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Halotolerant rhizobacterial community on the growth of tomato plants under salinity condition

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Abstract

Bio-fertilizer is a product used for improving soil quality. Microorganisms subsisting in bio-fertilizer have the ability to cycle nutrients in soils leading to enhanced soil fertility. Thus, the aim of this study was to investigate the ability of salt tolerant rhizobacteria to promote the growth of tomato plants in pot experiments under salinity conditions. Also, microbial communities within the cultivated soil samples were investigated. Three isolates of salt tolerant rhizobacteria including *Enterobacter aerogenes* P8, *Bacillus tequilensis* N15 and *Pseudomonas azotoformans* I2.1 were isolated from rhizosphere soil of rice plant and cultivated on soil supplemented with various salinity conditions. All isolates were able to live at the surface area of the tomato roots. Their abilities to promote tomato growth under different salinity conditions were investigated. The results showed that the inoculation of rhizobacteria which immobilized on carriers enhanced tomato plants biomass under two salinity levels (0%, 0.25% NaCl). Moreover, plants of both treatments showed an increase in total nitrogen, available phosphate and exchangeable potassium when compared to other treatments. According to the profile of rhizosphere bacteria community obtained using denaturing gradient gel electrophoresis (DGGE) analysis, eight species of bacteria including *Klebsiella aerogenes*, *Enterobacter* sp., *Caballeronia concitans* , *Raoultella planticola, Citrobacter freundii , Burkholderia thailandensis , Frateuria* sp., and *Fulvimonas* sp. were identified. This is the first report to show a member of *Fulvimonas* sp. in the tomato rhizosphere. These findings indicate that all of these bacteria might play a role in enhancing the growth of tomato plants.

Keywords: PGPR, Saline soil, Halotolerant rhizobacteria, Bio-fertilizer, Colonization

1. Introduction

Saline soil contains high soluble salts in soil solution rendering it unsuitable for plant growth.Thailand has large areas of saline soil causing serious problems to agricultural sectors. The salinity of soil can be determined by electrical conductivity (ECe) of ≥ 2 decisiemens/meter (dS/m), at 25 °C and the sodium adsorption ratio (SAR) of ≤ 13. Soil containing a high amount of salt ions affects the productivity of plants and the root area. It was found that when ECe was ≥ 4 dS/m or 40 mmol NaCl, the yield of many crops decreased [1]. Saltions commonly found in saline soils are chloride, sulfate, sodium, calcium and magnesium, while the most commonly-found form is sodium chloride ions (NaCl). High levels of sodium ions can affect soilstructure and cause the diffusion of soil particles. The pH of saline soil is lower than 8.5 and the elution of salinity from soil does not affect soil pH [2].

Salinity is a major problem for the growth of microorganisms residing in those environments. Salinity diminishes plant growth and productivity and inhibits enzymes' metabolic activity. Microorganisms that live in high salinity environments are called halophile and classified into four types: non halophile (tolerance to sodium chloride ≤ 0.2M); slight halophile (0.2–1.2M); moderate halophile (1.2–2.5M); and extreme halophile (≥ 2.5M).

To survive under stress conditions, microbes accumulate certain substances such as sugars, amino acids and amino acid derivatives in order to maintain osmotic pressure within cells [3].

Plant growth promoting rhizobacteria (PGPR) is a group of bacteria which reside around the rhizosphere zone of soil. These microorganisms exhibit symbiotic interaction with plants and play an important role in improving soil quality via plant growth promoting mechanisms.

The activity of these microorganisms affects the soil nutrient circulation such as enabling the availability of phosphorus and potassium. Some have the ability to fix nitrogen or produce phytohormones. For example, indole acetic acid (IAA) can aid root cell elongation resulting in the absorption of more nutrients under stress conditions [4]. An increase in the activity of these microorganisms is important to an ecosystem functioning. These activities can help plants to survive and live-in contaminated environments [5]. Moreover, soil enzymes produced by microorganisms could play an important role in organic matter decomposition and nutrient cycling.

Salinity has a negative effect on plant metabolisms, especially photosynthesis. In addition, salinity can change the plant metabolism process by inhibiting the accumulation of other nutrients andinfluencing the balance of sodium and potassium ions in high salinity conditions [6]. Plants are able to protect themselves from salinity by creating compatible solutes that help them preserve the structure and function of proteins, reduce the effect of salinity on enzymatic reactions, maintain the water balance, enhance the ability of the enzyme, and eliminate free radicals [7]. Compatible solutes contain both ions, proteins, sugars, and amino acids such as proline, ectoine. They can also protect and balance the osmotic pressure [8].

Tomatoes are an economic plant growing in tropical and lowland areas. Tomatoes are moderately tolerant to salinity. They can grow on soil that has electrical conductivity of 4-8 dS/m. Saline soil usually lacks the available nutrients for plant growth. Therefore, the use of bio-fertilizer, which is eco-friendly, can alleviate salt stress in saline soil resulting in improved soil quality. Bio-fertilizer containing microorganisms such as PGPR have benefits on the growth of plants. However, in order to apply the bio-fertilizer to saline soil, the survival of rhizobacterial cells must be maintained by incorporating supporting materials. Rice husk has been widely used a carrier material for bio-fertilizer because of its nutrient-rich nature as a food source for microorganisms, porous structure and its inexpensiveness. Therefore, rice husk was selected for immobilizing bacterial cells in this experiment. We focused on using microorganisms to promote the growth of tomato plants under salinity in pot conditions. Moreover, microbial community profiles in the rhizosphere of tomato plants that promote plant growth were investigated.

2. Materials and methods

2.1 PGPR traits

In this study, three strains of rhizobacteria including *Pseudomonas azotoformans* I2.1, *Enterobacter aerogenes* P8 and *Bacillus tequilensis* N15, were isolated from rice rhizosphere of saline soil in Khon Kaen, Thailand [9] and tested for PGPR properties. To determine nitrogen fixing activity, these microbes were inoculated on Ashby's agar without nitrogen source in the medium [10]. Bacteria were inoculated on National Botanical Research Institute's Phosphate growth medium (NBRIP) which was supplemented with tricalcium phosphate (inorganic phosphate source) [11] in order to investigate the phosphate solubilizing activity. IAA production was determined using the modified method of Mohite [12]. ACC deaminase activity was screened on Dworkin-Foster (DF) minimal salts medium supplemented ACC (1-aminocyclopropane-1-carboxylate) [13]. Tube method (TM) which is a qualitative assay for the detection of biofilm producing microorganisms was performed according to the method of Christensen et al. [14]. Screening of exopolysaccharide (EPS) was tested on a basal medium following the method of [15].

2.2 Detection of root colonization of microorganisms in tomato roots with SEM

Three isolates of the PGP rhizobacteria, *Enterobacter aerogenes* P8, *Bacillus tequilensis* N15 and *Pseudomonas azotoformans* I2.1., were each inoculated on nutrient broth and incubated at 30 °C for 24 h. Then, the bacterial cultures were centrifuged at 8000 rpm for 15 min and the cell pellet was washed with 0.85% NaCl. The cell pellets were re-suspended and the concentration adjusted to 10^8cfu/mL. After that, three stains of PGPR were mixed at a ratio of 1:1:1 (v/v/v). Hoagland's solution was prepared by varying the concentration of NaCl into three salinity levels of0, 2, 4 % (w/v) NaCl. Tomato plants were cultivated on Hoagland's solution. Then, 1 mL of the mixed inoculums was added to the medium before incubation for 15-20 days. The plant was sampled and separated into shoot part and root part. The root was fixed with 2.5% (v/v) glutaraldehyde for 2 h at 28 °C. Then, the root was washed three times with phosphate buffered saline (PBS, pH 7.2), and dehydrated with 50-100% gradient ethanol for 15 min. The samples were dried by using a critical point drying (CPD), Polaron Range, model CPD 7501. After that, the samples were mounted on aluminum stubs with double-coated carbon

conductive tape and sputtered with gold (Sputter coater machine of Cressingt on, model 108auto). Observation of root colonization was performed under the scanning electron microscope (SEM) (LEO, model 1450VP, England) with BE VPSE detector.

2.3 Effect of different salinity levels and halotolerant rhizobacteria on the growth of tomato plants

2.3.1 Bio-fertilizer

The inoculums of 3 isolates of rhizobacteria including *Enterobacter aerogenes* P8, *Bacillus tequilensis* N15 and *Pseudomonas azotoformans* I2.1 were prepared in the mixed inoculums at a ratio of 1:1:1 (v/v/v). Rice husk ash was used as a carrier and sterilized by autoclaving for 1 h at least 2-3 times. Sterility was double-checked after autoclaving by spread plate technique. Bio-fertilizer was produced by using 10% (v/w) of carrier inoculated with microorganisms and incubated at 30 °C for15 days.

2.3.2 Pot experiment tomato

Seedlings were cultivated on 104-well pots for2 weeks and then seedlings were transferred to a plastic pot. 1.5 kg of air-dried soil was added to the pot. Before transferring the seedlings, 5 g of dried bio-fertilizer carrier immobilized with microorganisms was added to the bottom of the pot beneath the seedlings. In the treatment with the liquid bio-fertilizer in the form of bacterial suspension, 5 mL of bacterial suspension at a concentration of 10^{8}cfu/mL was added into the pot after 3 days of seedling transplantation. This experiment was set up by using Randomized Complete Block Design (RCBD). There were 12 different types of treatments as follows: Control; without bacteria or carrier, B; bacterial suspension, BE; carriers immobilized with bacteria (bio-fertilizer), and C; carrier without bacteria, each of which was treated with 3 different concentrations of NaCl solutions; 0%, 0.25% and 0.5%. 150 mL of NaCl solution was added into the pot once a week before planting and then reapplied every week. Tomato plants were harvested after 40 days of cultivation to analyze plant growth parameters such as shoot length, root length, biomass, chlorophyll content and proline content. Also, the rhizosphere soil samples were collected and analyzed for soil enzymes, plant nutrients and microbial communities.

2.3.3 Growth parameters measurement and proline content detection

The shoots and root of tomato plants were separated to measure their lengths, and dried in a hot air oven at 80 °C. Biomass was also measured from the combination of shoot and root dry weight.

Moreover, tomato leaves were cut into small pieces having 0.05 g of fresh weight in total. 5 mL of 80% acetone was added into the test tube. Then, the sample was incubated in the dark at room temperature for 2 days and the chlorophyll content was determined by spectrophotometer at a wavelength of 645 and 663 nm. Total chlorophyll content was calculated following the method of Arnon, 1949 [16].Afterward, 0.5 g of fresh tomato plant was crushed and mixed with 10 mL of 3% sulfosalicylic acid and then filtered through filter paper Whatman No.1. Two mL of extracted sample was reacted with 2 mL of acid ninhydrin (1.25 g of ninhydrin dissolved in 30 mL of glacial acetic acid mixed with 20 mL of 6M phosphoric acid) and 2 mL of glacial acid (1:1:1). Subsequently, the sample was boiled at 100 °C for 1 h before being moved to an ice bath. Then, 4 mL of toluene was added and the mixture was allowed to stand at room temperature until the solvent layer was separated. Finally, the proline content was measured by spectrophotometer at a wave length of 520 nm and compared with proline standard [17].

2.3.4 Soil enzyme activity (Fluorescein diacetate hydrolysis analysis, FDA)

Total enzyme activity was determined by FDA. One gram of soil sample was mixed with 7.5 mL of 60 mmol sodium phosphate buffer (pH 7.6) and 0.1 mL fluorescein diacetate. The sample was shaken at 150 rpm for 30 min. Consequently, 7.5 mL of chloroform and methanol (2:1) was added and the mixture was centrifuged at 8000 rpm for 10 min. The amount of hydrolyzed FDA was determined at 490 nm and compared with the pattern of fluorescein standard curve [18].

2.4 Microbial community of rhizobacteria by denaturing gradient gel electrophoresis (DGGE) analysis

The sample was collected and 0.5 g of each rhizosphere soil sample was extracted by using ZR Soil Microbe deoxyribonucleic acid (DNA) MiniPrep™ (Zymoresearch, USA) following the manufacturer instructions. The

quantity and quality of genomic DNA were analyzed on 1% (w/v) agarose gel electrophoresis. PCR amplification of 16S ribosomal (rRNA) genes from genomic DNA was carried out using universal primer pairs 8F and 1512R. The PCR condition was set up as standard protocol. Then, the nested-PCR amplification of the V3 region of bacterial 16S rRNA gene was carried out using 338F (GC-clamp) and 518R. The PCR condition for this step was as follows: 10 min of pre-denaturation at 94 °C (1 cycle), 30s of denaturation at 94 °C, 30s of annealing at 55 °C, 45s of extension at 72 °C (total 30 cycles) and 7 min of final extension at 72 °C (1 cycle). Prior to DGGE analysis, the PCR products were visualized by electrophoresis on 1% agarose gel. The rhizobacterial communities were analyzed using DGGE analysis where 8% of the polyacrylamide gradient was used. The optimal separation was achieved by 55-60% denaturant gradient. Electrophoresis was performed for 20 min at 20 V and 16 h. at 70 V (60 °C) using the Tris-acetate-EDTA (TAE) buffer. For the visualization of DNA patterns, the gel was soaked in ethidium bromide and photos were taken using Gel Documentation (QUANTUM-ST4 1100/26MX, France)

Representative bands of interest were cut from the gels with a sterile blade. The gels were transferred into a microcentrifuge tube where deionized water was added before being incubated at 4 °C for 16-18 h. The DNA in the solution was re-amplified using a forward primer 338F and a reverse primer 518R. The PCR products were purified and then sent to First BASE Laboratories Sdn. Bhd., Malaysia for sequencing. Then, the sequences were compared to the 16S rRNA gene sequences available in the Gen Bank database.

2.5 Statistical analysis

Analysis of Variance (ANOVA) was performed using the Statistix 8 program to analyze the growth parameters of tomato plants (shoot and root length, biomass, chlorophyll content, proline content, soil enzyme and the content of macronutrients). Significant differences in the data were compared among treatments via the least significant difference (LSD) test ($p< 0.05$).

3. Results

3.1 PGPR traits

Rhizobacteria which were screened from rhizosphere of rice plants in saline soil, showed the abilities to promote plant growth including nitrogen fixation, produced indole-3-acetic acid, solubilized phosphate, produced ACC deaminase, bio-film and exopolysaccharide (EPS). The results are shown in Table 1.

Table 1 Plant growth promoting rhizobacteria (PGPR) properties.

Isolates	*Pseudomonas azotoformans*I2.1	*Enterobacter aerogenes* P8	*Bacillus tequilensis* N15
Nitrogen fixation	+	+	+
Phosphate solubilization(SI)	-	2.93 ± 1.1	-
ACC degradation	+	+	+
IAA production (µg/mL)	20.1 ± 1.8	40.5 ± 3.4	18.2 ± 4.2
Biofilm production	+	-	+
Exopolysaccharide production	+	+	+

3.2 Root colonization of rhizobacteria

Three species of rhizobacteria (*Enterobacter aerogenes* P8, *Bacillus tequilensis* N15, *Pseudomonas azotoformans* I2.1) exhibited plant growth promoting properties. All rhizobacteria were individually inoculated into the bottle containing Hoagland agar in which the salinity levels were adjusted to 0, 0.25 and 0.5% of NaCl. The results showed that these rhizobacteria were able to live on the surface of tomato plants under all the salinity conditions investigated here (Figure 1A-C). It seems that the inoculated rhizobacteria was able to colonize tomato plants by excreting exopolymers for attachment with the root surface.

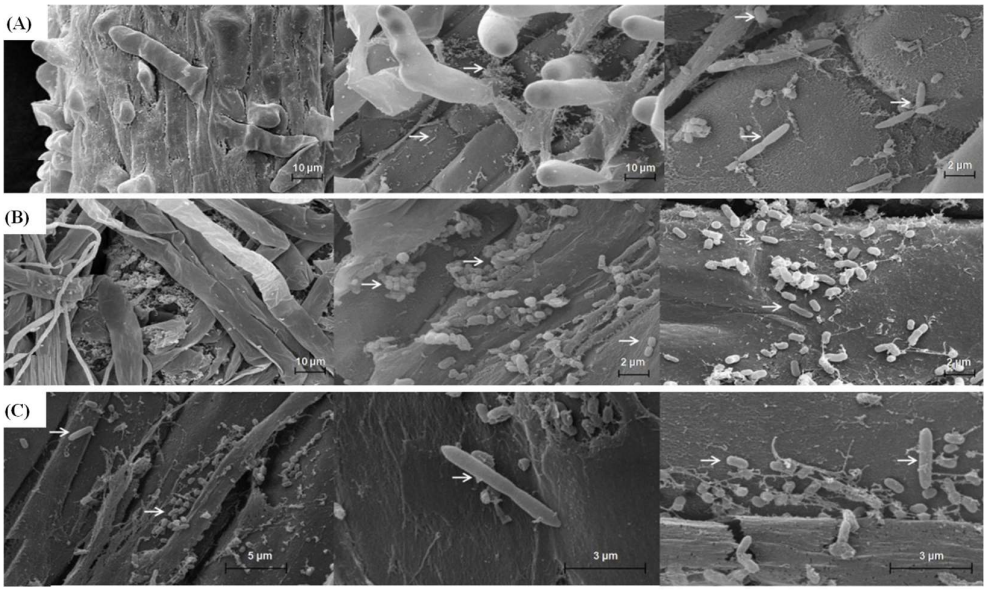

Figure 1 Colonization of rhizobacteria on tomato roots that was cultivated under different concentrations of NaCl under a scanning electron microscope. (A): 0% NaCl concentration, (B): 0.25% NaCl concentration and (C): 0.50% NaCl concentration.Scale bars are shown at the bottom right. Arrows indicated the shape of microbial cells.

3.3 Effect of various salinity concentrations on the growth of tomato plant

3.3.1 Plant analysis

40 days after transferring tomato plants into pots, the growth parameters of tomato plants including shoot length, root length and biomass were determined. The treatment of carriers immobilized with rhizobacteria in soil with 0 and 0.25% NaCl added showed significant differences compared to other treatments. The shoot lengths measured in these two treatments were 49.00 and 46.00 cm, respectively. Moreover, both treatments also showed relatively high amounts of biomass, of 4.88 and 4.83 g dry weight, respectively, compared to other treatments. The treatment of carriers immobilized with rhizobacteria where NaCl solution was not added showed significant difference in root length, with a highest value of 27.50 cm (Table 2).

Table 2 Effect of rhizobacterial suspension and rhizobacteria immobilized in carrier on the growth of tomato plants in soils with different salinity levels.

Salinity NaCl (%)	Treatment	Shoot length (cm)	Root length (cm)	Biomass (g dry weight)
0 %	Control	16.25^{c}	21.00^{d}	0.86^{c}
	Bacterial suspension	24.00 ^{cd}	24.25^{b}	3.31^{b}
	Carrier	32.75^{b}	24.25^{b}	3.17^{b}
	Bacteria + Carrier	49.00^{a}	27.50^{a}	4.88^{a}
0.25 %	Control	15.50^{e}	19.25^{d}	0.22^{d}
	Bacterial suspension	23.00^{d}	21.00^{cd}	0.67^{cd}
	Carrier	29.25^{bc}	19.25^{d}	3.18^{b}
	Bacteria + Carrier	46.00^{a}	23.75^{bc}	4.83^{a}
0.50 %	Control	14.00^{e}	18.25^{d}	0.37^{cd}
	Bacterial suspension	18.75^{de}	23.50^{bc}	0.33^{d}
	Carrier	29.50^{bc}	23.45^{bc}	0.67^{cd}
	Bacteria + Carrier	34.25^{b}	24.75^{ab}	0.58^{b}
F-test		**	*	*
% CV		15.03	8.59	15.77

*Significant different at 95% (p<0.05). **Significant different at 99% (p<0.01). Different letters in the column represented significant differences among treatments (p< 0.05) according to the LSD test.

The chlorophyll content in tomato leaves was determined. The highest chlorophyll content was found in tomato plants treated with carriers immobilized with rhizobacteria in soils under all levels of salinity. We found that chlorophyll content decreased as the concentration of NaCl solution increased. Carriers immobilized with bacteria without NaCl added showed the highest significant value of 0.14 mg/g FW. In cases of the soil that

0.25% NaCl and 0.50% NaCl solution was added, the values were found to be 0.11 mg/g FW and 0.10 mg/g FW, respectively (Figure 2A).

Proline content significantly increased with increasing NaCl concentration. Without adding NaCl solution, tomato plants accumulated the proline content at about 20-30 µM/g FW. When 0.25% and 0.50% NaCl solution was added, the proline content in tomato leaves was approximately 50-70 µM/g and 80-90 µM/g, respectively (Figure 2B).

FDA was analyzed in soil samples collected after harvesting. The treatment of carriers immobilized with rhizobacteria and the treatment of bacterial suspension added in the soil without NaCl solution showed significant differences in FDA activity, with values of 1.36 and 1.34 µg fluorescein g^{-1} soil h^{-1}, respectively. A similar result was found in the case of carriers immobilized with rhizobacteria supplemented with 0.25% NaCl solution and the bacterial suspension supplemented with 0.50% NaCl solution, yielding the highest FDA activity of 1.34 and 1.32 µg fluorescein g^{-1} soil h^{-1}, respectively (Figure 2C).

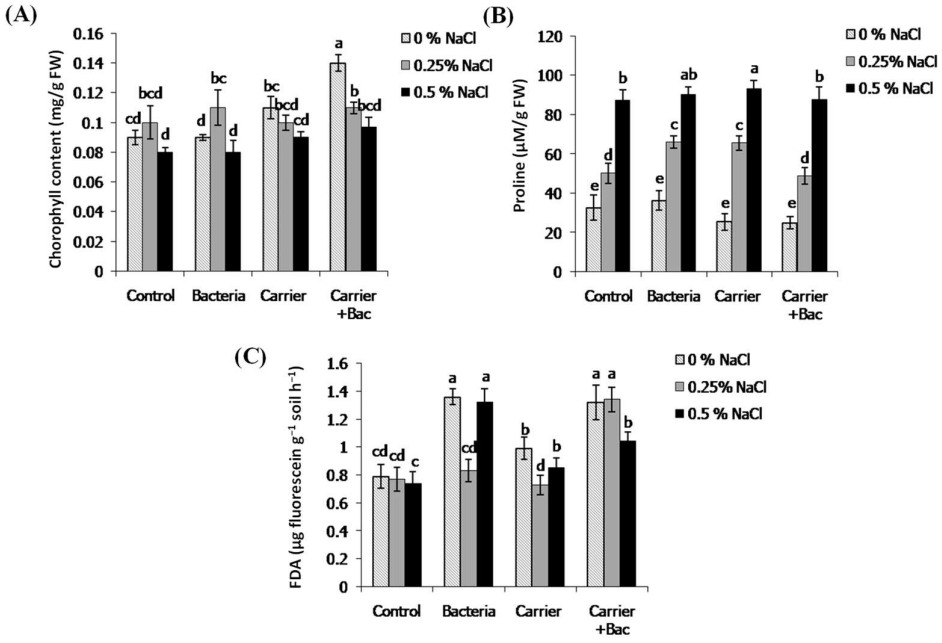

Figure 2 The chlorophyll content was determined in tomato leaves (A), the proline content was determined in tomato plants (B), FDA was carried out in soil samples (C), where plants were cultivated in soil supplemented with various concentrations of NaCl solution; 0%, 0.25% and 0.5% of NaCl solution. Error bars represent standard deviations of triplicate data. Different letters indicate significant differences among values.

3.3.2 Soil analysis

Soil properties were determined prior to plant cultivation and on harvesting day (40 days after transplantation). The properties of soil samples after harvesting day showed that when salinity level was increased, electrical conductivity rose as well. In this study, the initial EC value of soil sample was 0.02 dS/m. At the end of the experiment after treatment with 0.25% NaCl and 0.50% NaCl solution, the EC values were 1.57 dS/m and 2.34 dS/m, respectively. Moisture content increased in all treatments approximately 48-50% and pH value was 5-6.

Additionally, the macronutrients (total nitrogen, available phosphate and exchangeable potassium) in the soil samples were measured. The treatments with carriers immobilized with rhizobacteria supplemented with 0% NaCl and 0.25% NaCl showed an increase in total nitrogen content of 0.043% and 0.033%, respectively. The available phosphate was also found to be 27.33 and 24.91 mg/kg, respectively. Furthermore, threetreatments showed higher values of exchangeable potassium, which were the treatment of carriers immobilized with rhizobacteria at 0% NaCl (46.09 mg/kg) and 0.25% NaCl (44.52 mg/kg), and only rhizobacteria added at 0% NaCl (45.78 mg/kg) (Table 2).

Table 2 Macronutrients (Nitrogen, phosphorus and potassium) in the soil which was cultivated with tomato plants under different salinity concentrations.

Salinity NaCl (%)	Treatment	Total Nitrogen (%)	Available Phosphate (mg/kg)	Exchangeable Potassium (mg/kg)
	Control	0.025^{efg}	6.72^{a}	39.64^{c}
	Bacteria	0.029^{def}	15.05^{d}	45.78^{a}
0% NaCl	Carrier	0.033^{bc}	22.29^{c}	38.04^{c}
	Bacteria + Carrier	0.043^{a}	27.33^{a}	46.09^{a}
	Control	0.020^{g}	9.75^{e}	26.85^{e}
	Bacteria	0.029^{cde}	6.73^{g}	27.93^{e}
0.25%NaCl	Carrier	0.029^{cdef}	7.15^{fg}	28.18^{e}
	Bacteria + Carrier	0.034^{b}	24.91^{b}	44.52^{ab}
	Control	0.024^{fg}	6.67^{g}	35.12^{d}
	Bacteria	0.027^{ef}	6.03^{g}	35.96^{d}
0.50%NaCl	Carrier	0.028^{def}	8.01^{f}	24.33^{f}
	Bacteria + Carrier	0.032^{bcd}	22.22^{cf}	42.96^{b}
F-test		**	**	**
%CV		9.38	5.53	12.90

*Significant difference at 95% (p<0.05). ** Significant difference at 99% (p<0.01). Different letters in the column represented significant differences among treatments (p< 0.05) according to the LSD test.

3.3.3 Microbial community of rhizobacteria

The community of rhizobacteriain soil samples was detected using DGGE analysis at 20 days and 40 days after tomato seedlings were transferred into the pot. The DNA bands corresponding to 12 samples including 2T-12T (harvesting at 20 days) and 2F-12F (harvesting at 40 days) are presented in figure 3. The DNA bands (labeled with "P and I") were similar to the bands of a rhizobacterial strain P: *Enterobacter aerogenes* P8 and I: *Pseudomonas azotoformans* I2.1 from samples at 20 and 40 days. The DNA band was not found in the case of strain *Bacillus tequilensis* N15. It seems that both strains *Enterobacter aerogenes* P8 and *Pseudomonas azotoformans* I2.1 can colonizetomato roots. Moreover, eight species of bacteria were identified. The community profiles of rhizobacteria suggested that they were *Klebsiella aerogenes*, *Enterobacter* sp., *Caballeronia concitans*, *Raoultella planticola, Citrobacter freundii*, *Burkholderia thailandensis*, *Frateuria* sp., and *Fulvimonas* sp. In addition, these bacteria were previously reported to have the ability to promote plant growth (Table 3).

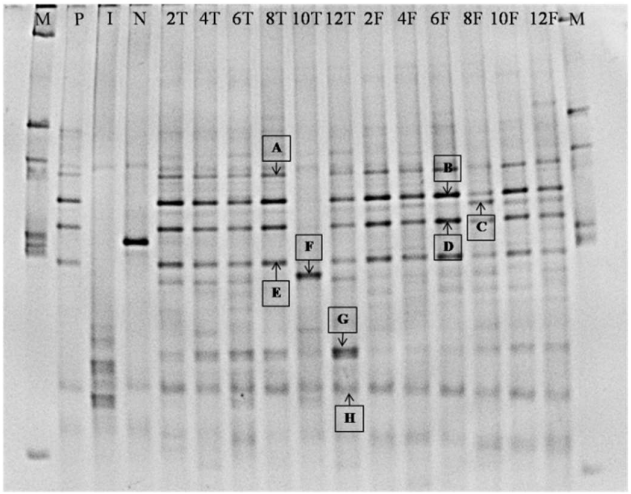

Figure 3 The microbial community profiles of the V3 region of the 16s rRNA genes, on 55%-60% Denaturing Gradient Gel Electrophoresis (DGGE).

The abbreviations shown in the picture are M: marker or DNA standard, P: *Enterobacter aerogenes* P8, I: *Pseudomonas azotoformans*I2.1 and N: *Bacillus tequilensis* N15, soil samples were sampled at 20 days and 40 days. The treatments included T2: bacterial suspension (0% NaCl), T4: carriers immobilized with rhizobacteria (0% NaCl), T6: bacterial suspension (0.25% NaCl), T8: carriers immobilized with rhizobacteria (0.25% NaCl), T10: bacterial suspension (0.5% NaCl), T12: carriers immobilized with rhizobacteria (0.5% NaCl). Eight species of rhizobacteria included A: *Klebsiella aerogenes*, B: *Enterobacter* sp., C: *Caballeronia concitans,* D: *Raoultella planticola*, E: *Citrobacter freundii*, F: *Burkholderia* sp., G: *Frateuria* sp. and H: *Fulvimonas* sp.

Table 3 Comparison of PGPR microbial communities in rhizosphere soil cultivated with tomato plants between this study and other previous reports.

Band	Species/ GenBank Accession no.	Characteristics of plant growth promoting properties
T8	*Klebsiella aerogenes*. CP044083.1	ACC deaminase activity [19], IAA production, the ability of increasing the cotton's absorption of the N, P, K, and Ca elements and decreasing on the absorption of the Na element under salt stress [20].
T6	*Enterobacter* sp. AM748792.1	ACC deaminase activity [19].
T8	*Caballeronia concitans.* MK000669.1	no data found
T6	*Raoultella planticola*. CP044121.1	ACC deaminase activity [19], Nitrogen fixation
T8	*Citrobacter freundii*. LR699006.1 (family*Enterobacter iaceae*.)	ACC deaminase activity [19], Tolerance to host plants under different abiotic stress environments [21].
T10	*Burkholderia* sp. CP020392.1	Tolerance to host plants under different abiotic stress environments [21], Nitrogen fixation, Phosphate solubilization (organic acid productions namely gluconic acid.
T12	*Frateuria* sp. MF478987.1 (*Pseudomonas* group)	ACC deaminase activity [19], Pseudomonas group are involved increasing the cotton's absorption of Mg^{2+} and Ca^{2+} and decreasing the absorption of the Na^{+}, IAA production, HCN production. Decreased in electrolyte leakage and increased in proline production and selected on uptake of K^{+}.
T10	*Fulvimonas* sp. KX350190.1	no data found; ourdata is the first report that this strain that might enhance tomato growth

4. Discussion

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria which consist of various species and strains. They showed an ability to promote plant growth including Nitrogen fixation, Phosphate solubilization, ACC degradation, IAA production, Biofilm formation, and Exopolysaccharide. Some reports found that *Streptomyces* sp. can produce proline and ACC deaminase to promote tomato (*Solanumly copersicum* L.) growth under salt stress [22]. Some plant growth-promoting rhizobacteria (PGPR) might provide a direct stimulation on plant growth and develop nitrogen activity, phytohormones (IAA, gibberellic acid), iron, and solubilize phosphate which helps to overcome the effects of salinity stress.

PGPR can live on the root surface and around the rhizosphere soil, promoting and enhancing plant growth. They could be classified into two categories: those living in the cortex, and those living in soil around the rhizosphere soil zone, stimulating plant growth [23]. In this study, we found that three rhizobacterial isolates including *Pseudomonas azotoformans*I2.1, *Enterobacter aerogenes* P8 and *Bacillus tequilensis*N15, which were salt tolerant, could colonize on tomato seeds under various salinity levels (0, 0.25 and 0.50 % NaCl). They could live on the surface of tomato roots (Figure 1). This is in agreement with Zhansheng et al. [24] who reported that *Klebsiella oxytoca* Rs-5 was colonized in each root segment, including the base, middle and tip of the cotton roots under salt stress and could also be observed using SEM. In addition, treating cotton plants with bacterial agents relieved the salinity stress and promoted cotton seedling growth. Colonization of these bacteria could help promote the ability of survival cells that were inoculated on seeds to respond to seed exudates, and attach to the root surface leading to a development of the root system. Salt-tolerant bacteria could survive in different salinity concentrations and adapt themselves to the effect of salt by their mechanisms, such as accumulation of compatible solutes for osmoregulation and the production of extracellular proteases. Das et al. reported [25] that *Staphylococcus epidermidis* P-30 was able to survive in high salt concentration (up to 20%) anddemonstrate plant growth-promoting properties.

Many factors affecting how microorganisms colonize at the root. These include water content, pH, nutrients such as N, P, and K, compositions of root exudates, and the presence of other microorganisms. Plant species are also another major determinant of overall microbial diversity. The roles of rhizosphere microbial populations for the maintenance of plant root health, nutrient uptake, and tolerance of environmental stress. Barnawal et al. [26] found that *Arthrobacter protophormiae benefitted* the colonized Pea (*Pisum sativum L.*) by creating a diverse bacterial population, increasing ACC deaminase activity, andproviding protection against salinity stress.

Tomato plants can naturally tolerate a moderate level of salinity in soils exhibiting salt ions of 0.25-0.50% andan EC value of 4-8 dS/m. Here, we also investigated the effects of salinity on the growth of tomato plants. The results showed that after 40 days of cultivation, soil supplemented with NaCl 0.25% treatment (46.00 cm) showed the highest plant shoots (46.00 cm). This was not significantly different to that found in normal soil treatment (49.00 cm) when inoculated with rhizobacteria immobilized with carriers (Table 2). Moreover, all of the treatments showed a significant difference in plant biomass. Due to the osmotic stress caused by salt accumulated in soil and the effects of ion toxicity in the form Na^{+} and Cl^{-} it was difficult for plants to absorb water. Also, salt in soil negatively affects nutrient uptake and the toxicity of ions. The balance of osmotic potential was an important factor when the ratio of potassium and sodium (K^{+}/Na^{+}) was higher. PGPR increased potassium content more than sodium, enhancing water and nutrient uptake into plant cells. Kim et al. [27] also reported that *Enterobacter* sp. EJ01 isolated from sea china pink in Korea was capable of ACC deaminase and IAA production, which increased fresh weight, dry weight, and plant height of tomato plants and Arabidopsis under both normal and high salinity conditions. Acuña et al. reported [28] that *Klebsiella* sp. and *Serratia* sp.

produced auxin and siderophores, improving the biomass of *Triticum aestivum* L. (wheat) under the salinity conditions by supplementation with 0.25M and 0.45M NaCl.

Osmotic adjustment was an important mechanism for the adaptation of plants grown on saline soils. The plants absorb ions into the vacuole and organic accumulation in the cytosol. Both mechanisms could help plants maintain water in their cells. Organic substances mostly accumulated in plants are called compatible solutes, for example, proline, glycine betaine, trehalose, and polyols. Compatible solutes act as an organic osmolyte to maintain normal cell functions. This is a mechanism of plant adaptation to reduce the impact of stress. In this experiment, tomato plants treated with 0.50% NaCl (2.34 dS/m) on harvesting day had high amounts of proline content at about 80-90 µmol/g FW. Tomatoes are moderately tolerant to salinity. They can grow in soil that has salinity of 0.25-0.50 percent of salts ion and the electrical conductivity of 4-8 dS/m. Tomato plants treated with 0.50% NaCl (2.34 dS/m) on harvesting day had high amounts of proline content of about 80-90 µmol/g FW. Tomato plants produced proline in order to protect themselves from osmotic pressure, as evidenced by an increase of the proline content in plant tissues when grown under high salt concentration conditions. According to figure 2A, proline content levels in plants treated with carriers immobilized with rhizobacteria were lower than that of plants treated with carriers alone. This might be because the rhizobacteria could help reduce proline. Marin et al. [29] reported that when *Prunus* rootstocks were cultured *in vitro* under increasing NaCl concentrations (0, 20, 60, 180 mmol), proline concentration in root tissues increased as the salt concentration in the medium.

Soil samples were analyzed for macronutrients such as the total nitrogen, available phosphate and exchangeable potassium. The results showed that plants treated with carriers immobilized with bacteria in soils at 0% NaCl and 0.25% NaCl had higher amounts of nitrogen, phosphorus and exchangeable potassium compared to other treatments (Table 2). Previously, there were reports of Ipek [30] that presented the plant growth promoting effects of *Alcaligenes* 637Ca, *Staphylococcus* MFDCa-1, MFDCa2, *Agrobacterium* A18, *Pantoea* FF1 and *Bacillus* M3 on strawberry plants. They found that concentrations of all nutrients including nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), iron (Fe), manganese (Mn), copper (Cu) and boron (B) could significantly increase with the bacterial treatments tested.

5. Conclusion

Finally, we identified 8 species of bacteria in the rhizosphere soil (Figure 3). Most bacteria are plant growth promoting rhizobacteria (PGPR) (Table 3), for example, *Klebsiella aerogenes*, *Enterobacter* sp., *Burkholderia thailandensis*, and *Fulvimonas* sp. *Citrobacter freundii* is a member of the family *Enterobacter iaceae* while *Frateuria* sp. is classified as Pseudomonas groups. Many studies have reported the ability of *Klebsiella aerogenes* on nitrogen fixation activity and degradation and hydrolysis of chitin (40). *Burkholderia* sp. could produce ACC-deaminase for synthesis of ammonia and α-ketobutyrate. Microbes in the Pseudomonas family have a major role in the carbon-cycle which could also produce IAA synthesis. *Enterobacter* sp. has the ability to produce ACC-deaminase, degrade and hydrolyze chitin. Last but not least, we found that the DNA bands of samples showed a similar DNA pattern (P and I) to 2 strains of PGPRs which were inoculated into soil, demonstrating that they could both survive and enhance tomato growth under salinity conditions.

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