

# Endophytic bacteria enhance the growth and salt tolerance of rice under saline conditions

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## Endophytic bacteria enhance the growth and salt tolerance of rice under saline conditions

**Abstract:** Developing biostimulants from salt-tolerant plant growth-promoting (PGP) bacteria is an emerging strategy for sustainable agriculture in the context of increasing soil salinization. This study aimed to isolate endophytic bacteria (EB) capable of promoting rice seed germination and seedling growth at different NaCl concentrations. Nine salt-tolerant EB strains were isolated and two, ST.6 and ST.8, with the rice seed promoting effect 99.3 and 99.7 %, respectively, were selected and identified as *Pantoea dispersa* and *Burkholderia cenocepacia*, respectively. ST.6 showed a higher value of the activity of phosphatase (617 mg P ml<sup>-1</sup>), production of indole-3-acetic acid (19.7 µg IAA ml<sup>-1</sup>), the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (13.5 µmol mg<sup>-1</sup> protein h<sup>-1</sup>), and production of siderophore (76.3 %). Especially, rice seedlings inoculated with strain ST.6 showed a significant improvement in root length (58.95 %), shoot length (16.6 %), dry biomass (7.0 %), the content of chlorophyll (46.2 and 57.1 % for chlorophyll a and b, respectively), carotenoids (22.2%), and proline (19.0 %). A decrease in antioxidant enzyme activities was also observed in the rice seedlings inoculated with either ST.6 or ST.8 strain under salt stress. Furthermore, the salt stress condition enhanced the colonization of roots by both studied endophytic bacteria. More experiments should be done to develop endophytic bacteria ST.6 and ST.8 as efficient bio-inoculants.

**Key words:** endophytes; salt stress; plant growth-promoting bacteria; antioxidant enzymes; seed germination; rice (*Oryza sativa*)

## Endofitske bakterije pospešujejo rast in toleranco na sol riža v razmerah slanosti

**Izvleček:** Razvoj biostimulantov iz bakterij, ki pospešujejo rast na sol tolerantnih rastlin (PGP) je razvijajoča se strategija za vzdržnostno kmetijstvo v času naraščajočega zasoljevanja tal. Namen raziskave je bil izolirati endofitske bakterije (EB), ki so sposobne pospeševati kalitev semen riža in rast sejank pri različnih koncentracijah NaCl. Izoliranih je bilo devet na sol tolerantnih sevov EB in dva seva, ST.6 in ST.8, ki sta pospeševala kalitev semen riža za 99,3 in 99,7 %. Ta dva seva sta bila izolirana in določena kot vrsti *Pantoea dispersa* in *Burkholderia cenocepacia*. Sev ST.6 je pokazal večjo vrednost v aktivnosti fosfataze (617 mg P ml<sup>-1</sup>), v produkciji indole-3-očetne kisline (19,7 µg IAA ml<sup>-1</sup>), v aktivnosti deaminaze 1-aminociklopropan-1-karboksilne kisline (ACC) (13,5 µmol mg<sup>-1</sup> protein h<sup>-1</sup>) in v tvorbi sideroforov (76,3 %). Še posebej so sejanke riža, inokulirane s sevom ST.6, pokazale značilno izboljšanje v dolžini korenin (58,95 %), dolžini poganjkov (16,6 %), suhi biomasi (7,0 %), v vsebnosti klorofila (46,2 in 57,1 % za klorofil a in b), karotenoidov (22,2 %) in prolina (19,0 %). V sejkah riža, inokuliranih s sevom ST.6 ali ST.8, je bil opazen tudi upad aktivnosti antioksidacijskih encimov v razmerah solnega stresa. Nadalje so razmere solnega stresa pospešile kolonizacijo korenin z obema sevoma preučevanih endofitskih bakterij. Še več poskusov bo potrebnih, da bi razvili seva endofitskih bakterij ST.6 in ST.8 kot učinkovita bioinokulanta.

**Ključne besede:** endofiti; solni stres; bakterije, ki pospešujejo rast rastlin; antioksidacijski encimi; kalitev semen; riž (*Oryza sativa*)

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## 1 INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop and is mainly cultivated in developing countries. It is also reported as one of the cultivated crops that required a high amount of water to grow. However, climate change is one of the factors that cause drought, and soil salinity more often. These led to a strong reduction in rice productivity (Kumar et al., 2020). The salt stresses mainly affect plant growth and development through changes in morphology, biochemistry, and physiology such as plants' ability to uptake nutrients and water (Liang et al., 2018; Bistgani et al., 2019; Hao et al., 2021), and/or reduction of photosynthesis efficiency (Wang et al., 2019a). Further, during soil salinization, some plants could potentially be adapted to changing salinity in soil by adjusting their metabolism through the regulation of signal transduction and gene expressions (Daliakopoulos et al., 2016; Hao et al., 2021); or ion-selective absorption and compartmentalization (Liang et al., 2018; Hao et al., 2021); or through the production of antioxidants and inhibition of reactive oxygen species (ROS) generation (Abbas et al., 2019; Hao et al., 2021). The microbial supplement of halotolerant microorganisms to the soil is an emerging method that assists salt stress tolerance in plants (Etesami and Glick, 2020).

The important group of plant growth-promoting bacteria (PGPB) are endophytic bacteria that live inside the plant tissues and do not cause plant disease (Schulz and Boyle, 2007). Under salinity stress conditions, endophytic bacteria could improve plant growth under salinity conditions by producing exopolysaccharides (EPS), and/or enhancing the activity of enzymes like ACC deaminase and antioxidative enzyme and/or accumulating proline in the plant (Wang et al., 2019b). In addition, it was reported that endophytic bacteria could promote plant development through many mechanisms such as the production of indole acetic acid (IAA), siderophores, and phosphate solubilization (Trung et al., 2022). There were several studies that demonstrated the plant growth-promoting functions of endophytic bacteria isolated from wild rice and trade rice (Chu et al., 2021). For example, the endophytic bacteria isolated from wild rice such as *Bacillus*, *Microbacterium*, *Pantoea ananatis* D1, *Herbaspirillum*, *Ideonella*, *Enterobacter*, and *Azospirillum* were able to produce IAA, which showed roles in promoting plant growth (Lu et al., 2021; Zhang et al., 2021). These results suggest that endophytic bacteria could be developed into biofertilizers used in organic cultivation to promote the growth and development of plants in normal as well as saline conditions.

It is a fact that the farmers used an extensive amount of chemical fertilizers and pesticides in modern agriculture to increase crop productivity causing a change in nutrient supply, a decrease in microbial diversity, limitation of productivity, and deterioration of soil health (Etesami and Glick, 2020). Recently, the organic rice cultivation model produced a positive shift in rice production and has been effectively applied by many farmers in Nam Dinh, Viet Nam. Using natural systems and avoiding the use of synthetic substances increased biodiversity in soil by up to 30 % on organic farms compared to conventional farming (Rundlöf et al., 2016). Hence, exploiting the salt-stress plant growth-promoting bacteria (ST-PGPB) strains as a safe bioinoculant to improve crop productivity in organic agriculture is an alternative strategy. However, there are not many studies to exploit endophytic bacteria isolated from rice grown under organic cultivation as potential biofertilizers in organic agriculture. Therefore, this research aimed to isolate native endophytic bacteria from rice tissue grown in organic farming and investigated their plant growth promotion abilities under salt stress conditions. These endophytic bacteria with multiple plant growth promotion abilities could be used to develop biofertilizers for organic agriculture in Nam Dinh province.

## 2 MATERIAL AND METHODS

### 2.1 ISOLATING RICE ENDOPHYTIC BACTERIA

Samples of rice roots were collected from farms in Nam Dinh, Viet Nam. The soil adhered to the root samples was removed under running tap water. The endophytic bacteria were isolated by the method described by Trung et al. (2022). Briefly, the root samples were sterilized by soaking for 2 minutes in 70 % ethanol; then immersed in 3 % NaOCl solution for 5 minutes with the addition of 2-3 drops of Tween 20, and finally washed five times with sterile distilled water. Plating 100 µl of the final rinse on LB (Luria-Bertani) agar plates and incubating them at 28 °C for 72 h was used to verify the effectiveness of surface sterilization. The surface-sterilized roots were dried on sterile filter paper and placed on LB media plates supplemented with 3 % NaCl, adjusted pH = 8. These plates were cultured for 3 days at 28 °C. Single colonies on each plate were observed for their morphology and color before being separately transferred to new plates to purify and finally stored at -80 °C in LB broth containing 18 % glycerol.

## 2.2 SCREENING THE ISOLATED BACTERIA FOR IMPROVING RICE SEED GERMINATION UNDER SALT STRESS

The selected bacterial strains were grown on LB overnight at 28 °C. The rice seeds (*Oryza sativa* L.) variety ST24 were sterilized as the method described by Sarkar et al. (2018). The surface-sterilized rice seeds were bacterized by soaking at room temperature for 24 h in prepared bacterial culture ( $10^6$  CFU ml<sup>-1</sup>). In the negative control experiment, the sterilized rice seed was soaked in sterile distilled water. The experiment was designed randomly with 3 repetitions, each experiment included 30 bacterized rice seeds placed in a plastic container, which had a double layer of sterile filter paper moistened with 100 mM NaCl solution. The plastic containers containing inoculated seeds were incubated in a dark at 28 °C for 7 days. The parameters such as germination rate, shoot, and root length were measured. The bacteria strains with the highest efficiency were selected for further studies.

## 2.3 MOLECULAR IDENTIFICATION OF ISOLATES

The genomic DNA of selected strains was extracted by using the Rapid Bacteria Genomic DNA Isolation Kit (Biobasic, Canada) as per the kit instructions. The PCR amplification of the 16S rRNA gene fragment (1.5 kb) was done by using universal primers 27 F (5'-AGA GTT TGA TCC TGG CTC AG-3'), and 1492 R (5'-TAC GGT TAC CTT GTT ACG ACT T-3'). The obtained PCR products were cleaned and sequenced by First Base Company (Singapore). The nucleotide sequence was checked for quality before being used to blast on the BLAST server (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to find the closest species. The high-similarity sequences (more than 99 %) were selected and used to regenerate a phylogenetic tree by using MEGA software (v.7.2) (Kumar et al., 2016). The obtained sequences of isolates were deposited on GenBank (accession numbers ON326555 and ON326556).

## 2.4 ASSAY FOR PLANT GROWTH-PROMOTING ACTIVITIES UNDER IN VITRO CONDITIONS

The plant growth promotion (PGP) traits (production of indole-3-acetic acid (IAA), siderophore, ACC deaminase; and phosphate solubilization) of isolates were investigated under *in vitro* conditions.

IAA production of isolates was measured by the method described by Patten and Glick (2002). Briefly, 20 µl of bacterial culture ( $OD_{600} = 1$ ) were pipetted and

cultured in flash containing LB media supplemented with 1 g l<sup>-1</sup> L-Tryptophan at 28 °C for 24 h. After incubation, the culture was centrifuged to remove bacteria cells and a 1 ml aliquot of the supernatant was added with 4 ml of Salkowski's reagent (150 ml of 95–98 % H<sub>2</sub>SO<sub>4</sub>, 75 ml of 0.5 mol l<sup>-1</sup> FeCl<sub>3</sub>·6H<sub>2</sub>O and 250 ml of distilled H<sub>2</sub>O). The mixture solution was incubated at room temperature and measured the absorbance at 535 nm. Finally, the IAA amount in the mixture was calculated based on the standard curve of IAA.

The siderophore production of isolated strains was measured by the method described by Arora and Verma (2017). A single colony was taken to culture in the Tryptone Soy Agar (TSA) broth media at 28 °C for 3 days. After that, the culture was centrifuged at 8000 rpm for 10 min to get the supernatant. Then, the collected supernatant (1 ml) was mixed with prepared the chrome azurol S (CAS) assay solution (1 ml) and incubated at room temperature for 1 h. For the control, the autoclaved TSA medium was used instead. The absorbance of the sample (As) and the control (Ac) at 630 nm was determined and the amount of siderophore was calculated according to the formula:

$$\% \text{ siderophore units} = \frac{Ar - As}{Ar} \times 100$$

The isolates were also investigated for their ability of phosphate solubilization on the NBRIP (National Botanical Research Institute's Phosphate) medium. The bacterium was cultured at 28 °C on the shaker. After 3 days of incubation, the pH of the culture broth was determined by a pH meter and the phosphate amount released in the culture supernatant was measured by the molybdenum blue method (Murphy and Riley, 1962).

The 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production of the isolates was carried out as the method described by Penrose and Glick (2003).

All of the above experiments were designed in triplicate and were repeated three times.

## 2.5 EVALUATING THE PLANT GROWTH-PROMOTING CAPACITY OF ISOLATED BACTERIA UNDER SALINE STRESS

Surface-sterilized rice seeds were inoculated by soaking for 1 h in bacterial solution ( $10^6$  CFU/ml) or in sterile water for control. 6 bacterized rice seeds were grown in plastic pots (25 x 30 cm) containing 400 g of non-sterile soil collected from Ca Mau, Vietnam, which has characteristics as follow: clayey soil, pH 7.3, available-P (P<sub>2</sub>O<sub>5</sub>) 16 mg kg<sup>-1</sup>, available K 218 mg kg<sup>-1</sup>, total nitrogen 28 mg kg<sup>-1</sup>, E.C (0.72 ds m<sup>-1</sup>), CaCO<sub>3</sub> 3.3 %, organic matter 1.1 %. The bacterial solution ( $10^6$  CFU ml

1) or sterile water was added to the base of seedlings 7 days after transplantation, respectively for the treatment and control. After that, the salt solution (30 ml of 150 mM NaCl) was used to water the seedlings at 48 h intervals while the water with a similar volume was applied to the ones with no salinity stress. The experiment was designed randomly with 3 repetitions.

All the pots were kept in the grow chamber for 20 days (light/dark cycle: 16h/8h). At the harvest, plant growth parameters were recorded including shoot and root length, the number of roots; the dry mass of plants after drying at 60 °C for 24 h (mg/plant), and chlorophyll content/fresh mass was determined by the colorimetric method as described by Ben et al. (1980) on spectrophotometers at different wavelengths: chlorophyll a (OD = 663 nm), chlorophyll b (OD = 645 nm) and carotenoids (OD = 440.5 nm).

To explore the response of plant under salt stress, the proline content was also measured as the method described by Bates et al. (1973); the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were determined by using the suitable kits as the manufacturer's instructions (College of Forestry Biotechnology, Chuong My, Vietnam).

## 2.6 DETERMINATION OF INOCULATED BACTERIA IN THE PLANTS

The inoculated bacteria were screened for their presence in the inoculated rice plants under normal and salt stress conditions to investigate the effect of abiotic conditions on their colonization ability. The surface sterilization of plant samples was done as the method described by Trung et al. (2022). The sterilized samples

were homogenized in 0.9 % NaCl solution and 1 ml of the homogenized solution was plated on the LB agar plates. The inoculated plates were cultured overnight at 37 °C. Then the presence of inoculated bacteria was done by screening 100 isolated colonies using Restriction Fragment Length Polymorphism (RFLP) method, in which the identity of the isolated bacteria was compared with the inoculated strains.

## 2.7 DATA ANALYSIS

All the experiments were conducted in triplicate. Data analysis was done by applying the SPSS ver. 17 package (SPSS Inc., Chicago, IL), and statistically significant differences were done by using one-way ANOVA followed by Duncan's test ( $p < 0.05$ ).

## 3 RESULTS AND DISCUSSION

### 3.1 ISOLATION AND EVALUATION OF ENDOPHYTIC BACTERIA FROM RICE ROOTS FOR ENHANCING RICE SEED GERMINATION UNDER SALT STRESS

From rice root and shoot samples, a total of nine endophytic strains capable of forming colonies on the salt stress media were isolated and distinguished from others by colony morphological characteristics. All of them were used to evaluate their abilities in improving rice seed germination under salt-stress conditions. The results are shown in Table 1.

As can be seen from Table 1, the results indicated the ability to enhance the seed germination of most iso-

**Table 1:** Effect of isolated endophytic bacteria on germination rate and growth parameters of rice seedlings after 7 days

No.	Bacterial strains	Germination rate (%)	Shoot length (mm)	Root length (mm)
1	ST.1	93.67 ± 0.5 <sup>ab</sup>	81.89 ± 0.7 <sup>b</sup>	75.54 ± 0.7 <sup>b</sup>
2	ST.2	94.33 ± 0.9 <sup>ab</sup>	83.92 ± 0.4 <sup>b</sup>	74.32 ± 0.4 <sup>b</sup>
3	ST.3	83.13 ± 0.8 <sup>c</sup>	63.43 ± 0.5 <sup>c</sup>	58.18 ± 0.6 <sup>c</sup>
4	ST.4	94.67 ± 1.2 <sup>ab</sup>	64.14 ± 0.9 <sup>c</sup>	57.69 ± 0.7 <sup>c</sup>
5	ST.5	82.31 ± 0.7 <sup>c</sup>	74.41 ± 0.3 <sup>bc</sup>	66.18 ± 0.9 <sup>bc</sup>
6	ST.6	99.33 ± 0.8 <sup>a</sup>	99.47 ± 0.6 <sup>a</sup>	82.16 ± 0.8 <sup>a</sup>
7	ST.7	92.71 ± 0.9 <sup>ab</sup>	82.32 ± 0.7 <sup>b</sup>	76.31 ± 0.5 <sup>b</sup>
8	ST.8	99.67 ± 0.6 <sup>a</sup>	98.87 ± 0.6 <sup>a</sup>	81.26 ± 0.6 <sup>a</sup>
9	ST.9	81.33 ± 0.9 <sup>c</sup>	75.34 ± 0.8 <sup>bc</sup>	67.19 ± 0.7 <sup>bc</sup>
10	No bacteria	81.24 ± 0.9 <sup>c</sup>	63.14 ± 0.5 <sup>c</sup>	56.43 ± 0.6 <sup>c</sup>

The data represent the mean ± standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ( $p < 0.05$ )

lates. Among those, two bacteria strains, ST.6 and ST.8 produced significant improvements in germination rates, shoot and root length, and statistically significant differences ( $p < 0.05$ ) compared to control tests (no bacteria).

The experimental results showed that two lines of isolated endophytic bacteria, ST.6 and ST.8 at densities of  $10^6$  CFU ml<sup>-1</sup>, were highly effective in supporting germination stimulation, helping to support the growth and biomass of rice crops. Hence, the isolates ST.6 and ST.8 were chosen for further studies.

The results of molecular identification indicated the ST.6 and ST.8 strains were close to *Pantoea dispersa* Gavini et al. 1989 (accession number MT275631.1) and *Burkholderia cenocepacia* AU16956 (accession number CP034545.1), respectively. The 16S rDNA sequences of two strains ST.6 and ST.8 were deposited on GenBank with the accession numbers ON326555 and ON326556.

### 3.2 EVALUATION OF THE *IN VITRO* PLANT GROWTH-PROMOTING ABILITIES OF SELECTED ENDOPHYTIC BACTERIA

Two selected endophytic bacteria were investigated for the plant growth-promoting (PGP) traits. The results were presented in Table 2.

Based on the data shown in Table 2, the endophytic bacteria strain ST6 presented the ability in producing all tested plant growth-promoting traits, while strain ST.8

only presented three of four but not the siderophore production trait under *in vitro* conditions. Notably, a remarkable decrease in pH was observed during the phosphate solubilization process of these two endophytic bacteria strains.

### 3.3 INOCULATION WITH SELECTED ENDOPHYTIC BACTERIA ENHANCED RICE GROWTH UNDER NORMAL AND SALINE CONDITIONS

The results of greenhouse experiments were carried out to investigate the capacity of isolated endophytic bacteria in improving rice growth under stress conditions. The results were illustrated in Figure 1 and shown in Table 3.

The data indicated a significant decrease in the analyzed growth parameters (shoot and root length, number of roots per plant, and plant biomass) when watered seedlings with 150 mM NaCl solution. Interestingly, the inoculation with both selected strains, ST.6 and ST.8, resulted in a significant improvement in plant growth parameters compared with the control ( $p < 0.05$ ). Presumably, the results showed that the isolates, ST.6 and ST.8, are beneficial for rice seedling growth promotion and improve the salt tolerance of rice seedlings as well (Figure 1).

In Viet Nam, rice is one of the important crops that

**Table 2:** Characterization of *in vitro* plant growth-promoting traits of endophytic bacteria isolated from rice root

Bacterial strain	IAA ( $\mu\text{g ml}^{-1}$ )	ACC Deaminase	Siderophore (%)	Soluble phosphate amount ( $\text{mg ml}^{-1}$ )
ST.6	$19.71 \pm 1.83$	$13.52 \pm 0.14$	76.32	$617.35 \pm 0.23$
ST.8	$15.67 \pm 1.79$	$12.72 \pm 0.47$	-	$579.75 \pm 0.45$

The data represent the mean  $\pm$  standard error (SE) based on three replicates. The ACC deaminase activity was measured spectrophotometrically at 590 nm and expressed as  $\mu\text{mol mg}^{-1}$  protein h<sup>-1</sup>

**Table 3:** Enhancement of growth parameters of rice seedlings inoculated with selected endophytic bacteria under normal and saline conditions

Watering solution	Treatment	Root length (cm)	Shoot length (cm)	Average number of roots/plant	Plant dry weight (mg)
Water	No bacteria	$3.12 \pm 0.4^b$	$21.4 \pm 1.2^b$	$8.12 \pm 0.3^a$	$40.35 \pm 0.3^b$
	Strain ST.6	$4.83 \pm 0.5^a$	$26.8 \pm 2.3^a$	$7.95 \pm 0.5^a$	$43.21 \pm 0.2^a$
	Strain ST.8	$4.21 \pm 0.7^a$	$25.7 \pm 1.8^a$	$8.13 \pm 0.3^a$	$42.57 \pm 0.3^a$
150 mM NaCl	No bacteria	$2.29 \pm 0.5^c$	$17.5 \pm 1.6^d$	$7.1 \pm 0.5^b$	$36.78 \pm 0.5^c$
	Strain ST.6	$3.64 \pm 0.7^{ab}$	$20.4 \pm 1.3^{bc}$	$7.6 \pm 0.4^{ab}$	$39.36 \pm 0.2^b$
	Strain ST.8	$2.78 \pm 0.4^{bc}$	$19.1 \pm 1.5^c$	$7.4 \pm 0.7^{ab}$	$38.14 \pm 0.3^{bc}$

The data represent the mean  $\pm$  standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ( $p < 0.05$ )



**Figure 1.** Inoculation of strains ST.6 and ST.8 improves rice growth under normal conditions (A) and in the presence of 150 mmol l<sup>-1</sup> NaCl (B). Scale bar: 5 cm

provide food for domestic consumption and international export as well. However, it is a plant sensitive to salination that appeared more often in some areas in Viet Nam including Ca Mau, Vietnam due to climate change. Among methods that have been applied to reduce the effect of salination on crop yield, the one using plant growth-promoting bacteria is emerging as an alternative method that could alleviate biotic and abiotic stresses in plants (Vaishnav et al., 2019). There were several studies have been carried out to explore the endophytic bacterial community colonizing the internal tissues of healthy rice plants (Chu et al., 2021; Zhang et al., 2021). An example is endophytic bacteria, strain Fse28, obtained from the surface-sterilized seeds of Dongxiang wild rice presented a significant improvement in the rice seed germination (86 %) compared with the control (70 %) after 5 days of germination (Zhang et al., 2021). The results of this study showed that two strains ST.6 and ST.8 presented the strongest abilities in enhancing the germination rate of rice seed under salt stress (100 mM NaCl). There were several studies also presented the beneficial effect of endophytes on seed germination. Wang et al. (2019b) reported that the *Epichloë bromicola* Leuchtm. & Scharndl endophyte significantly increased root length, coleoptile length, and germination rate of *Hordeum brevisubulatum* (Trin.) Link. seeds at high NaCl concentrations (200 and 300 mM). In another report, Lu et al. (2021) showed that *Pantoea ananatis* D1 endophyte clearly improved rice seed germination percentage in comparison to the negative control at 100 mM NaCl. These findings suggest that the endophyte plays an important role in seed germination under different conditions. In this research,

two endophytic strains ST.6 and ST.8 enhanced germination under salt stress, and this may ensure more seedling establishment and enable rice plant associations to withstand saline conditions.

It was reported that the PGPB could enhance the salt tolerance of plants through several direct and indirect mechanisms such as the production of PGP compounds, the modulation of nutrients metabolism, and/or the accumulation of osmolytes (Ilangumaran and Smith, 2017; Otlewska et al., 2020). Otlewska et al. (2020) demonstrated that IAA produced by endophytic bacteria could improve the stress tolerance of plants by increasing root length, promoting nutrient access, and enhancing root exudation. Another PGP compound is ACC that also been proven able in reducing the accumulation of ethylene that was induced by salt stress, hence improving the plant salt resistance (Vaishnav et al., 2019). In this study, two selected endophytic bacterial strains, ST.6 and ST.8, generated two PGP compounds (including IAA and ACC) and promoted rice development under normal and salt stress conditions compared to the control. These might be due to the strain ST.6 and ST.8 still producing IAA in high NaCl concentration (150 mM NaCl) to enhance the rice root development. These results were consistent with other studies, in which the bacteria strains produced IAA at high NaCl concentrations. For example, Kumar et al. (2021) demonstrated that *Bacillus pumilus* Meyer and Gottheil 1901 (Approved Lists 1980) JPVS11 could produce IAA at high NaCl concentrations (up to 1200 mM NaCl) and inoculation with *B. pumilus* JPVS11 significantly improved the agronomic traits of rice at 50 mM NaCl compared to the control. Presumably, the pro-

duction of IAA and/or ACC may be a strategy of endophytic bacteria to adapt to stress conditions.

Moreover, salinity stress caused a low P availability limiting crop production. Hence, an alternative method to improve crop productivity is the increase of P availability in soil by using salt-tolerant phosphate-solubilizing bacteria (ST-PSB). In this study, endophytic strains ST.6 and ST.8 showed a higher amount of soluble P ( $617.35 \pm 0.23$  and  $579.75 \pm 0.45$ , respectively) in the supernatant. Hence, inoculation of these two isolates may enhance the solubilization of insoluble phosphate increasing the level of P availability in soil that is easily absorbed by plants (Vaishnav et al., 2019). There were several studies demonstrated that the inoculation with ST-PSB increased the N, P, and K uptake and enhanced the salt tolerance efficiency in different plants such as rice (*Oryza sativa* L.), wheat (*Triticum* sp.), tomato (*Solanum lycopersicum* L.), maize (*Zea mays* L.), and quinoa (*Chenopodium quinoa* Willd.) (Vaishnav et al., 2019; Lu et al., 2021).

Furthermore, it was reported that in iron-limited conditions the PGPB could produce siderophore to chelate iron from the environment transforming insoluble iron into plant-accessible iron-siderophore complexes (Vaishnav et al., 2019). In this study, endophytic bacteria strain ST.6 secreted siderophore into the supernatant, while strain ST.8 did not; and better rice growth was observed when seedlings were inoculated with strain ST.6 but not with strain ST.8. These results could be the ST.6 produced organic acids (with hydroxyl or carboxyl groups), and siderophores, which further interact with different cationic metals of insoluble Pi to generate the bioavailable phosphate and metal-chelating complex that is easily uptaken by plants. These results suggest that the endophytic bacteria strain ST.6 and ST.8 could be developed into biofertilizer to apply in acidic or alkaline soil (Rfaki et al., 2019).

#### 3.4 EFFECT OF INOCULATED ENDOPHYTIC BACTERIA ON THE CONTENT OF CHLOROPHYLL AND PROLINE IN RICE LEAVES EXPOSED TO SALINE CONDITIONS

Chlorophyll plays an important role during plant development. Hence, the chlorophyll composition: chlorophyll a, chlorophyll b, and carotenoids contents in the rice leaves exposed to the saline condition were determined in the interaction with inoculated bacteria strains. The results were shown in Table 4.

There were statistically significant differences between bacterial strains applied (Table 4). The results showed that in all treatments of rice seedlings with bacterial strains under normal and saline conditions, the content of chlorophyll a, chlorophyll b, and carotenoids was higher, statistically significant difference compared to the control without bacterial inoculation. In which, treatment with bacterial strain ST.6 had the highest chlorophyll a, chlorophyll b, and carotenoid contents ( $0.27$ ;  $0.19$ , and  $0.18 \text{ mg g}^{-1}$ ). The results are in agreement with the results of Sun et al. (2020), in which rice seedlings inoculated with *Pantoea alhagi* NX-11 showed a 32.6 % higher fresh mass, a 30.6 % greater root length, a 26.4 % greater shoot length, and a 42.5 % higher chlorophyll compared with the control under salt stress conditions. These results showed that the bacterial strains were effective in synthesizing chlorophyll a, chlorophyll b, and carotenoids in rice seedlings during the seedling stage under normal or stress conditions.

In addition, proline was reported as an important osmotic adjustment substance, which exists in plant cells. The accumulation of proline was one of the mechanisms to alleviate the salt stress in plants (Liang et al., 2018). Our results showed that the uninoculated and inoculated seedlings treated with 150 mM NaCl solution resulted

**Table 4:** Effect of bacterial inoculation on chlorophyll content of rice seedlings under salt stress

Watering solution	Treatment	Chlorophyll a ( $\text{mg g}^{-1}$ )	Chlorophyll b ( $\text{mg g}^{-1}$ )	Carotenoid ( $\text{mg g}^{-1}$ )	Proline ( $\mu\text{g g}^{-1}$ FM)
Water	No bacteria	$0.15 \pm 0.1^{\text{d}}$	$0.16 \pm 0.2^{\text{ab}}$	$0.15 \pm 0.3^{\text{a}}$	$17.61 \pm 0.4^{\text{c}}$
	Strain ST.6	$0.27 \pm 0.4^{\text{a}}$	$0.19 \pm 0.2^{\text{a}}$	$0.18 \pm 0.2^{\text{a}}$	$29.32 \pm 0.5^{\text{cd}}$
	Strain ST.8	$0.22 \pm 0.3^{\text{b}}$	$0.18 \pm 0.3^{\text{b}}$	$0.17 \pm 0.3^{\text{ab}}$	$27.15 \pm 0.3^{\text{d}}$
150 mM NaCl	No bacteria	$0.13 \pm 0.2^{\text{e}}$	$0.07 \pm 0.4^{\text{d}}$	$0.09 \pm 0.2^{\text{c}}$	$76.83 \pm 0.7^{\text{c}}$
	Strain ST.6	$0.19 \pm 0.4^{\text{c}}$	$0.11 \pm 0.1^{\text{c}}$	$0.11 \pm 0.4^{\text{b}}$	$91.51 \pm 0.6^{\text{a}}$
	Strain ST.8	$0.17 \pm 0.3^{\text{cd}}$	$0.09 \pm 0.2^{\text{cd}}$	$0.10 \pm 0.3^{\text{bc}}$	$87.95 \pm 0.5^{\text{b}}$

The data represent the mean  $\pm$  standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ( $p < 0.05$ )

in a 3 to 5 times higher amount of proline than that of seedlings watered with water only. These results suggest two isolated endophytic bacteria, ST6 and ST.8, could enhance the proline synthesis in rice under normal and salt conditions.

### 3.5 EFFECT OF INOCULATED ENDOPHYTIC BACTERIA ON THE ACTIVITIES OF ANTI-OXIDANT ENZYME IN RICE SEEDLINGS EXPOSED TO SALINE CONDITIONS

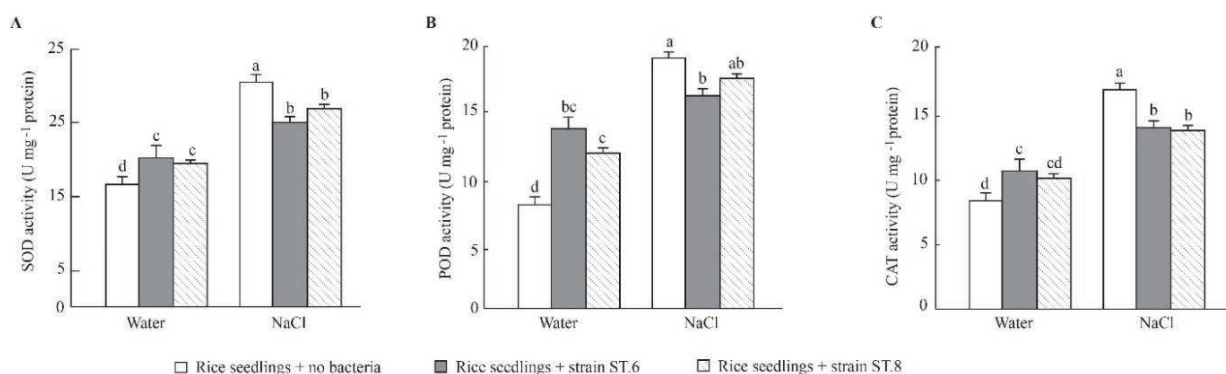
As can be seen from Figure 2, the salt treatment caused significant changes in the activities of antioxidant enzymes in rice seedlings. Three tested enzymes (SOD, POD, and CAT) showed a rapid increase in activities under saline conditions and their activities were significantly higher than that in rice grown under normal conditions. The seedlings inoculated with strain ST.6 had higher activities of those enzymes than the ones treated with strain ST.8 under normal conditions, but under salt stress conditions, the opposite results were observed (Figure 2).

Moreover, the results also indicated a significant decrease in SOD, POD, and CAT activities in the rice seedling treated with either strain ST.6 or strain ST.8 compared to the control under salt conditions. These data suggest the endophytic bacteria strain ST.6 and ST.8 isolated from rice roots play roles in alleviating oxidative stress.

It was reported that plant under salt stress produces many changes in physiological and biochemical properties such as a decrease in photosynthetic efficiency, osmotic imbalance, ion toxicity, and generation of ROS (Otlewska et al., 2020). In this study, the increase of chlorophyll and carotenoids amount in seedlings treated with a salt solution was observed (Table 4), meaning the pho-

tosynthetic efficiency of seedlings was improved. There were several studies that reported the chlorophyll content of rice seedlings inoculated with PGPB such as *Pantoea alhagi* NX-11 (Sun et al., 2020) and *P. ananatis* D1 (Lu et al., 2020) significantly increased (42.5 and 45 %, respectively) in comparison with control seedlings. In addition, the result of this study also demonstrated that seedlings inoculated with endophytic bacteria also significantly increased the proline content in plants compared to the control. These results were in agreement with the report of Sun et al. (2020), in which rice seedlings inoculated *Pantoea alhagi* NX-11 presented an improved growth after 7 days under salt stress compared to the control with a 37.5 % lower malondialdehyde content, a 133 % higher  $K^+/Na^+$  ratio, and a 52.8 % higher proline content. Later, Lu et al. (2020) reported that a significant accumulation of proline ( $98.25 \mu\text{g g}^{-1}$  of fresh mass) in rice seedlings inoculated with *Pantoea ananatis* D1 compared to the uninoculated seedlings grown under saline conditions was observed. These could be proline played as an osmoprotectant to stabilize proteins and cell membranes of plant cells when plants were grown under salt stress (Radhakrishnan and Baek, 2017). Hence, the accumulation of proline by plants aided the balance of the osmotic pressure under salt stress. Further, the generated proline could serve as nitrogen and carbon sources for plants freed from salt stress and could play roles in detoxing the freed radicals from plant cells (Mohammadkhani and Heidari, 2008). Therefore, inoculation of strain ST.6 or ST.8 could promote rice seedlings to adapt to the rapid change of osmotic pressure caused by salt stress through proline accumulation.

In addition, it was documented that plants could alleviate the harmful effects caused by salinization through the production of antioxidant enzymes (Radhakrishnan and Baek, 2017; Lu et al., 2020). The results of this study showed that the seedlings cultivated under salt condi-



**Figure 2:** Effect of strain ST.6 and ST.8 inoculation on antioxidant enzyme activities in rice under normal (water) and saline (NaCl) conditions. (A) SOD; (B) POD; (C) CAT. Different letters show statistically significant differences ( $p < 0.05$ )



tions produced a significant increase in the antioxidant enzymes (SOD, POD, and CAT) compared with plants grown in non-saline conditions. These results were supported by the report of Lu et al. (2020), who demonstrated that rice seedlings treated with NaCl resulted in an increase of 92.4 % in SOD activity, 97.2 % in CAT activity, and 139.2 % in POD activity when compared with the water group. Especially, this study also exhibited a reduction in antioxidant enzyme activities observed in seedlings inoculated with both endophytic bacteria strain ST.6 and ST.8 under salt stress, especially, since strain ST.6 presented a better effect than strain ST.8 did. These results were consistent with reports of Sarkar et al. (2018) and Lu et al. (2020), in which salt-stressed plants also presented a decrease in the activity of antioxidant enzymes when they were inoculated with PGPB. Hence, the results suggest both strains, ST.6 and ST.8 could help rice tolerant with salinity.

### 3.6 DETERMINATION OF THE INOCULATED ENDOPHYTIC BACTERIA IN THE RICE SEEDLINGS

It was reported that plant-bacteria synergism play important role in improving the salt tolerance of plants. In this study, higher numbers of inoculated endophytes were observed in the root, and shoot of the inoculated rice seedlings than in the non-inoculated ones under normal or salt stress conditions (Table 5).

In addition, the screening experiment also showed significantly higher numbers of inoculated bacteria in the root interior than that in the shoot interior. Interestingly, the results also showed a relatively higher number of inoculated bacteria in the plant sample collected from salt treatment than that in the water treatment.

It was reported that the plants produced an optimal

microenvironment surrounding their root for the growth of the inoculated and indigenous microbes, subsequently increasing the number of inoculated endophytes (Tara et al., 2019). Our data showed an agreement with this report. Moreover, higher numbers of inoculated bacteria in the root interior than in the shoot interior. This might be because the inoculated bacteria were isolated from rice roots, hence they preferred locating in the root. Especially, the inoculated bacteria in the rice seedling under normal conditions were lower than the ones in rice seedlings grown under salt stress. This might be because the salt conditions could generate a benefit for the development of inoculated bacteria in the root rhizosphere and for their root colonization.

## 4 CONCLUSIONS

Endophytic bacteria have been exploited as bioinoculant to improve plant growth under abiotic and biotic stress. Two salt-tolerant endophytic bacteria, ST.6 and ST.8, produce the highest value of germination rate (99.33 and 99.67 %, respectively) and were close to *Pantoea dispersa* (accession number MT275631.1) and *Burkholderia cenocepacia* (accession number CP034545.1), respectively. They were capable of dissolving P insoluble, producing IAA, exhibiting ACC deaminase activities, and only ST.6 could produce siderophores. Inoculation of rice seedlings with strains ST.6 and ST.8 promoted rice growth under both normal and salt-stressed conditions. Moreover, ST.6 and ST.8 could have roles in reducing the salt stress on rice plants by penetrating into the plant and then inducing chlorophyll and proline accumulation, decreasing antioxidation activity, and maintaining ionic balance. The results suggest strains ST.6 and ST.8 could be used to develop the biofertilizer for sustainable agriculture in the future.

**Table 5:** Identification of the inoculated endophytic bacteria in the root interior, and shoot interior of rice seedlings under salt stress

Watering solution	Treatment	Root sample ( $\times 10^3$ CFU $g^{-1}$ )	Shoot sample ( $\times 10^3$ CFU $g^{-1}$ )
Water	No bacteria	$0.82 \pm 0.3^a$	$0.21 \pm 0.6^a$
	Strain ST.6	$10.21 \pm 0.4^a$	$6.4 \pm 1.5^a$
	Strain ST.8	$9.25 \pm 0.9^{bc}$	$5.3 \pm 1.1^a$
150 mM NaCl	No bacteria	$1.02 \pm 0.7^a$	$0.31 \pm 0.7^a$
	Strain ST.6	$10.64 \pm 0.7^b$	$6.8 \pm 1.3^{bc}$
	Strain ST.8	$9.78 \pm 0.4^c$	$6.1 \pm 0.7^c$

The data represent the mean  $\pm$  standard error (SE) based on three replicates. Values in the same column with the same letter(s) are not significantly different as determined by Duncan's test ( $p < 0.05$ )

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