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Contributed Paper

Plant Growth Promoting Properties of Salt Tolerant Bacteria and Their Application as Microbial Inoculant for Seed Germination

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ABSTRACT

A total of 29 bacterial strains were isolated from salt-affected paddy fields in Khon Kaen province, Thailand. These isolated bacterial strains were evaluated for qualitative parameters such as nitrogen fixation on Ashby's agar, phosphorus (P) and potassium (K) solubilization on NBRIP and Aleksandrov media, respectively under sodium chloride concentrations at electrical conductivity (EC) from 0 to 16 dS m^{-1}. The resulted showed that most of the isolates were nitrogen fixers and positively promoted the plant growth at saline levels EC 2-8 S m^{1}. The ability of P and K solubilization, in addition to the production of Indole-3-acetic acid (IAA) varied among bacterial strains and saline concentrations. The highest production of IAA (46.30 μg mL^{-1}) was reported by isolate 18 at EC 8 dS m^{-1}. The potential antagonism of 26 isolates to plant pathogenic fungi was tested according to dual culture assay. Isolate 1 was the most effective; being able to inhibit more than 50% to *Sclerotium rolfsii* and 18.93% to *Fusarium oxysporum*. Strains 1, 22, and 29 demonstrated multiple traits of plant growth promoting and were used to test corn (*Zea mays*) seed germination. It was found that they could activate salt tolerance and germination of corn seeds as efficiently as commercial biofertilizer -effective microorganisms (EM) at salinity level 8 dS m^{-1}; while previous research reported that corn could not grow well at salinity level higher than 4 dS m^{-1}. Thus, the selected isolates in this study have the potential concerned be utilized as a bio-remedy for improving plant growth in saline soil.

Keywords: salt tolerant bacteria, plant growth promoting, biofertilizer, corn seed germination.

1. INTRODUCTION

In recent years, saline soil is one of the most stressful conditions which impacts directly to crop growth and productivity [1]. Saline soil is defined as the soil having electrical conductivity (EC) of saturated soil extracts exceeding 4 dS m^{-1}. Soils having EC values between 0 to 2 dS m^{-1} are considered non-saline, while those having EC of 2-4, 4-8, 8-16 and >16 dS m^{-1} are classified as slightly, moderately, highly and extremely saline, respectively [2]. Saline soils contain a high

concentration of ions in the form of Na^+, Mg^{2+} and Ca^{2+} bound with Cl^- and is the main component of saline soil. The high salinity induces osmotic imbalance or ion toxicity in plant tissue expressed by negative impacts on seed germination, flower, fruit, root, and shoot growth, etc [3]. Especially, seed germination is known as a decisive and also the most salt-sensitive stage of plant growth.

Reclamation of saline soil to make it suitable for growing crops is therefore essential and necessary. This can be carried out through the application of various techniques, such as leaching, using halophytes vegetation, application organic or mineral amendments, utilizing excavation and removal of the salt-affected soil. However, such chemical or mechanical remediations are quite costly. Plant growth-promoting rhizobacteria (PGPR) has been suggested as a safe and economic strategy for restore saline soil and enhancing plant salt tolerance. PGPR are known as soil living bacteria that are capable to promote plant growth in their interaction with the rhizophere [4]. Bio-fertilizer is produced from living beneficial microbes that can solubilize the nutrients, enhance the stress tolerance, produce plant hormones, and inhibit plant pathogen through particular mechanisms [5]. The purpose of this study was to screen salt tolerant bacteria as environmentally friendly chemical fertilizers for promoting plant growth and for improving the quality of saline soils.

2. MATERIALS AND METHODS

2.1 Collection of Saline Soil Samples

Six saline soil samples were collected from 2 rice fields in Dang Yai village, Khon Kaen province, Thailand. Soil samples were tested for EC, pH and moisture content. Then they were kept in sterile bags and stored at 4 ^oC for further analysis.

2.2 Isolation of Bacteria in Saline Soil

Bacterial strains in soil samples were isolated on soil extract agar (SEA) by using spread plates technique. Soil extract was obtained when mixing all soil samples having an average EC of 3.28 dS m^{-1} in the distilled water. For each soil sample was prepared 10 grams of soil in 90 mL of 0.85% NaCl to obtain soil solution. Serial ten-fold dilution from 10^{-1} to 10^{-5} of soil solution with 0.85% NaCl was conducted. From each dilution, i.e. 10^{-3}, 10^{-4} and 10^{-5}, 0.1 mL of aliquot was spread on agar plates to determine the number of microbes which can cultivate in saline condition. The plates were incubated at 30 ^oC for 2 days in an incubator. Viable cells were calculated in colony-forming unit CFU mL^{-1} of diluted solution or log CFU mL^{-1} Bacterial isolates with different morphological characters were considered and streaked on Nutrient agar (NA) plates and incubated for 1-2 days at 30 ^oC to get pure colonies. The pure isolates were re-streaked on new NA plates, SEA slants and store at 4 ^oC for further assays.

2.3 Nitrogen Fixation

The selected isolates which were different in the character were tested by point inoculating on nitrogen-free Ashby's Mannitol Agar (Mannitol 20 g; Agar 15 g; $CaCO_3$ 5 g; K_2HPO_4 0.2 g; $MgSO_4.7H_2O$ 0.2 g; NaCl 0.2 g; K_2SO_4 0.1 g for 1 L and pH 7.4 $\pm$ 0.2 at 25 ^oC) [6]. These isolates with three replicates were tested under five salinity levels 0, 2, 4, 8 and 16 dS m^{-1} by adding sodium chloride into the medium. The inoculated petri plates were incubated at 30 ^oC for 3 days. After incubation period, the appearance of bacterial colonies indicated that they could use atmospheric nitrogen as a source of nitrogen.

2.4 Phosphate Solubilization

The isolates with different characters were point inoculated on National Botanical Research Institute phosphate growth (NBRIP) agar to screen for phosphate solubilizing ability. The selective medium contains the following components ($g.L^{-1}$): glucose 10; $Ca_3(PO_4)_2$ (tricalcium phosphate) 5; $MgCl_2.6H_2O$ 5; $MgSO_4.7H_2O$ 0.25; KCl 0.2; $(NH_4)_2SO_4$ 0.1; agar 15 and pH is adjusted at 7.0 [4]. The phosphate solubilization mechanism occurs

in bacteria which are able to produce organic acids to dissolve insoluble tricalcium phosphate [7]. Various salinity levels 0, 2, 4, 8 and 16 dS m^{-1} were applied to medium by adding sodium chloride. The test was conducted in triplicate for each isolate at each salinity level. The inoculated petri dishes were incubated at 30 °C for 5 days. The formation of clear zones around colonies indicated positive results and solubilization index (SI) was determined according to the formula (1):

$$SI = \frac{Colony\ diameter + Halozone\ diameter}{Colony\ diameter} \quad (1)$$

2.5 Potassium Solubilization

Potential isolates were point inoculated on modified selective Aleksandrov medium containing insoluble potassium to determine potassium solubilization ability components in Aleksandrov medium ($g.L^{-1}$) are glucose 5; $MgSO_4$. $7H_20$ 5; $CaCO_3$ 0.1; potassium aluminium silicate 2; $FeCl_3$ 0.005; $Ca_3(PO_4)_2$ 2; $(NH_4)_2SO_4$ 0.1; agar 15 and pH was adjusted at 7.0. The possible solubilizing mechanism in this assay was considered the production of organic acids to dissolve insoluble forms of potassium i.e. potassium aluminium silicate [8]. Each isolate was tested in triplicate. However, only 3 salinity levels 5, 8 and 16 dS m^{-1} were applied because original EC value of medium was already quite high at 5 dS m^{-1}. The inoculated petri dish were incubated at 30 °C for 5 days. The clear zones around colonies indicated the positive results and SI values were recorded as equation above (1).

2.6 IAA Production

The assay of Indole-3-acetic acid (IAA) production was conducted by inoculating bacterial isolates into test tubes containing 6 mL TSB (Trypticase Soybean Broth) supplemented L-tryptophan 1 $g.L^{-1}$. The control was TSB without supplement of tryptophan and bacteria. This test was investigated under 5 salinity levels-0, 2, 4, 8 and 16 dS m^{-1} by adding sodium chloride into the broth solution. A full-loop of each isolate was inoculated in test tube and was replicated 3 times. After 3 days of shaking in the dark, 5 mL of bacterial culture broth was collected and centrifuged at 4000 rpm for 8 minutes at 4 °C. The production of IAA was determined color formation after the supernatant reacted with Salkowski reagent [9]. The amount of IAA was quantified from absorbance at 530 nm against IAA standard curve and expressed in $\mu g\ mL^{-1}$[10].

2.7 Antagonistic Test

To evaluate the antagonistic ability of bacterial isolates, dual culture method with plant pathogens was used [11]. Two plant pathogenic fungi, *Sclerotium rolfsii* and *Fusarium oxysporum*, were used and initially grown on potato dextrose agar (PDA) medium for 7 days at the room temperature. After culturing period, the 5-mm plugs of fungal mycelium were picked up from the margin of each fungal colony by cork borer. One plug was transferred to the center of Petri plate containing mixed media of soil extract agar and nutrient agar with 3 streaking lines of each bacterial isolate at a distance of 2 cm from the margin of the plate. A control plate was a mycelial plug that placed at the center without inoculation of bacteria. The plates were incubated at 30 °C for 2-5 days. The antagonistic activity between bacteria and fungus was observed after this period. The percentage inhibition of radial growth (PIRG) following the formula of Skidmore and Dickinson in 1976 [12] was calculated as:

$$\text{PIRG (\%)} = \frac{R1 - R2}{R1} \times 100 \quad (2)$$

Where R1 is the radius of pathogenic colony in control and R2 is the radius of pathogenic colony in paired cultures.

2.8 Seed Germination

Based on the modified method of Pastor-Bueis *et al.* [13], corn (*Zea mays*) was used in this study.

Seeds were sterilized by soaking in 6% sodium hypochlorite for 5 minutes, then washed 3 times with sterile distilled water (DW). Next, seeds were soaked for 30 minutes in the following 5 treatments: Control treatment - sterile DW; BC treatment - Bacterial cells after centrifuging of isolates 1, 22 and 29 suspended in 0.85% NaCl; SN treatment - Supernatant of the mixture of 3 isolates; MB treatment – Medium broth; and EM treatment - Commercial effective microorganism solution diluted in DW at 1:100 ratio. As for bacterial mixture, three selected strains were cultured separately and then mixed after centrifugation. The treated seeds were placed on petri plates lined with damp tissue papers (three 10-seed replications). All treatments were arranged in a randomized complete design and watered per 2 days by 5 saline levels: sterilized water and NaCl solutions at EC 2, 4, 8 and 16 dS m^{-1}. After 5 days, seed germination was recorded and seed germination rate (GR), germination index (GI), seed vigor indexes (SVI) were calculated basing on Emino and Warman [14] as follow:

$$GI = \frac{\text{Germination rate (\%)} \times \text{Relative growth index (\%)}}{100} \quad (4)$$

In which:

$$\text{Germination rate (\%)} = \frac{\text{Number of seeds germinated in extract}}{\text{Number of seeds germinated in distilled water}} \times 100$$

$$\text{Relative growth index (\%)} = \frac{\text{Mean root length in extract}}{\text{Mean root length in distilled water}} \times 100$$

And,

$$SVI = GP \times \text{seedling length} \quad (5)$$

In which:

$$GP\ (\text{germination percentage})(\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

$$\text{Seedling length} = \text{root length} + \text{shoot length}$$

2.9 Study of Root and Salt Tolerant Bacteria Interaction by Scanning Electron Microscope

Corn seeds were soaked in 6% of sodium hypochlorite for 5 minutes for surface sterilizing and washed 3 times by distilled water. Sterilized seeds were transferred to sterile petri dishes lined with tissue paper wetted by sterilized DW and incubated at 30 ^{o}C for pre-germination in dark for 1-2 days. Subsequently, sprouted seeds were soaked in suspension of selected plant growth promoting bacterial isolates (1×10^{8} CFU mL^{-1}) for 2 hours, and transferred to sterile bottles of semi-solid Hoagland nutrient agar (containing NaCl at 4 dS m^{-1}, 8 dS m^{-1}, and 16 dS m^{-1}) under aseptic condition. Sterile DW was used as a negative control and bacterial suspension as a positive control. Seedlings were incubated for 5 days at room temperature.

Preparation of roots for observation under Scanning Electron Microscope (SEM) was based on Karcz [15]. The roots were excised from the seedlings, rinsed twice in sterile distilled water and fixed with 2.5 % (v/v) glutaraldehyde in 0.1M phosphate buffer. Phosphate buffer (0.075 M) was used to wash the samples 3 times, each for 15 minutes. Samples were dehydrated at 4 °C in ascending concentrations of ethanol (50%, 60% 70%, 80%, 90% for 15min each and 100%, two times for 30 min each.. Next step, samples were dried in a critical point dryer. Resulting of SEM was observed under a field emission scanning electron microscope after coating the sample in 10 mÅ of gold-palladium for 2.5 min.

2.10 Statistical Analysis

Data was performed statistic calculation using the Statistics 8 program. Analysis of Variance (ANOVA) and it was expressed for ability of plant growth promoting bacteria (phosphate solubilizing, potassium solubilizing, IAA production , antagonistic and seed germination). To detect the significant differences ($P < 0.05$) between means, the Least-Significant Different (LSD) test was performed.

3. Results and Discussion

3.1 Microbial Population

A total of 6 soil samples were collected at 3 locations each from 2 paddy fields (DY1 and DY2) in Dang Yai village (Table 1). The soils were slightly saline having EC in the range of 2 to 5 dS m^{-1}, except for the sample DY1.3 which was classified as normal non-saline soil due to its low EC (1.50 dS m^{-1}). It was found that the soil in the DY1 field was more alkaline than that from DY2, and had a higher pH of approximately 7.6. According to Shin *et al.*[16], saline soil also was defined that EC is larger than 4 dS m^{-1} and pH lower than 8.5. The average microbial populations were 6.32 and 6.13 log CFU mL^{-1}, respectively for DY1 and DY2. There was no significant difference in moisture content among soil samples. The moisture contents of the 6 soil samples were in the range of 13-16% (Table 1). The moisture of soil samples was lower than normal soil. Normally, saline soil was considered nutrient-poor, low in water content and toxic for microbial and plant growth [17]. A total of 29 bacterial strains were isolated from these soil samples (Table 2) and they were expected to have halotolerance potential and

Table 1. The characters of Dang Yai (DY) soil samples.

Soil samples	Microbial population (Log CFU mL^{-1})	Moisture content (%)	pH	EC (dS m^{-1})	Salinity level
DY1.1	6.25	15.3	7.56	2.68	Slightly saline
DY1.2	6.32	13.6	7.37	3.66	Slightly saline
DY1.3	6.380	14.5	7.86	1.50	Non saline
DY2.1	6.13	14.4	5.72	4.60	Moderately saline
DY2.2	6.29	12.9	5.76	3.01	Slightly saline
DY2.3	5.96	13.2	5.37	4.22	Moderately saline

Table 2. Classification of isolated bacteria in Dang Yai (DY) soil samples.

Soil sampls	Number of isolated bacteria	Bacterial code
DY1.1	6	1, 2, 3, 4, 5, 6
DY1.2	3	23, 25, 29
DY1.3	7	7, 21, 22, 24, 26, 27, 28
DY2.1	6	8, 9, 10, 14, 16, 20
DY2.2	4	11,12, 18, 19
DY2.3	3	13, 15, 17

plant growth promoting activities. However, after repeated culturing in salinized media, isolates 10, 16 and 17 showed slower growth than the others. Thus, only 26 isolates were used for microbial properties testing on the next step of the study.

Similar to the results of previous reports of Shin *et al.* [16] and Nan Yan *et al.* [18], soil salinity in our research was mainly evaluated due to electrical conductivity and found that most soil samples in Dang Yai rice fields were salt-affected. Salinity mostly depended on the presence of soluble salts, and if the soil was more alkaline, the soluble salt will be less [19]. Due to its higher alkalinity, DY1 tended to be less saline than DY2. Thus, DY2 was the more stressful site for plant growth and soil microbes. However, even with low water content and high salinity condition, a large number of microbes were found in both fields. These bacteria could possibly have salt-tolerant ability to survive.

3.2 Microbial Properties in Term of PGPB in Saline Soil

To evaluate the adaptation under high salt stress and the ability to promote plant growth of these isolated microorganisms, our study investigated their activities in fixing nitrogen, solubilizing insoluble phosphate and potassium (data not shown), and IAA production (Table 4) in saline media. The growth of isolates on Ashby's agar in various salinity conditions showed their ability to fix nitrogen under salt stress. In this experiment, most isolates were able to grow on the selective medium with or without supplement of NaCl solution at 2, 4, 8 and 16 dS m^{-1}. However, isolate 2 and 6 indicated negative. We observed that they tended to fix nitrogen on medium with EC ranged 2-8 dS m^{-1} better than medium with EC 0 and 16 dS m^{-1}. For example, isolate 1, 3, 11, 13, 19, 28 and 29 have shown that their nitrogen-fixing activities increased at high salt concentrations. Especially, isolate 11 was halotolerant on Ashby's agar supplied with NaCl solution at EC 8 and 16dS m^{-1}, but not on medium with low salinity levels. Most of other isolates were depressed by NaCl at EC 16 dS m^{-1}. It showed that salt concentration at EC 2, 4 and 8 dS m^{-1} were most suitable for stimulating nitrogen fixing capacity of these bacteria. The experiment was conducted on Ashby's agar, positive results mainly based on the growth of bacteria; it indicated the close relationship between cell growth and nitrogen fixation. Thus, under optimal salt conditions, some bacteria grew well and fix nitrogen immediately. This current research was also in agreement with the report of Tripathi in 1998 [20] on the responses of *Azospirillum* under salinity stress. The sensitivity of nitrogen fixing ability could be different between bacteria and even among isolates of the same species.

For promoting plant growth, solubilization of insoluble form of phosphate (P) and potassium (K) in soil are essential criteria to evaluate the bio-fertilizing efficiency of soil bacteria. The result of solubilizing tricalcium phosphate ($Ca_3(PO_4)_2$) showed that 9 isolates were able to create clear zones on NBRIP agar at all saline conditions (EC 5, 8 and 16 dS m^{-1}) The P-solubilization index (SI) ranged from 2 to 3.83. However, phosphate solubilizing activity of the bacteria was lower when they grew on medium with EC 8 dS m^{-1}, but no significant differences between salt concentrations. This might be explained that salt concentration at 8 dS m^{-1} could not stimulate almost bacteria to produce organic acids for dissolving phosphate. However, some isolates such as isolate 9, 14 and 20 were able to solubilize phosphate effectively at this concentration. The highest SI of 3.83 was shown by the clear zone of isolate 9, but it seemed to be inactive at higher salinity at 16 dS m^{-1}.

Halozone formation on Aleksandrov agar medium for testing K-solubilization also was recorded and presented through solubilization index. The positive results were obtained on 11 isolates under various NaCl concentrations with EC 0-16 dS m^{-1}. K-solubilizing bacteria were reported their presence in many types of soils and were isolated in saline soil [8]. Generally, the isolates referred to solubilize K at high salinity

conditions but efficiency of solubilizing was highest at 8 dS m^{-1}. The range of SI among isolates varied from 1.33 to 2.33. Isolates 1, 22 and 29 were able to solubilize insoluble potassium in medium at all salinity levels with high SI (>2). Thus, we selected salt-tolerant bacteria in all tests with the multifunctional activities on plant growth such as isolates 1, 22 and 29 to perform the next experiments.

According to Mahmood and coworkers [21], phosphate was mostly declined in salt-affected soil containing a high concentration of Ca^{2+} ions because it was precipitated into poorly soluble form. Salinity also affected the plants potassium uptake, which plays a critical role in plant metabolism due to the high concentration of soluble sodium (Na^{+}) ions [16]. Specially, isolates found in our research could increase plant uptake of these macronutrients due to solubilizing P and K activities under salinity condition. The SI values for solubilization of tricalcium phosphate in this study i.e. 2 to 3.83, were relatively high compared with previous reports. Mukri *et al.* [22] showed that SI of phosphate solubilizing bacteria (PSB) from the non-saline rice fields in Perlis, Malaysia ranged from 1.16 to 1.87. Bacterial strains in rhizophere of palm tree showed SI from 1.36 to 2.33 in solubilizing tricalcium phosphate [23]. Conversely, the range of SI in K-solubilization among isolates in our research varied from 1.33 to 2.33 which were much lower than SI for P-solubilization. Variations in mineral solubilization capacities depend on several factors including the levels of salt stress, bacterial strains and growth conditions. Bacteria in saline soil of Dang Yai were found that they had salt-tolerance and multiple potential activities in promoting plant growth. Isolates had more than 2 or 3 plant growth promoting traits that may induce plant growth directly or indirectly or synergistically [27]. Their antifungal activity is also important for biological control and indirectly promotes plant growth [28]. Table 3 and 4 showed characteristics of four potential bacteria (isolates 1, 18, 22 and 29) having good behaviors on fixing nitrogen, solubilizing insoluble phosphate and potassium, producing phytohormone IAA and antifungal activity against to *Sclerotium rolfsii* and *Fusarium oxysporum*. However, before seed germination testing and root colonization by SEM technique, these isolates were screened due to the dual cultures test. Three out of them did

Table 3. Plant growth promoting activities of potential isolates in various salinity levels.

	Plant promoting growth activity								
	N_2 fixation	P solubilization			K solubilization				
		Salinity level (dS m^{-1})							
Isolate		**5**	**8**	**16**	**0**	**2**	**4**	**8**	**16**
1	+	2.18 ± 0.06^{a}	2.10 ± 0.10^{a}	2.19 ± 0.02^{a}	-	2.04 ± 0.22^{a}	2.00 ± 0.03^{b}	2.12 ± 0.05^{a}	2.03 ± 0.04^{b}
18	+	-	-	-	-	-	-	-	-
22	+	2.15 ± 0.04^{a}	2.04 ± 0.04^{a}	2.14 ± 0.07^{a}	2.11 ± 0.04^{a}	2.02 ± 0.03^{a}	2.08 ± 0.05^{ab}	2.11 ± 0.06^{a}	2.12 ± 0.02^{a}
29	+	2.16 ± 0.05^{a}	2.06 ± 0.01^{a}	2.09 ± 0.04^{a}	2.12 ± 0.07^{a}	2.05 ± 0.05^{a}	2.14 ± 0.02^{a}	2.13 ± 0.05^{a}	2.08 ± 0.03^{ab}
F-test		ns	ns	ns	ns	ns	**	ns	*
%CV		2.29	3.01	2.27	4.02	6.37	1.8	2.43	1.68

Remark: + Positive result; - Negative result. P and K solubilization index (SI) were expressed as mean value of 3 replicates ± STDEV and followed by F-test at $P < 0.01$ with small alphabetic letters indicated significant differences among isolates by LSD at $P \leq 0.05$.

Table 4. IAA production of potential isolates in various salinity levels.

Isolates	IAA concentration (μg mL^{-1})				
	Salinity level (dS m^{-1})				
	0	2	4	8	16
1	19.56± 2.69^{b}	13.57 ± 1.43^{b}	10.07 ± 1.86^{b}	11.48 ± 1.75^{b}	7.21±3.32^{bc}
18	45.10 ± 6.99^{a}	33.11 ± 1.00^{a}	28.21 ± 1.39^{a}	46.30 ± 5.19^{a}	21.92 ± 7.59^{a}
22	8.68 ± 0.40^{c}	7.61 ± 1.09^{b}	9.16 ± 1.02^{b}	8.29 ± 4.02^{bc}	6.84 ± 3.40^{c}
29	4.82 ± 0.97^{c}	6.45 ± 1.82^{b}	4.34 ± 0.87^{c}	2.93 ± 0.94^{c}	17.29 ± 1.40^{ab}
F-test	**	**	**	**	**
%CV	19.51	10.48	10.38	19.88	33.87

IAA concentration was expressed by Mean value of 3 replicates ± STDEV and followed by F-test at $P \leq 0.01$ with small alphabetic letters indicated significant differences among isolates by LSD at $P \leq 0.05$.

not inhibit each other but isolate 18 could inhibit strongly the growth of the others. Although isolate 18 had the highest IAA producing activity, we used a mixture of isolates 1, 22 and 29 for corn seed germination test under salinity.

Amount of IAA were produced by isolated bacteria varied among saline concentrations (Table 4). The highest IAA amount produced by isolate 18 and was 46.30 μg/mL at EC 8 dS m^{-1}, while 45.10 μg/mL under normal conditions without NaCl solution supplement. Similarly, isolates 1 and 22 only showed high amount of IAA at EC 2-8 dS m^{-1} and EC 4-8 dS m^{-1} respectively. However, almost isolates were depressed and produced low concentration of IAA at EC 16 dS m^{-1}. According to Mirza *et al.* [24], IAA production by PGPR can vary among different species and influenced by culture condition and growth stage of isolates. The variation was also affected by various biosynthetic pathways, involved gene locations, regulatory sequences, and the presence of enzymes to change form of free IAA [25]. Tripathi reported that low salinity level in fact increased the production of IAA [26]. However, the results of this current study suggested that the IAA synthesis of bacteria could be activated at suitable salinity levels when some isolates showed high capacity at medium amended with salt solution at EC levels of 2, 4 and 8 dS m^{-1} respectively.

Ten out of 26 isolates indicated their inhibition to the growth of *Sclerotium rolfsii* (32.22 to 65.56%) while *Fusarium oxysporum* was inhibited by 8 isolates (4.07 to 18.93%) (Table 5). However, most isolates were able to control the growth of both plant pathogens at varying efficiency. Three of 26 isolates (isolate 1, 6 and 14) had the antagonistic activity to both *S.rolfsii* and *F.oxysporum*. Table 5 showed 15 isolates which could inhibit the growth of *S. rolfsii* and *F. oxysporum*. There were 5 isolates having the best ability to inhibit the growth of *S. rolfsii* (higher than 50%), such as isolates 1, 6, 9, 23 and 27. Moreover, isolate 1 showed the highest growth inhibitory activity (18.93%) on *F. Oxysporum*.

3.3 Seed Germination

In general, root and shoot length were reduced when the concentration of NaCl increased. The result of corn seed germinated for 5 days at various salt concentrations (0-16 dS m^{-1}) showed that corn seeds soaked with EM treatment had a high significant difference of root and shoot length when compared with all treatments. The relative germination index (RGI) increased following the increase of salt concentration. When the corn seed soaked with EM treatment, it presented a high

Table 5. Antagonistic activity of saline soil bacteria against to *Sclerotium rolfsii* and *Fusarium oxysporum*.

Isolates	Percentage Inbition (%)	
	Sclerotium rolfsii	***Fusarium oxysporum***
1	65.56± 1.92[a]	18.93 ± 3.51 [a]
3	43.33± 0.00[e]	-
4	32.22± 1.92[f]	-
6	52.22± 1.92[cd]	8.12 ± 2.34 [cd]
9	46.67 ± 5.77[de]	-
12	-	8.12 ± 2.34 [cd]
13	36.67± 5.77[f]	
14	36.67± 5.77[f]	14.20 ± 2.34 [b]
15	33.33± 0.00[f]	-
18	-	8.79 ± 3.51 [c]
23	54.44± 3.85[bc]	-
25	-	8.12 ± 2.34 [cd]
27	58.89± 1.92[b]	-
28	-	4.07 ± 2.34 [d]
29	-	6.77 ± 0.00 [cd]
F test	**	**
% CV	7.83	26.45

Remark: - Negative result. The antagonistic activity was expressed by mean value of percentage inhibition of radial growth (PIRG) of 3 replicates ± STDEV, followed by F-test at $P < 0.01$ with small alphabetic letters indicated significant differences among isolates by LSD at $P \leq 0.05$.

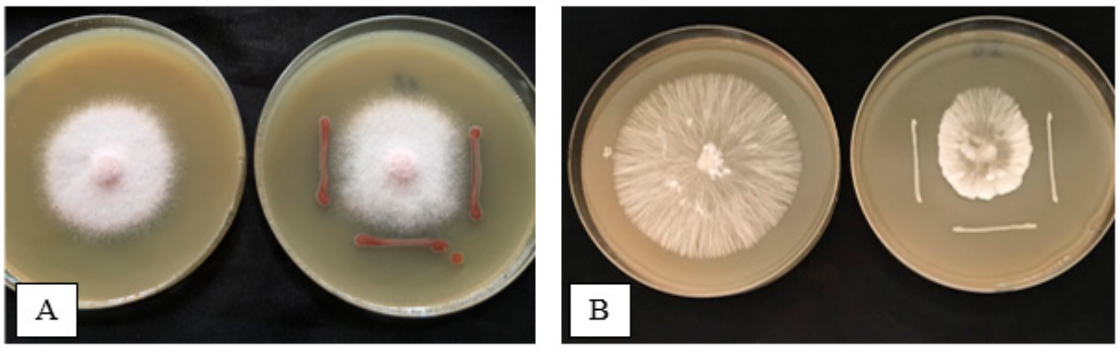

Figure 1. Dual cultures of Isolate 12 and *F. oxysporum* (A); Isolate 6 and *S. rolfsii* (B).

significant difference of RGI as compared with other treatments. Except for the salinity level 4 dS m^{-1}, the BC and SN treatments presented a high significant difference of RGI as compared with control treatment. The results of this research reported that increasing of salinity level showed the decreasing of seed germination. Germination Rate (GR%) and Germination Percentage (GP%)

at the salinity 2,4 and 16 dS m^{-1} did not show any significant effects on the GR% and GP% of corn seeds. During the period of seed germination, the sterile distilled water supplemented with NaCl solution at the salinity 2, 4, 8 and 16 dS m^{-1} were watered the seed on the petri dish. This study demonstrates that at the salinity 2, 4, and 16 dS m^{-1} showed a significant effect on GR% and GP%,

Table 6. Effect of saline concentrations in germination of corn **(***Zea mays***)** at day 5.

Treatments	Root (cm)	Shoot (cm)	RGI	SVI	GR (%)	GP (%)
0 dS m^{-1}						
Control	6.19±0.83^{b}	6.60±0.40^{b}	83.21^{bc}	1108.90^{a}	100.88^{c}	86.67^{ab}
BC	5.49±0.83^{b}	5.73±0.70^{c}	88.66^{b}	1050.31^{a}	122.81^{a}	93.33^{a}
SN	4.90±0.40^{bc}	5.33±0.20^{cd}	79.20^{bc}	820.36^{a}	105.26^{bc}	80.00^{b}
MB	3.42±0.35^{c}	4.57±0.40^{d}	55.28^{c}	719.00^{a}	118.26^{ab}	90.00^{ab}
EM	9.88±1.56^{a}	10.30±0.50^{a}	159.65^{a}	1951.16^{a}	127.19^{a}	96.67^{a}
2 dS m^{-1}						
Control	3.24±0.07^{b}	3.57±0.35^{b}	93.18^{b}	634.28^{a}	94.21^{a}	93.33^{a}
BC	3.02±0.47^{b}	3.70±0.35^{b}	102.53^{b}	626.85^{a}	101.45^{a}	93.33^{a}
SN	2.91±0.76^{b}	3.70±0.77^{b}	99.14^{b}	593.67^{a}	97.83^{a}	90.00^{ab}
MB	2.06±0.74^{b}	2.93±0.81^{b}	70.19^{b}	432.56^{a}	94.21^{a}	86.67^{ab}
EM	9.96±1.83^{a}	10.07±1.7^{a}	338.75^{a}	1802.00^{a}	97.83^{a}	90.00^{ab}
4 dS m^{-1}						
Control	3.47±1.10^{a}	4.00±1.06^{a}	81.07^{bc}	745.67^{a}	97.22^{a}	100.00^{a}
BC	3.35±0.41^{a}	3.90±0.36^{a}	107.72^{ab}	651.59^{a}	97.22^{a}	90.00^{ab}
SN	3.46±0.33^{a}	4.03±0.30^{a}	115.77^{a}	672.67^{a}	97.22^{a}	90.00^{ab}
MB	1.49±0.24^{b}	2.40±0.24^{b}	49.97^{d}	375.46^{a}	100.70^{a}	96.67^{a}
EM	2.21±0.62^{b}	3.10±0.70^{ab}	74.08^{cd}	509.72^{a}	100.70^{a}	96.67^{a}
8 dS m^{-1}						
Control	2.11±0.18^{b}	2.87±0.14^{bc}	101.46^{b}	479.75^{a}	106.06^{a}	96.67^{a}
BC	2.55±0.30^{b}	3.20±0.13^{b}	135.39^{b}	496.97^{a}	98.48^{ab}	86.67^{ab}
SN	2.62±0.38^{b}	3.27±0.33^{b}	139.12^{b}	570.98^{a}	109.85^{a}	96.67^{a}
MB	1.85±0.08^{b}	2.60±0.17^{c}	91.75^{b}	343.30^{a}	90.91^{b}	76.67^{bc}
EM	7.33±0.88^{a}	7.50±0.53^{a}	390.01^{a}	1433.14^{a}	109.85^{a}	96.67^{a}
16 dS m^{-1}						
Control	1.87±0.28^{b}	2.37±0.33^{c}	110.20^{b}	423.33^{a}	100.00^{a}	100.00^{a}
BC	1.81±0.18^{b}	2.80±0.30^{b}	106.36^{b}	425.91^{a}	93.33^{a}	93.33^{a}
SN	2.13±0.24^{b}	2.83±0.22^{b}	124.95^{b}	463.23^{a}	93.33^{a}	93.33^{a}
MB	1.19±0.09^{c}	2.13±0.11^{c}	70.26^{c}	309.04^{a}	93.33^{a}	93.33^{a}
EM	3.48±0.27^{a}	4.03±0.09^{a}	204.90^{a}	809.11^{a}	100.00^{a}	100.00^{a}

Small letters a, b, c, d perform significant differences ($p<0.05$); RGI: Relative Germination Index; SVI: Seed Vigor Index; GR: Germination Rate; GP: Germination Percentage; Control: Sterile distilled water; BC: Bacterial cells of Isolates 1, 22 and 29 suspended in 0.85% NaCl; SN: Supernatant of the mixture of Isolates 1, 22 and 29; EM: Commercial Effective Microorganisms solution in water ratio 1:100.

except for the salinity at level 8 dS m^{-1}. The result showed no significant difference in BC and SN treatments which was similar to the control and EM treatments (Table 6). Furthermore, salt stress had a negative impact on germination percentage.

The result in Figure 2 showed that in the salinity level 8 dS m^{-1}, corn seeds could grow well. It was demonstrated that the corn variety used in this experiment had the growth ability in the high saline concentration, exactly 8 dS m^{-1} (about 68.4 mM of NaCl). This unit of salinity was determined to have a higher value compared to previous reports by Turan *et al.* that presented the corn seeds were inhibited when the level of salinity was greater than 50 mM of NaCl (around 5 to 6 dS m^{-1}) [29]. 8 dS m^{-1} was demonstrated to be a suitable concentration for this corn as 0 dS m^{-1} and 2 dS m^{-1} were observed to be effective for corn seeds growth. Bacteria in the treatment of cell dissolved in distilled water (BD) could

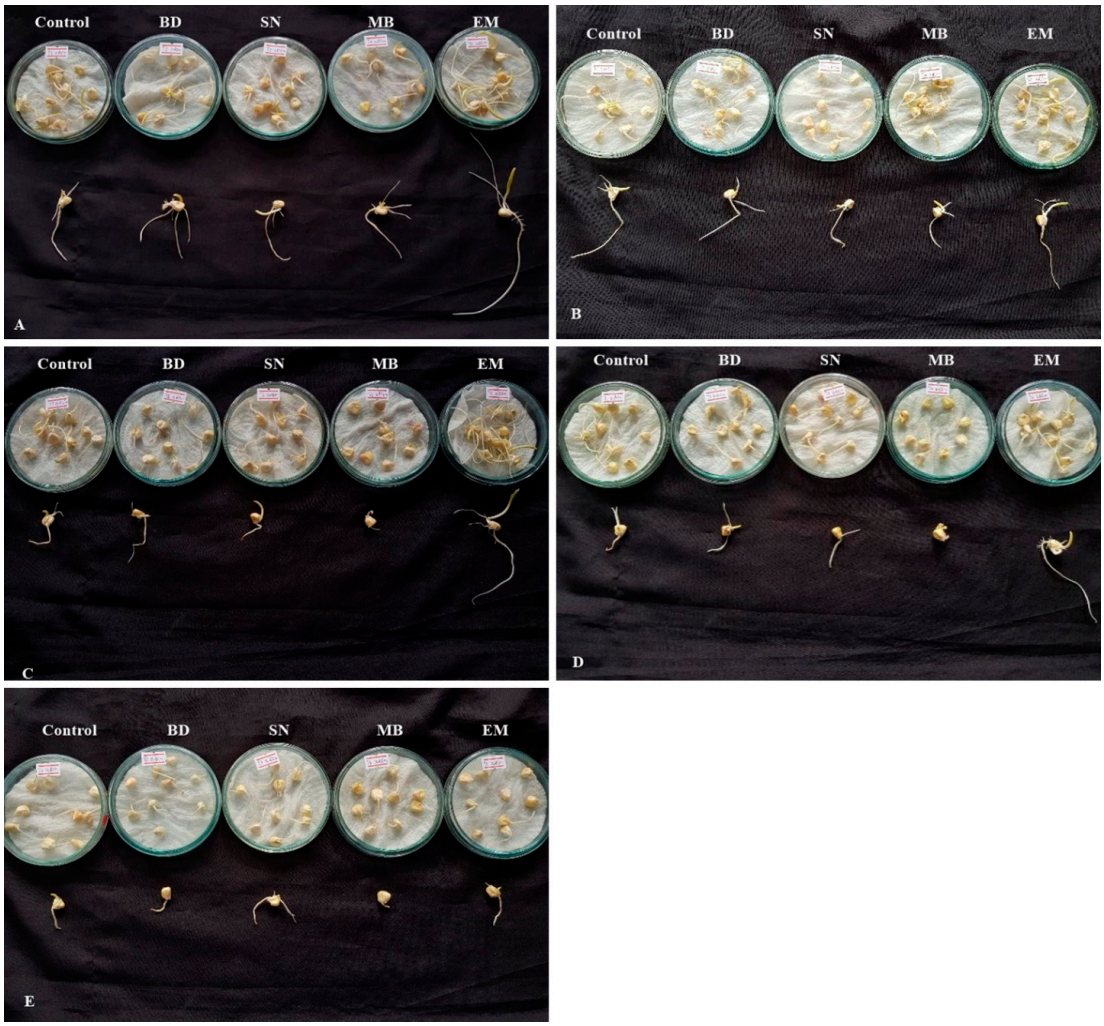

Figure 2. Germination of corn seeds at different salinity levels after 5 days. Five treatments **(**control, BC, SN, MB and EM**)** of corn seeds were watered with NaCl solution at the salinity 0 dS m^{-1}**- A**, 2 dS m^{-1}**- B**, 4 dS m^{-1}**- C**, 8 dS m^{-1}**- D** and 16 dS m^{-1}**- E.**

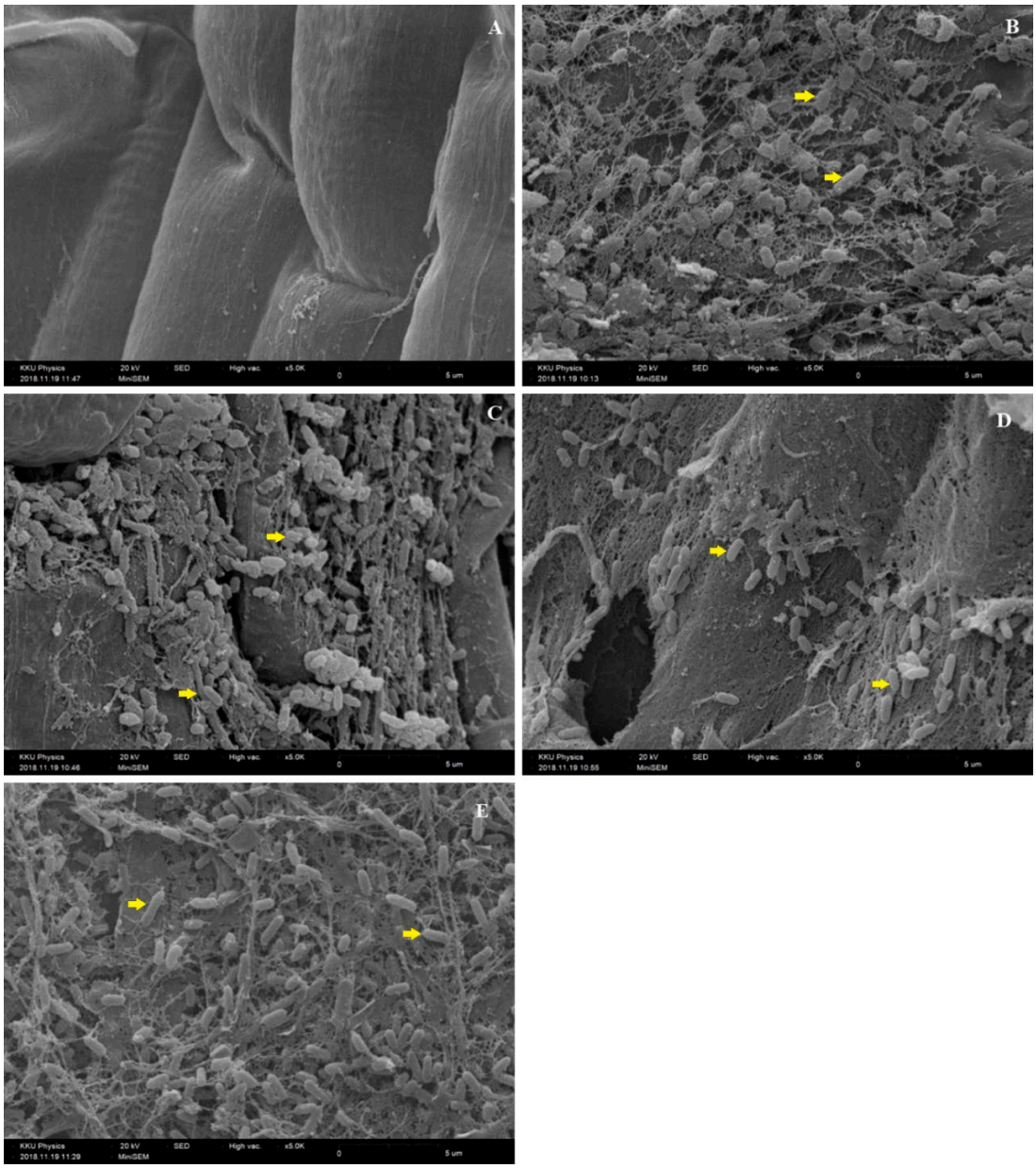

Figure 3. Interaction between salt tolerant bacteria and corn seed after 5 days on *in vitro* aseptic growth conditions.
A: Inoculated without salt tolerant bacteria and salt, **B:** inoculated with salt tolerant bacteria and without salt, **C:** inoculated with salt tolerant bacteria supplemented with NaCl solution at the salinity 4 dS m^{-1}, **D:** inoculated with salt tolerant bacteria supplemented with NaCl solution at the salinity 8 dS m^{-1} and **E:** inoculated with salt tolerant bacteria supplemented with NaCl solution at the salinity 16 dS m^{-1}.

colonize plant root and using carbon sources, nitrogen sources from the root exudates of plant. In addition, bacteria isolate 8 was able to produce IAA hormone up to 46.30 μg mL^{-1} at salinity 8 dS m^{-1}. IAA production was important to root. It could enhance the growth of root with cell division, cell elongation and cell development. These significant advantages demonstrate the potential for BD treatment to provide a positive effect on the growth seed. For supernatant treatment (SN), under salinity stress, the salt tolerant bacteria interacted with each other, producing their own beneficial compounds such as IAA and unknown compounds [2]. Such treatments helped the plant to grow and the overall efficiency was similar to that of EM.

3.4 Study of Root and Salt Tolerant Bacteria Interaction by SEM

The investigation on the ability of rhizobacteria to adhere to the surface of corn roots by using SEM was studied. Corn seeds were cultured on Hoagland medium supplemented with various concentrations of NaCl solution with the salinity (4-16 dS m^{-1}). The corn seed was inoculated with and without salt tolerant bacteria. The results showed that at the different salinity levels, the inoculation with salt tolerant bacteria clearly showed the colonization at the root surface (Figure 3B, 3C, 3D and 3E). The colonization pattern clearly showed that bacterial cells were located mainly on the root surface of corn. Even if, the culture medium has a high salt concentration at 16 dS m^{-1}, good root colonization was apparent (Figure. 3E).

Root colonization is an important requirement for interaction between microorganisms and plants. These bacteria adhere to the root surface and have the ability of colonizing and most importantly, these bacteria promote the growth of plants. In addition, the bacteria are beneficial successful applications in controlled environment and increase biotic and abiotic stresses [30]. The colonization of PGPR in the rhizospheric region increases root exudates including organic acid, amino acid and carbohydrates, which promote the growth and other microbial activities.

4. Conclusions

Bio-fertilizers that are microbial inoculants with the properties to promote plant growth have been developed. They are able to solubilize nutrients in the soil and convert poor soil become fertile soil. Based on the screening in this study, these microorganisms are nitrogen fixing microorganisms, phosphate solubilizing microorganisms and potassium solubilizing microorganisms. The results showed that isolated bacteria had high salt tolerance but some isolates were inhibited by salt concentration 16 dS m^{-1}. Most could promote plant growth efficiently at salinity levels from 2-8 dS m^{-1}. These isolates possessed multiple PGP properties such as nitrogen fixation, IAA production, P and K-solubilization, and antifungal activity. Nine isolates could solubilize tricalcium phosphate with solubilization index ranged from 2 to 3.83. Eleven isolates were able to solubilize K with the range of SI varied from 1.33 to 2.33. Isolate 18 produced the highest amount of IAA (46.30 μg/mL) at salinity level 8 dS m^{-1}. Isolate 1 showed high antagonistic activity against to both *S. rolfsii* and *F.oxysporum*. According to report of He *et al.* [4], they suggested that co-inoculation of three or four microbes was a good strategy for healthy crop production. It was also found that the mixture of isolates 1, 22 and 29 could induce corn seed germination as efficiently as commercial biofertilizer – EM. Moreover, the corn plants were salt-tolerant under high concentration of NaCl (EC 4-8 dS m^{-1}). Salt tolerant bacteria which adhered on surface of corn roots could possibly induce secretion of root exudates beneficial for further bacterial growth in rhizopheric region. Thus, four bacterial strains 1, 18, 22 and 29 were indicated as promising isolates to increase production of crop plants such as *Zea mays* and also improve quality of soil under salt stress.

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