	10	2.876 ± 0.132^{bc}	1.252 ± 0.124^{c}	-
ND06	18	3.021 ± 0.211^{b}	1.239 ± 0.102^{c}	+
ND07	10	2.417 ± 0.215^{c}	1.532 ± 0.079^{b}	+
ND09	22	3.549 ± 0.115^{a}	1.635 ± 0.063^{ab}	+
ND10	12	2.437 ± 0.102^{c}	1.781 ± 0.023^{ab}	-

Data are means ± SD (n = 3). Values in the same column with the same letter(s) are not significantly different as determined by Tukey's honestly significant difference test ($p < 0.005$). '-' mean no media color; '+' means media color changed

lated strains were capable of growth on the Nitrogen Free Mineral Medium (MNFM), excepted for ND01, ND04, and ND10. The MNFM is a media with no nitrogen sources, hence, the formation of colonies on this media demonstrates that bacterial strains were capable of using air nitrogen sources for cellular processes. In an MNFM, there was a supplement of blue bromophenol as a pH indicator, which is yellow when the pH < 7, green at pH = 7 and turns blue when the pH > 7. In this experiment, the environment changed from green to blue because nitrogen-fixed bacteria created NH_4^+ products that increased the pH of the media.

All 7 isolated strains of bacteria showed the phosphate solubilization capacity when produced a clear zone around colonies after 7 days (Table 1). The PSI ranged from 1.239 to 2.013. The mechanisms for dissolving phosphate by bacteria vary widely, but according to Sharma et al. (2013), there are three main mechanisms: organic acid production, inorganic acid release, and extracellular polymeric substances (EPSs) (Fatima and Arora, 2021).

3.3 EVALUATION OF ACC DEAMINASE PRODUCTION AND COLONIZATION OF BACTERIAL ISOLATES

In order to identify the presence of ACC deaminase in potential strains, the PCR to amplify the specific DNA sequence of this gene in *Pseudomonas* was performed as the method described by Sheehy et al. (1991). In addition, Saravanakumar and Samiyappan (2007) proved that this pair of primers is specific to characterize *Pseudomonas fluorescens* (Flügge 1886) Migula, 1895. Electrophoresis results showed that the target product of about 750 bp appeared in all two selected strains.

Moreover, the biofilm formation ability of isolates under salt stress conditions was also studied. The results were shown in Figure 1.

As can be seen from Figure 1A, under normal conditions, the biofilm formation dynamics of bacterial isolates were different. The ND09 presented the late production of biofilm formation compared to the ND06 strain, showing a lower OD_{540nm} value at the beginning and reaching a higher OD_{540nm} value after 24 hours while the ND06 presented a decline of OD_{540nm} value during the experiment. The results also indicated the delay effect of salt stress on the bacterial biofilm formation, which reached a maximum OD_{540nm} value after 6 hours and higher than the maximum OD_{540nm} value in normal conditions (Figure 1B). These results are consistent with some previous studies on the formation of bacterial biofilm under salt stress (Costa-Gutierrez et al., 2020b; Costa-Gutierrezet al., 2021).

3.4 EVALUATE THE ABILITY TO STIMULATE PEANUT GROWTH UNDER SALT STRESS

Based on the results of the growth-stimulating characteristics of the isolated strains, among the strains isolated from the soil rhizosphere, ND03, ND06, ND07, and

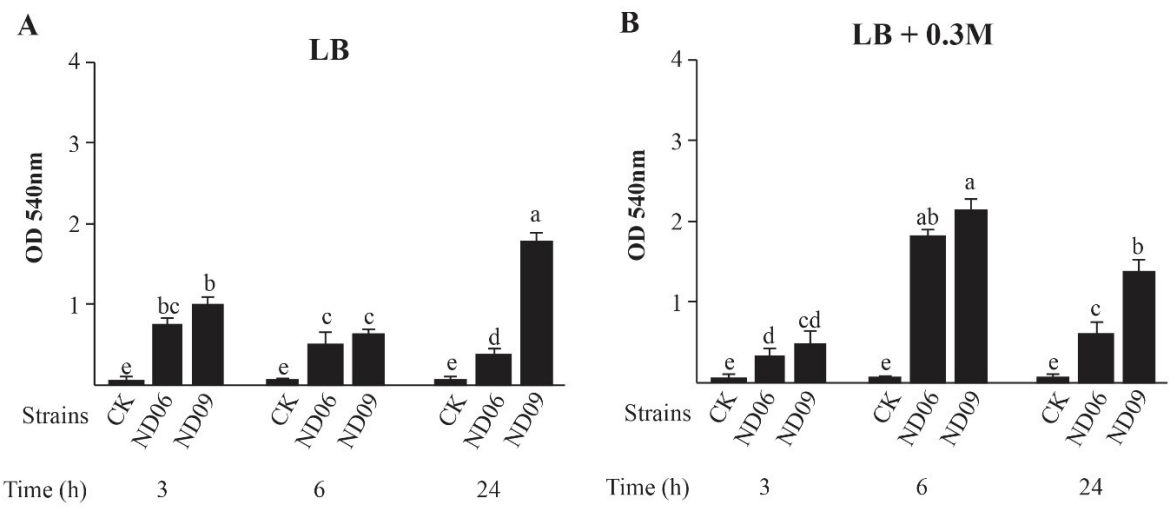

Figure 1: Biofilm formation ability of bacterial isolates (ND06 and ND09) in LB (A) and LB + 0.3M NaCl (B) in different periods of time. CK: media only. Plotted data are means ± SD (n = *3).* The same letter(s) are not significantly different as determined by Tukey's honestly significant difference test ($p < 0.05$)

ND09 are full of characteristics such as IAA production, nitrogen fixation, and phosphate solubilization. However, when considering the results of IAA production, salt-resistance, and phosphate solubility index, 2 strains ND06 and ND09 were selected for further experiments. All two strains gave positive PCR results with primers specific for *Pseudomonas*. *Pseudomonas* strains isolated from rhizosphere and agricultural soil samples were assessed to be relatively safe for humans and animals. These two strains were selected for investigating their ability in promoting plant growth development under *in vitro* and *in vivo* conditions.

In this experiment, the potential of bacterial strains in supporting plants to withstand salty stress will be evaluated on agricultural plant models. Compared to corn, which is only sensitive to salty stress at an average of degrees (Zörb et al., 2004), peanut plants are a very sensitive type to salty stress (Goswami et al., 2014; Sharma et al., 2016). Therefore, although 2 strains were selected from the root rhizosphere of corn and peanut, in this experiment peanut seedlings were selected as models to conduct stress response tests.

In *in vitro* experiments, the results were illustrated in Figure 2 and Table 2. The results indicated that under normal conditions, peanut seedlings treated with the ND09 strain showed growth stimulation expressed in an increase in total plant biomass (34.63 %), shoot biomass (35.22 %), and root biomass (32.87 %) compared to the control. In contrast, the ND06 strain presented no difference from the control plant (Figure 2A and Table 2). It is notable that the IAA concentration reaching from 0.1 to 1 μg ml^{-1} could produce beneficial effects on plant growth (Bui, 2016). It implies that if co-inoculation of isolated strains with plants for a long time, these strains could provide enough exogenous IAA for plant growth by increasing root growth through root elongation and reducing ethylene.

However, under salty stress, all seedlings treated with bacteria showed an increase in the biomass of the plants compared to the control. In particular, the seedling treated with the ND09 strain presented the highest efficiency (Figure 2B and Table 2). This might be because the bacterial isolates produced the ACC deaminase under salt stress to degrade ACC (the precursor of ethylene in all higher plants) and hence prevented the over-accumulation of ethylene in plants under salt stress conditions; subsequently enhancing plant development. This suggests that bacteria have the ability to reduce the effect of salty stress on the growth of peanut plants.

The results of greenhouse experiments were consistent with the *in vitro* results and were illustrated in Figure 3 and presented in Table 3. As can be seen, under normal conditions, seedlings treated with the ND09 strain showed an increase in shoot and root biomass respectively 30.37 % and 32.87 % compared to control seedlings. In contrast, seedlings treated with ND06 strain presented a slight decrease in biomass compared to control seedlings (Figure 3A and Table 3). Under salty stress conditions, all seedlings treated with bacteria had higher fresh biomass than control (Figure 3B and Table 3).

The results of the greenhouse experiments showed a match with ones under the *in vitro* conditions. These results indicated that the ND09 strain not only effectively stimulated peanut seedling growth under normal condi-

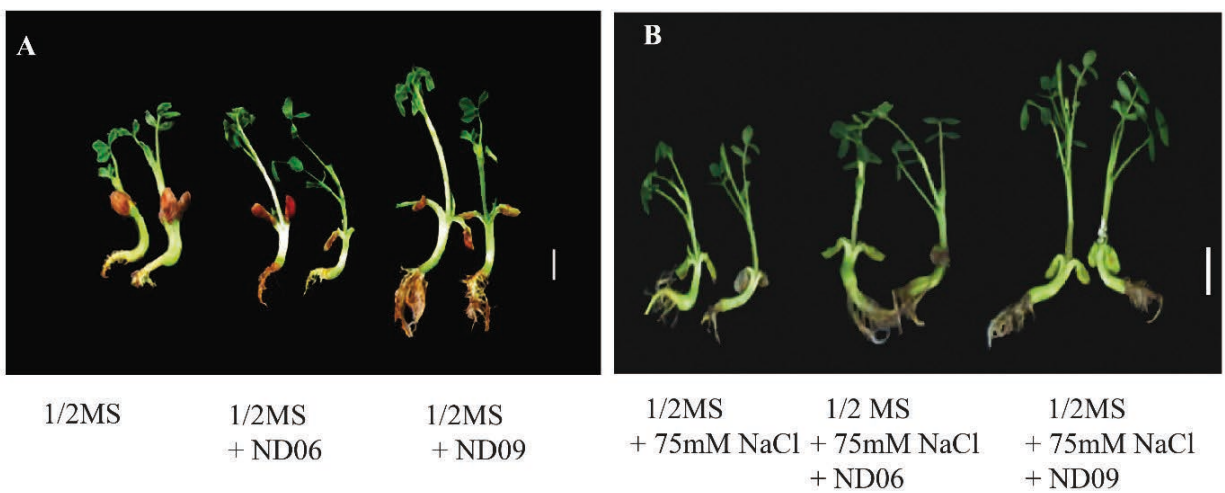

Figure 2: Bacteria isolates (ND06 and ND09) enhances the peanut seedling growth under normal condition (A) and 100 mM NaCl (B) after 4 weeks of sowing under in vitro conditions

Table 2: Effect of selective bacteria on fresh biomass of peanut seedlings grown on different media after 4 weeks under in vitro conditions

	Total plant biomass (mg)		Shoot biomass (mg)		Root biomass (mg)	
Experiments	0 mM NaCl	100 mM NaCl	0 mM NaCl	100 mM NaCl	0 mM NaCl	100 mM NaCl
Peanut seedlings (control)	2213.3 ± 61.2^{b}	1969.5 ± 91.3^{c}	1634.5 ± 101.2^{b}	1612.5 ± 132.1^{c}	579.1 ± 125.0^{b}	356.8 ± 82.3^{c}
Peanut seedlings + ND06	2268.2 ± 75.8^{b}	2410.3 ± 151.3^{b}	1681.7 ± 87.2^{b}	1897.5 ± 121.2^{b}	586.2 ± 72.4^{b}	512.7 ± 64.3^{b}
Peanut seedlings + ND09	2979.7 ± 135.5^{a}	2707.7 ± 35.2^{a}	2210.2 ± 112.3^{a}	2102.3 ± 53.1^{a}	769.5 ± 187.5^{a}	605.3 ± 67.2^{a}

Data are means ± SD (n = 3). Values in the same column with the same letter(s) are not significantly different as determined by Tukey's honestly significant difference test ($p < 0.05$)

tions but also had the potential to improve plant growth under salty tress conditions. Meanwhile, the remaining strain had only a positive effect under salty stresses but not under normal conditions. All of these results suggest the ND09 strain has shown to be a potential strain in the production of probiotic fertilizers that could improve crop yields, whether under normal conditions or salty stress conditions.

These results were in agreement with previous studies that also investigated the alleviation of salt stress by *Pseudomonas* in plant development. Cai et al. (2021) reported that *Chenopodium quinoa* Willd. inoculated with *Pseudomonas* sp. strain M30-35 significantly improved the dry mass of roots by 51.97 % at 150 mM NaCl treatments for 7 d. Another example is the report of Fatima and Arora (2021), who proved that *P. entomophila* PE3 in cobination with 2 % EPS enhanced the growth and resilience of sunflower in saline soil (increment in root and shoot length was 49 % and 85 % respectively in comparison to control). Although each plant species has different selective effects on rhizosphere diversity, *P. fluorescens* and *P. putida* Trevisan, 1889 are still the most dominant species (Egamberdieva, 2015). Many commercial preparations from these two species have been widely used. Several cases of *P. putida* causing disease in humans have been reported, however, these are rare and mostly occur in immunocompromised individuals (Fernández et al., 2015). Another member of this genus, *P. aeruginosa* (Schröter 1872) Migula 1900, has great potential for promoting plant growth and potent antagonism against rhizosphere pathogens. Unlike *P. fluorescens* and *P. putida*, some strains of *P. aeruginosa* are opportunistic pathogens in humans (Fernández et al., 2015). This bacterium is widely distributed in water, soil, and even in some foods. However, the level of risk posed by this bacterium is not large and is only classified as a Class II biosafety risk. Furthermore, not all strains of this species are pathogenic due to the absence of pathogenic genes in the genome. In summary, PGPR strains belonging to the genus *Pseudomonas* can be widely applied in agricultural practices with low risk and controllability.

4 CONCLUSIONS

In this study, 7 bacteria strains belonging to the *Pseudomonas* genus and capable of living in salty conditions were isolated from soil in Nam Dinh. The two strains of bacteria ND06 and ND09 were selected based on phosphate solubilization, nitrogen fixation, and IAA production. The ND09 strain had the potential to stimulate peanut seedling growth under both normal and salty stress conditions, indicating the potential of this strain in sustainable agricultural practices. However, before being widely adopted or commercialized, the ND09 strain

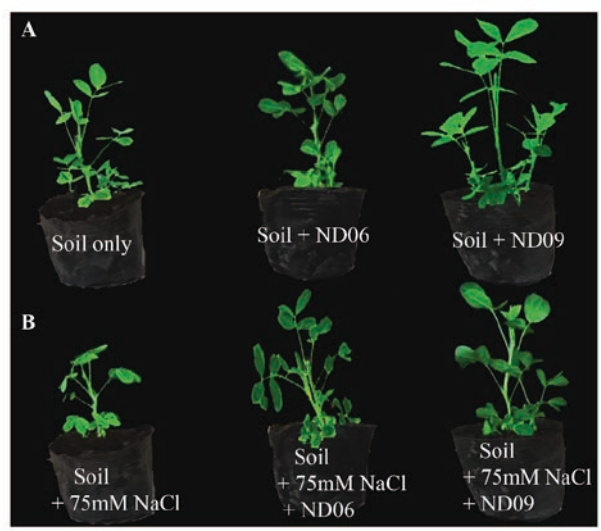

Figure 3: Illustration of a positive effect of bacteria isolates (ND06 and ND09) on the peanut seedling growth under normal condition (A) and 75mM NaCl (B) after 40 days of sowing

Table 3: Bacterial isolate enhance peanut plant growth under salty stress condition after 40 days of Sowing under greenhouse conditions

	Total plant biomass (mg)		Shoot biomass (mg)		Root biomass (mg)	
Experiments	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl	0 mM NaCl	75 mM NaCl
Peanut seedlings (control)	2722.8 ± 31.7^{b}	1559.8 ± 110.1^{c}	2173.7 ± 102.3^{b}	1231.2 ± 111.3^{c}	548.7 ± 62.7^{b}	328.2 ± 101.2^{b}
Peanut seedlings + ND06	2634.2 ± 56.2^{b}	2302.5 ± 102.1^{b}	2091.2 ± 35.7^{b}	1497.3. ± 109.1^{b}	542.6 ± 61.2^{b}	803.7 ± 113.7^{a}
Peanut seedlings + ND09	5396.1 ± 52.1^{a}	2688.9 ± 91.1^{a}	3372.5 ± 115.7^{a}	1895.5 ± 115.7^{a}	2023.4 ± 94.5^{a}	792.7 ± 82.3^{a}

Data are means ± SD (n = 3). Values in the same column with the same letter(s) are not significantly different as determined by the Tukey's honestly significant difference test ($p < 0.005$)

should be evaluated on the risk of disease in humans and animals, as well as the impact on the ecological environment.

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6 REFERENCES

Bui, T.V. (2016). *Plant physiology*. Ho Chi Minh National University Publishing House, p. 198. (in Vietnamese)

Botelho, G.R., Mendonça-hagler, L.C. (2006). Fluorescent *Pseudomonas* associated with the rhizosphere of crops- An overview. *Brazilian Journal of Microbiology*, *37*, 401-416. https://doi.org/10.1590/S1517-83822006000400001

Kang, S.M., Joo, G.J., Hamayun, M., Na, C.I., Shin, D.H., Kim, Y.K., Hong, J.K., Lee, I.J. (2009). Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnology Letters*, *31*(2), 277-281. https://doi.org/10.1007/s10529-008-9867-2

Cai, D., Xu, Y., Zhao, F., Zhang, Y., Duan, H., Guo, X. (2021). Improved salt tolerance of *Chenopodium quinoa* Willd. contributed by *Pseudomonas* sp. strain M30-35. *Peer Journal, 9*, e10702. https://doi.org/10.7717/peerj.10702

Chu, T.N., Tran, B.T.H., Bui, V.L., Hoang, M. (2019). Plant growth-promoting rhizobacterium *Pseudomonas* PS01 induces salt tolerance in *Arabidopsis thaliana*. *BMC Research Notes*, *12*(1), 1-7. https://doi.org/10.1186/s13104-019-4046-1

Costa-Gutierrez, S.B., Raimondo, E.E., Lami, M.J., Vincent, P.A., Espinosa-Urgel, M., de Cristóbal, R.E. (2020a). Inoculation of *Pseudomonas* mutant strains can improve growth of soybean and corn plants in soils under salt stress. *Rhizosphere*, *16*, 100255. https://doi.org/10.1016/j.rhisph.2020.100255

Costa-Gutierrez, S.B., Lami, M.J., Caram-Di Santo, M.C., Zenoff, A.M., Vincent, P.A., Molina-Henares, M.A., Espinosa-Urgel, M., de Cristóbal, R.E. (2020b). Plant growth promotion by *Pseudomonas putida* KT2440 under saline stress: Role of *eptA*. *Applied Microbiology and Biotechnology*, *104*, 4577-4592. https://doi.org/10.1007/s00253-020-10516-z

Costa-Gutierrez, S.B., Caram-Di Santo, M.C.d.V., Zenoff, A.M., Espinosa-Urgel, M., de Cristóbal, R.E., Vincent, P.A. (2021). Isolation of *Pseudomonas* strains with potential for protection of soybean plants against saline stress. *Agronomy*, *11*, 2236. https://doi.org/10.3390/agronomy11112236

Damodaran, T., Sah, V., Rai, R.B., Sharma, D.K., Mishra, V.K., Jha, S.K., Kannan, R. (2013). Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria (PGPR) and growth vigour in tomato under sodic environment. *African Journal of Microbiology Research*, *7*(44), 5082-5089.

Egamberdieva, D. (2009). Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiologiae Plantarum*, *31*(4), 861-864. doi: 10.1007/s11738-009-0297-0. https://doi.org/10.1007/s11738-009-0297-0

Egamberdieva, D. (2011). Survival of *Pseudomonas extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 in the rhizosphere of common bean (*Phaseolus vulgaris*) under saline conditions. *Plant, Soil and Environment*, *57*(3), 122–127. https://doi.org/10.17221/316/2010-PSE

Egamberdieva, D. (2015). *The role of phytohormone producing bacteria in alleviating salt stress in crop plants*. In: *Biotechnological techniques of stress tolerance in plants* (Editors: M. Miransari), Stadium Press LLC, USA, p. 20–39.

El-Nahrawy, S., Yassin, M. (2020). Response of different cultivars of wheat plants (*Triticum aestivum* L.) to inoculation by *Azotobacter* sp. under salinity stress conditions. *Journal of Advances in Microbiology*, *20*, 59-79. https://doi.org/10.9734/jamb/2020/v20i130209

Fatima, T., Arora, N.K. (2021). *Pseudomonas entomophila* PE3 and its exopolysaccharides as biostimulants for enhancing growth, yield and tolerance responses of sunflower under saline conditions. *Microbiological Research*, *244*, 126671. https://doi.org/10.1016/j.micres.2020.126671

Fernández, M., Porcel, M., de la Torre, J., Molina-Henares, M.A., Daddaoua, A., Llamas, M.A., Roca, A., Carriel, V., Garzón, I., Ramos, J.L., Alaminos, M., Duque, E. (2015). Analysis of the pathogenic potential of nosocomial *Pseudomonas putida* strains. *Frontiers in Microbiology*, *6*, 871. https://doi.org/10.3389/fmicb.2015.00871

Goswami, D., Dhandhukia, P., Patel, P., Thakker, J.N. (2014). Screening of PGPR from saline desert of Kutch: Growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. *Microbiological Research*, *169*(1), 66-75. https://doi.org/10.1016/j.micres.2013.07.004

Glickmann, E., Dessaux, Y. (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology*, *61*(2), 793-796. https://doi.org/10.1128/aem.61.2.793-796.1995

Kim, J., Mele, P., Crowley, D. (2013). Application of PCR primer sets for detection of *Pseudomonas* sp. functional genes in the plant rhizosphere. *Journal of Agricultural Chemistry and Environment*, *2*(1), 8-15. https://doi.org/10.4236/jacen.2013.21002

Malik, D.K., Sindhu, S.S. (2011). Production of indole acetic acid by *Pseudomonas* sp.: Effect of co-inoculation with *Mesorhizobium* sp. on nodulation and plant growth of chickpea (*Cicer arietinum*). *Physiology and Molecular Biology of Plants*, *17*(1), 25-32. https://doi.org/10.1007/s12298-010-0041-7

O'Toole, G.A., Kolter, R. (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. *Molecular Microbiology*, *28*, 449-61. https://doi.org/10.1046/j.1365-2958.1998.00797.x.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya*, *17*, 362–370.

Sharma, S., Kulkarni, J., Jha, B. (2016). Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut. *Frontiers in Microbiology*, *7*, 1600. https://doi.org/10.3389/fmicb.2016.01600

Singh, R.P., Jha, P., Jha, P.N. (2015). The plant-growth-promoting bacterium *Klebsiella* sp. SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. *Journal of Plant Physiology*, *184*, 57-67. https://doi.org/10.1016/j.jplph.2015.07.002

Shafi, J., Tian, H., Ji, M. (2017). *Bacillus* species as versatile weapons for plant pathogens: a review. *Biotechnology & Biotechnological Equipment*, *31*, 446–459. https://doi.org/10.1080/13102818.2017.1286950

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A. (2013). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus*, *2*(1), 1-14. https://doi.org/10.1186/2193-1801-2-587

Saravanakumar, D., Samiyappan, R. (2007). ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. *Journal of Applied Microbiology*, *102*(5), 1283-1292. https://doi.org/10.1111/j.1365-2672.2006.03179.x

Sheehy, R.E., Honma, M., Yamada, M., Sasaki, T., Martineau, B., Hiatt, W. (1991). Isolation, sequence, and expression in *Escherichia Coli* of the *Pseudomonas* sp. strain ACP gene encoding 1-aminocyclopropane-1-carboxylate deaminase. *Journal of Bacteriology*, *173*(17), 5260-5265. https://doi.org/10.1128/jb.173.17.5260-5265.1991

Upadhyay, S.K., Singh, D.P. (2015). Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment. *Plant Biology*, *17(1)*, 288-293. https://doi.org/10.1111/plb.12173

Widmer, F., Seidler, R.J., Gillevet, P.M., Watrud, L.S., Giovanni, G.D. (1998). A highly selective PCR protocol for detecting 16S rRNA genes of the genus *Pseudomonas* (sensu stricto) in environmental samples. *Applied and Environmental Microbiology*, *64*(7), 2545-2553. https://doi.org/10.1128/AEM.64.7.2545-2553.1998

Wright, S.F., Weaver, R.W. (1981). Enumeration and identification of nitrogen-fixing bacteria from forage grass roots. *Applied and Environmental Microbiology*, *42*(1), 97-101. https://doi.org/10.1128/aem.42.1.97-101.1981

Yadav, S., Yadav, S., Kaushik, R., Saxena, A.K., Arora, D.K. (2014). Genetic and functional diversity of fluorescent *Pseudomonas* from rhizospheric soils of wheat crop. *Journal of Basic Microbiology*, *54*(5), 425-437. https://doi.org/10.1002/jobm.201200384

Zörb, C., Schmitt, S., Neeb, A., Karl, S., Linder, M., Schubert, S. (2004). The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by a mitigation of symptoms and not by a specific adaptation. *Plant Science*, *167*(1), 91-100. https://doi.org/10.1016/j.plantsci.2004.03.004

Zörb, C., Geilfus, C.M., Dietz, K.J. (2019). Salinity and crop yield. *Plant Biology*, *21*, 31-38. https://doi.org/10.1111/plb.12884