



Isolation and Selection of Plant Growth Promoting Endophytic Bacteria Associated with Healthy *Hevea brasiliensis* for Use as Plant Growth Promoters in Rubber Seedlings under Salinity Stress

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ABSTRACT

Endophytic bacteria are defined as microorganisms that live in plant tissues for the whole or part of their lives without causing adverse effect on the host plant. Bacterial endophytes have been reported as plant growth promoters in several kinds of plants under normal and stress conditions. The aims of this study were to isolate plant growth-promoting endophytic bacteria from healthy Para rubber trees (*Hevea brasiliensis*) and investigate their effects on the growth of Para rubber seedlings grown under salinity stress. Among the 415 endophytic isolates obtained, isolates AP6A3 and ER212 were chosen for *in vivo* study under greenhouse conditions. Isolate AP6A3 produced a phytohormone indole acetic acid (IAA) at 58.39 µg/ml and ACC deaminase; it was able to fix nitrogen, having a nitrogenase activity at 64.14 µmol C₂H₄/tube/h determined using acetylene reduction assay but could not solubilize inorganic phosphate. Isolate ER212 strongly solubilized tricalcium phosphate and released soluble phosphate at 197.01 µg/ml; it produced only trace amounts of IAA, was absent of ACC deaminase and did not fix nitrogen. Isolates AP6A3 and ER212 tolerated salinity well, at least 7% and 10% NaCl, respectively. Characterization of these two isolates by conventional methods displayed that isolates AP6A3 and ER212 belonged to the genus *Enterobacter* and *Bacillus*, respectively. These two endophytes were then employed in greenhouse experiments with Para rubber seedlings grown under salinity stress (500 mM NaCl). Interestingly, both endophytic isolates significantly enhanced the growth (based on shoot height, stem diameter, root length, fresh and dry weight) of Para rubber seedlings when compared to the un-inoculated seedlings. These results demonstrated that the endophytic *Enterobacter* sp. strain AP6A3 and *Bacillus* sp. strain ER212 had a potential application as bio-fertilizer for plants grown in saline environments.

Keywords: endophyte, plant growth, Para rubber, seedling, salt stress

1. INTRODUCTION

Thailand is one of the world's top producers of natural rubber (*Hevea brasiliensis*), exporting over 4.8 million metric tons in the year 2018 [1]. Traditionally, rubber plantations has been predominately in the southern part of Thailand, but recently cultivation has been extended to the northeast region. However, it has been estimated that around 20% of land in northeast Thailand is affected by saline soils [2], and soil salinity is a critical agricultural problem, causing lower rates of seed germination, plant growth and crop yield [3]. Indeed, this problem significantly affects plant cultivation on a global scale. Plants employ some mechanisms to overcome salinity stress such as accumulation of compatible solutes, synthesizing membrane transporters and antioxidant enzymes, producing secondary metabolites and phytohormones [4].

Plant growth-promoting bacteria including bacterial endophytes have been reported to play important roles in alleviating salinity stress in plants [5]. Plant growth-promoting bacterial endophytes (PGPE) colonize plant tissues and confer beneficial effects on plant growth [6]. Plant growth promotion by several mechanisms have been reported, including phosphate solubilization activity, production of phytohormones, nitrogen fixation and expression of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity [7]. Endophytes can colonize plants by entering through cuttings or other plant parts including seeds. Rhizospheric bacteria can also penetrate and colonize within root tissues from which they can move to other parts of the plant, assuming endophytic behavior [6].

There have been reports on PGPE playing important roles in growth stimulation for various plants e.g. rice, currant tomato, chickpea, rape and many more [4, 8-10]. However, there has been no report yet on PGPE stimulating growth in rubber plants. Therefore, the objectives of this study were to (i) isolate endophytic bacteria from healthy rubber plants, (ii) screen them for

their plant growth-promoting properties and stress tolerance, and (iii) evaluate their effects on stimulating growth of rubber seedlings under salt stress.

2. MATERIALS AND METHODS

2.1 Sampling and Isolation of Endophytic Bacteria

Plant tissues of healthy Para rubber trees were collected from various locations in Northeastern Thailand i.e. Khon Kean, Chaiyaphum, Roi Et, Sakon Nakhon, and Nong Khai provinces. Roots, leaves, petioles and flowers of healthy rubber trees were cut, packed in polythene zip-lock bags, and transported to the laboratory within 24 h. Plant samples were surface-sterilized using the methods described by Qin *et al.* [11]. The sterility check carried out by plating the last washed water confirmed that bacteria obtained on the plates were endophytes. Isolation of endophytic bacteria from the surface-sterilized rubber tissues was performed employing the methods described by Araújo *et al.* [12] with slight modifications. The surface sterilized sample was aseptically cut into small pieces (5.0 x 5.0 mm), placed onto Trypticase Soy Agar (TSA) plates containing benomyl (50 µg/ml) to inhibit fungal growth. Samples were incubated at 28°C for 7 days. Meanwhile, the surface sterilized sample was aseptically crushed using a mortar and pestle and serially diluted in phosphate-buffered saline (8 g/l NaCl, 0.2 g/l KCl, 1.4 g/l Na₂HPO₄, 0.24 g/l KH₂PO₄) prior to plating on TSA containing benomyl followed by 7 days of incubation at 28°C. Bacterial colonies emerging from plant tissues or appearing on TSA plates were purified by cross-streaking and considered to be endophytic bacteria. The pure isolates were maintained on Nutrient agar (NA) slant at 4°C for further experiments.

2.2 In Vitro Screening of Endophytic Bacteria for Their Growth-Promoting Activities

2.2.1 Screening for IAA production

The ability of bacterial endophytes to produce IAA was measured using the colorimetric method

described by Glickmann and Dessaux [13] with some modifications. Bacterial isolates were grown in Trypticase Soy Broth (TSB) supplemented with tryptophan (0.2 mg/ml) and incubated at 28°C for 48 h. Broth culture was centrifuged (10,000 x g, 5 min) to remove cell pellets. One milliliter of culture supernatant was mixed with 1 ml of Salkowski's reagent (12 g/l FeCl₃, 7.9 M H₂SO₄) and kept at room temperature in the dark for 30 min before absorbance was measured at 530 nm. IAA concentration in culture supernatant was determined by comparison with a standard curve of pure IAA (Sigma-Aldrich).

2.2.2 Screening for nitrogen-fixing ability

Qualitative evaluation of nitrogen-fixing ability was carried out by growing bacterial isolates on nitrogen-free agar plates (Burk's and Ashby's media) at 28°C for 7 days. Isolates that developed colonies on agar plates were selected as nitrogen fixers for confirmation by acetylene reduction assay (ARA) according to the methods described by Hardy *et al.* [14] Bacterial isolates were grown in 10-ml airtight vials containing 5 ml Burk's medium at 28°C for 3 days. Then, 10% (v/v) of air in the headspace was replaced with acetylene through a syringe and the cultures were further incubated for 3 h. Ethylene in the headspace was analyzed by gas chromatography (GC) using a Shimadzu GC14A gas chromatograph (Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID). Injector and detector were set at 110 °C and 120 °C, respectively. The Porapak N column used (i.d. 80–100 mesh, 6ft x 1/8") was set at 65°C and helium was used as a carrier gas with 40 ml/min flow rate. All experiments were conducted in triplicate together with a control (without bacterial inoculation). The mean value of N₂ fixation was calculated and expressed as $\mu\text{moles C}_2\text{H}_4/\text{tube/h}$.

2.2.3 Screening for phosphate solubilizing ability

Bacterial isolates were point-inoculated on

Pikovskaya agar containing 0.5% (w/v) tricalcium phosphate (TCP) and incubated at 28°C for 7 days. The clear haloes around colonies were measured and the solubilization index (SI) calculated according to the equation suggested by Premono *et al.* [15] as follows:

$$\text{SI} = \text{diameter of halo zone} / \text{diameter of colony}$$

Phosphate solubilization was further confirmed by analyzing the soluble phosphate content in the culture supernatant using the Fiske and Subbarow method [16]. Bacterial isolates were grown in the National Botanical Research Institute's phosphate (NBRIP) broth and incubated at 28°C for 10 days. The culture supernatant was obtained by centrifugation at 8,000 rpm for 10 min. An aliquot of 500 μl culture supernatant was mixed with 500 μl of 10% (w/v) trichloroacetic acid in a test tube to which 4 ml of color developing reagent (3 M H₂SO₄ : 2.5% (w/v) ammonium molybdate : 10% (w/v) ascorbic acid : distilled water; 1:1:1:2) was added, and the solution was left at room temperature for 15 min before the absorbance of the developing blue color was measured at 820 nm. The concentration of soluble phosphate was obtained using the standard curve of KH₂PO₄ (Sigma, USA). Sterile NBRIP medium was employed as a control. All experiments were performed in triplicate.

2.3 Characterization of the Selected Endophytic Bacteria

Two bacterial endophytes i.e. isolates AP6A3 and ER212 displaying the best properties of IAA production, nitrogen fixation and phosphorus solubilization were further characterized. Taxonomic studies of the isolates were determined as described in Bergey's Manual of Systematic Bacteriology [17-18].

2.4 Evaluation of 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Activity

Bacterial isolates, AP6A3 and ER212, were

determined for ACC deaminase production according to the methods of Ali *et al.* [19] with slight modification. Both isolates were preliminarily grown in 5 ml of TSB medium at 28 °C for 24 h, centrifuged (8,000 x g, 10 min), washed twice with 0.1 M Tris–HCl (pH 7.5) and resuspended in 1 ml of 0.1 M Tris–HCl (pH 7.5). Bacterial suspension was then spot-inoculated on agar plates containing a modified DF minimal salts medium [glucose, 2.0 g; gluconic acid, 2.0 g; citric acid, 2.0 g; KH₂PO₄, 4.0 g; Na₂HPO₄, 6.0 g; MgSO₄·7H₂O, 0.2 g; micro-nutrient solution (CaCl₂, 200 mg; FeSO₄·7H₂O, 200 mg; H₃BO₃, 15 mg; ZnSO₄·7H₂O, 20 mg; Na₂MoO₄, 10 mg; KI, 10 mg; NaBr, 10 mg; MnCl₂, 10 mg; COCl₂, 5 mg; CuCl₂, 5 mg; AlCl₃, 2 mg; NiSO₄, 2 mg; distilled water, 1,000 ml), 10 ml; distilled water, 990 ml] supplemented with 3 mM ACC as a nitrogen source.

Plates containing only DF minimal salts medium without ACC were used as the negative control and those with 0.2 % (w/v) (NH₄)₂SO₄ were used as the positive control. ACC deaminase activity was determined qualitatively based on bacterial ability to use ACC as a sole nitrogen source for growth. Next, the agar plates were incubated at 28°C for 3 days. The isolates were considered positive for ACC deaminase production if they grew in the medium with ACC but not in the medium without ACC.

2.5 Stress Tolerance of Endophytic Bacteria

2.5.1 Salt tolerance

Salt tolerance test was carried out at different NaCl concentrations (0, 2, 5, 6, 7, 8 and 10% w/v) in NB. The overnight-grown cultures were inoculated in the respective liquid media and incubated at 28°C for 28 h. Bacterial growth was determined every 2 h by measuring optical density at 600 nm.

2.5.2 Drought tolerance

Evaluation of bacterial tolerance to drought was performed in NB containing various concentrations (0, 2, 4 and 6%) of polyethylene glycol (PEG)

6000 representing an osmotic potential of 0, -0.29, -0.58 and -0.88 MPa, respectively [19]. The overnight-grown cultures were inoculated in the respective liquid media and incubated at 28°C for 24 h. Bacterial growth was determined by measuring optical density at 600 nm (OD₆₀₀) comparing with the OD₆₀₀ in the medium without PEG.

2.6 Effects of Endophytic Bacteria on Para Rubber Seedlings under Greenhouse Conditions

Rubber seeds clone RRIM 600 were surface-sterilized with 5% NaOCl for 10 min and rinsed thoroughly with sterile distilled water. Rubber seeds were then spread over the germination beds in a single layer, with the ventral part of the seeds placed downward and press gently into moist coconut husk. The seeds, covered with a thin layer of coconut husk to prevent excessive moisture loss, were kept for germination for 7 days. Then, the germinated seeds were transferred to polythene bags (12.7 × 8.89 cm) containing 0.5 kg of peat. At the same time, 10 ml aliquot of an overnight-grown isolate AP6A3 (10⁸ CFU/ml) was inoculated into the bag once and later once a week for 3 weeks; it was assigned as T1. Similarly, 10 ml aliquot of an overnight-grown isolate ER212 was added to another set of rubber seedling and assigned as T2. Un-inoculated seedlings were used as the controls. All seedlings were watered twice a day with tap water. The ability of endophytic isolates to enhance rubber seedlings growth under salt stress was evaluated by irrigating seedlings T1 and T2 at 44 days old with a 500 mM NaCl solution (50 ml) once every 4 days, 4 times. For the un-inoculated controls, one set of the control seedlings (C1; negative control) received 50 ml of 500 mM NaCl solution once every 4 days, 4 times; another set of the control seedlings (C2; positive control) received 50 ml of distilled water. The experimental plots were arranged in a randomized block design with three independent replications per treatment and each replication containing ten seedlings. At the of greenhouse experiments, growth was determined

for all seedlings by measuring shoot height, stem diameter, root length, fresh weight and dry weight

2.7 Statistical Analysis

Data were analyzed with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was employed for means comparison using Statistical Analysis System (SAS).

3. RESULTS AND DISCUSSION

3.1 Isolation of Endophytic Bacteria from Para Rubber Trees

A total of 415 morphologically different endophytic bacteria were isolated from the interior tissues of healthy rubber plants including 244, 71, 95 and 5 isolates obtained from roots, leaves, petioles and flowers, respectively (Table 1). The highest diversity of endophytic bacteria was found in roots and the bacterial densities are usually decreased progressively from the roots to the flowers [20] as observed in the present study. Similar results were reported in *Echinacea purpurea*, *Echinacea angustifolia* [21] and *Lavandula dentata* [22]. This can be explained by the exudates secreted from plant roots behave as nutrients for soil microorganisms and attract those in root vicinity – resulting in more microbes entering and living in plant roots [7].

3.2 Plant Growth-Promoting Properties of the Isolates

The abilities of the isolates to produce IAA and ACC deaminase, solubilize inorganic phosphate and fix nitrogen are shown in Table 2. Of 415 isolates tested, 335 isolates (80.7%) produced IAA in the range of 0.52 ± 0.1 to 68.45 ± 0.3 $\mu\text{g/ml}$. The top three isolates that produced high IAA were AL6A1, AP6A3, and AP611 resulting in 68.45 ± 0.3 , 58.39 ± 0.7 and 49.23 ± 0.5 $\mu\text{g IAA/ml}$, respectively. For nitrogen fixing ability, 220 isolates were able to grow on nitrogen-free medium producing colonies with diameters ranging from 2 to 20 mm. Twenty-seven large colonies exhibited nitrogenase enzyme activity by acetylene reduction assay ranging from 53.15 to 74.24 $\mu\text{mol C}_2\text{H}_4/\text{tube/h}$, in which isolate BR4A2 displayed the highest activity.

Seventeen isolates were found capable of producing a clear zone on an inorganic phosphate-containing agar medium. The phosphate solubilization index (SI) of the isolates varied from 1.1 to 2.0 (Table 2). Six isolates with high SI were further investigated for the soluble phosphate content released in the culture supernatant. It was found that isolate ER212 had released the highest amount of soluble phosphate (197.01 $\mu\text{g/ml}$) at 10 days of incubation which was concomitant with the

Table 1. Sources and locations of isolated bacterial endophytes obtained from the interior tissues of healthy rubber trees.

Province	Number of endophytic bacterial isolates				Total
	Root	Leaf	Petiole	Flower	
Khon Kean	35	31	23	-	89
Chaiyaphum	56	-	-	-	56
Roi Et	23	17	21	-	61
Sakon Nakhon	45	3	8	-	56
Nong Khai	85	20	43	5	153
Total isolates	244	71	95	5	415

Table 2. Evaluation of PGP characteristics: IAA production, phosphate solubilization, Nitrogen fixation and ACC deaminase in endophytic bacterial isolates associated with rubber trees grown in the northeastern Thailand.

Isolate from plant tissue	IAA production (µg/ml)	Phosphate solubilization		Nitrogen fixation (µmolC ₂ H ₄ /tube/hr)	ACC deaminase
		SI	(µg/ml)		
AL111 (leaf)	1.44±0.7	-	ND	65.56±4.7	ND
AL512 (leaf)	2.00±0.1	-	ND	69.09±1.0	ND
AL6A1 (leaf)	68.45±0.3	-	ND	ND	ND
AP6A3(petiole)	58.39±0.7	-	ND	64.14±2.8	+
AP611(petiole)	49.23±0.5	-	ND	62.89±4.0	ND
BR3A1(root)	1.37±0.1	-	ND	69.89±2.9	ND
BR4A2(root)	2.21±0.1	-	ND	74.24±3.5	ND
BR4A4(root)	-	-	ND	64.40±7.5	ND
EL111(leaf)	2.26±0.4	1.25±0.04	189.58±6.1	ND	ND
EL1A11(leaf)	2.66±0.1	1.20±0.03	132.54±5.9	ND	ND
EL1A12(leaf)	2.60±0.1	1.25±0.04	166.67±6.0	ND	ND
EP122(petiole)	0.81±0.5	2.00±0.07	111.95±3.3	57.31±3.1	ND
EP3B3(petiole)	0.89±0.1	1.67±0.09	113.26±1.1	ND	ND
ER212(root)	2.00±0.8	1.33±0.04	197.01±1.3	ND	-
FP1A2(petiole)	45.35±0.3	-	ND	ND	ND
FR214(root)	33.74±0.2	-	ND	ND	ND
FR421(root)	48.77±0.1	-	ND	63.68±9.6	ND

“ - ”, absence; “ + ”, presence; ND, not determined

Values are presented as the mean of triplicates ± standard deviation (SD).

decrease of culture broth pH from 6.3 to 4.7 (data not shown). In this study, all 6 phosphate solubilizing isolates could solubilize calcium phosphate contained in both liquid broth and agar medium (Table 2). Similarly, consistent phosphate solubilization by phosphate solubilizing bacteria in both agar and broth assay were observed earlier [23].

Plant growth-promoting bacteria are of great interest in enhancing growth of plants when grown under either normal or stress conditions. There have been studies reporting endophytic bacteria possess plant growth promoting traits i.e. ability

to produce IAA and ACC deaminase, fix nitrogen and solubilize inorganic phosphate. Some of those bacterial endophytes were *Bacillus subtilis* LK14 [24], *Enterobacter* sp. K3-2 [25], *Lysinibacillus xylanilyticus* DSM23493, *Brevibacterium sediminis* FXJ8269 [26], *Bacillus cereus*, *Pseudomonas aureginosa*, *Brachybacterium paraconglomeratum*, and *Providencia vermicola* [27]. In this study, two endophytic bacteria were chosen for further study. The isolate AP6A3 was chosen based on its IAA production and nitrogen fixation and isolate ER212 was chosen based on its ability to solubilize inorganic phosphates.

3.3 Characterization of the Selected Endophytic Bacteria

The isolate AP6A3 was a Gram-negative, motile rod. This bacterium was tested positive for catalase, Voges-Proskauer and citrate utilization. It did not produce oxidase, indole, H₂S, phenylalanine deaminase, gelatinase or deoxyribonuclease and gave negative result on methyl red test. Characterization of isolate AP6A3 revealed that it most likely belonged to the genus *Enterobacter* [17]. The isolate ER212 was a gram-positive, endospore-forming rod, oxidase and catalase positive. These properties indicated that it belonged to the genus *Bacillus* [18].

3.4 ACC Deaminase Activity of *Enterobacter* sp. AP6A3 and *Bacillus* sp. ER212

The selected bacterial endophytes were examined qualitatively for ACC deaminase production on agar plates. Only *Enterobacter* AP6A3 successfully grew in DF salt minimal medium containing ACC as the sole carbon source, indicating its ability to produce ACC deaminase enzyme. This deaminase helps to decrease the stress-induced ethylene produced in plants under stress conditions. The beneficial effect of growth-promoting ACC deaminase-producing bacteria in salt tolerance has been well documented in several reports [19, 28].

3.5 Stress Tolerance of *Enterobacter* AP6A3 and *Bacillus* ER212

Salt tolerance of both bacterial endophytes was evaluated in NB containing various concentrations of NaCl. As shown in Figure. 1, it was found that *Enterobacter* AP6A3 could tolerate up to 7% NaCl (Figure 1A) whereas *Bacillus* ER212 could grow in NB with 10% NaCl. The bacterial endophytes isolated from rubber plant petioles and roots were not expected to be halophilic because they were not taken from salty soil. Interestingly, the results of this study revealed that both chosen endophytes tolerated saline condition well similar to other identified halophilic strains did [29]. In contrast, evaluation of drought tolerance of both bacterial

strains in NB containing various concentrations of PEG 6000 revealed that they did not tolerate drought (data not shown).

3.6 Effects of *Enterobacter* AP6A3 and *Bacillus* ER212 on the Growth of Para Rubber Seedlings under Salt Stress

To evaluate the growth promoting ability of the selected bacterial endophytes on Para rubber seedlings grown under salt stress, seedlings were inoculated with *Enterobacter* AP6A3 and *Bacillus* ER212, exposed to salt stress (500 mM) and grown under greenhouse conditions; results are shown in Table 3. When comparing seedlings which received bacterial endophytes grown under salt stress, *Enterobacter* AP6A3 (T1) showed significantly ($p < 0.05$) greater beneficial effects than *Bacillus* ER212 (T2) on seedling growth based on shoot height and stem diameter but similar effects on root length, seedling fresh and dry weight. Interestingly, seedlings receiving either *Enterobacter* AP6A3 (T1) or *Bacillus* ER212 (T2) had significantly ($p < 0.05$) higher growth in all parameters (stem height, stem diameter, root length, seedling fresh and dry weight) than seedlings without bacterial addition (C1). These results clearly confirm the plant growth-promoting properties of both endophytic strains. Furthermore, there were no significant differences in stem height and root length between seedlings in T1 and C2 which indicated that *Enterobacter* AP6A3 alleviated the salinity stress that the Para rubber seedlings encountered.

The results in this study are similar to those previously reported on a significant improvement of plant growth due to inoculation with plant growth-promoting endophytic bacteria. Yaish *et al.* [28] reported that *Paenibacillus xylanexedens* PD-R6 and *Enterobacter cloacae* PD-P6 enhanced the root elongation of canola grown under normal and saline conditions. Abd_Allah *et al.* [3] described the beneficial effect of *B. subtilis* BERA 71 on significant increase of shoot dry weight of chickpea grown under saline conditions. This findings are similar to those of the study conducted by

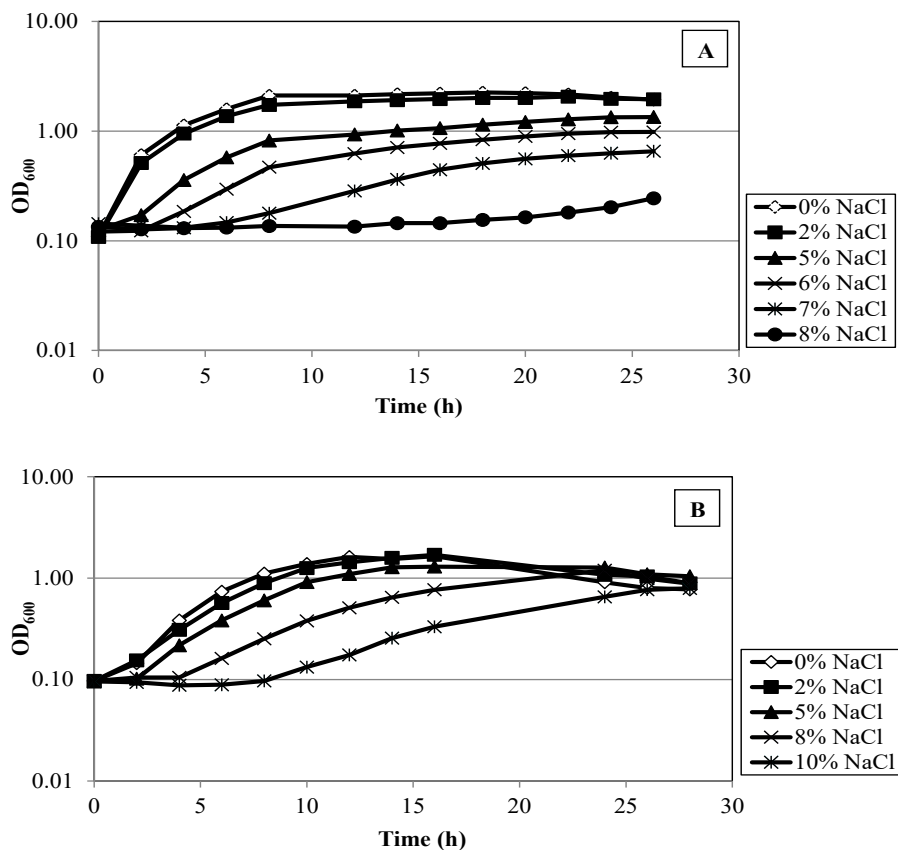


Figure 1. Growth of bacterial endophyte AP6A3 (A) and ER212 (B) in NB containing various NaCl concentrations.

Table 3. Effect of endophytic bacterial application on the growth of Para rubber seedlings after 44 days.

Treatment	S.H. (cm)	S.D. (mm)	R.L. (cm)	F.W. (g)	D.W. (g)
T1: AP6A3+NaCl	47.4±0.51 ^a	3.8±0.07 ^a	17.8±0.37 ^{ab}	6.5489±1.03 ^b	1.3322±0.13 ^b
T2: ER212+NaCl	41.0±0.33 ^b	3.5±0.07 ^b	17.3±0.29 ^b	7.2311±0.05 ^b	1.3733±0.34 ^b
C1: Negative control	32.5±0.34 ^c	3.2±0.19 ^c	12.2±0.30 ^c	4.2211±0.08 ^d	0.9178±0.07 ^c
C2: Positive control	48.2±0.19 ^a	3.6±0.04 ^b	18.1±0.88 ^a	9.1067±0.07 ^a	2.2611±0.05 ^a

S.H. = Shoot height, S.D. = Stem diameter, R.L. = Root length, F.W. = Fresh weight and D.W. = dry weight

Abbreviations for each experimental group are as follows: T1: AP6A3+NaCl, seedlings grown under salt stress and received the isolate AP6A3; T2: ER212+NaCl, seedlings grown under salt stress and received the isolate ER212; C1: Negative control, seedlings grown under salt stress without bacterial inoculation; C2: Positive control, seedlings grown under normal condition without bacterial inoculation. Values in each column with different superscripts are significantly different ($p < 0.05$).

Soldan *et al.* [30] that *Gordonia terrae* KMP456-M40 increased the root length of mangrove seedlings and the biomass of salt-stressed rice under axenic conditions.

The present study demonstrated for the first time that endophytic bacteria alleviated salt stress in rubber plants when grown under salinity conditions.

4. CONCLUSIONS

This study collected 415 endophytic bacteria isolated from roots, leaves, petioles, and flowers of healthy Para rubber trees in Northeastern Thailand and examined their plant growth-promoting properties. Overall, 80.7% of the isolates were IAA producers, 53% nitrogen fixers and 4.1% phosphate solubilizers. The *Enterobacter* AP6A3 produced IAA, ACC deaminase enzyme, fixed nitrogen but did not solubilize inorganic phosphate, whereas *Bacillus* ER212 poorly produced IAA, strongly solubilized inorganic phosphate, but could neither produce ACC deaminase nor fix nitrogen. The *Enterobacter* AP6A3 and *Bacillus* ER212 tolerated saline conditions of at least 7% and 10% NaCl, respectively. Greenhouse experiments revealed that inoculation with *Enterobacter* AP6A3 or *Bacillus* ER212 alleviated salinity stress in rubber seedlings and demonstrated promising growth promoting potential in Para rubber plants. The evaluation of these plant growth-promoting endophytic bacteria under salinity stress in field conditions should be the next area of study.

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