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Contributed Paper

Effects of Agricultural Residues as Carriers for Bio-fertilizer Production to Promote Tomato Growth in Saline Soil

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ABSTRACT

The detection of plant growth promoting properties of three isolates of rhizobacteria (*Enterobacter aerogenes* P8*, Bacillus tequilensis* N15*, Pseudomonas azotoformans* I2.1) showed positive results for nitrogen fixing, phosphate solubilization, ACC deaminase activity, cellulolytic activity and IAA production but activity declined when the concentration of NaCl in the medium was increased. Among the four types of carrier tested (rice husk ash, rice straw, sugarcane leaves and coconut coir), rice husk ash was the best carrier, able to support the highest growth and survival of rhizobacteria under the temperature range of 30-50 °C. The maximum number of survival rhizobacteria cells was found (10^9-10^{10} CFU g^{-1}) on day 15 of incubation, at 30 °C. The growth of tomato plants in two different soils (normal and saline soil) showed that biomass was significantly highest with the treatment of chemical fertilizer supplemented with rhizobacteria. In normal soil, the biomass was 4.46 g pot^{-1}, while it was 5.15 g pot^{-1} in saline soil. The accumulation of proline content in the tissue of tomato in the control treatment (without supplemented rhizobacteria and without supplemented with carrier in both normal and saline soil conditions) was higher than with other treatments. The highest total enzyme activity in soil samples detected by using the fluorescein diacetate hydrolysis (FDA) method was found in the treatment containing rice husk ash as carrier, supplemented with rhizobacteria in saline soil.

Keywords: agricultural residues, carrier, bio-fertilizer, tomato, saline soil

1. INTRODUCTION

Salt-affected soils contain large amounts of soluble salts which are toxic to salt sensitive plants, inhibiting growth. High salinity in soil also causes problems for growth, production and quality of plants due to osmotic stress [1]. Normally, plants growing in saline soils have to use increased energy for the up-take of water and nutrients necessary for growth.

Salt in the soil has the effect of increasing osmotic pressure and decreasing water potential. This causes plants to be dehydrated, and this lack of water may result in plant death. In addition, ion toxicity and nutrient imbalance occurr in saline soils. [1]. Soil salinity is evaluated by measuring the electrical conductivity (EC/salinity) of the total soluble salts of saturated soil extracts prepared by mixing the soil samples with distilled water at ratio of 1:5. Salinity levels that affect plants are classified as follows; soils having EC in the range of 0 to 2 dS/m is considered non-saline, 2 to 4 dS/m slightly saline, 4 to 8 dS/m moderately saline, and 8 to 16 dS/m highly saline [2]. Mostly, yield reduction occurrs when salinity levels in the soils show electrical conductivity (salinity/EC) greater than 2 dS/m. Some plants can tolerate and grow at EC 4-8 dS/m but in the case of salinity level greater than 16 dS/m, most plants show severe symptoms. The tomato plant is moderately tolerant to salinity and can tolerate salinity levels of 8-12 ds/m [2]. It is one of the most commercially important crop plants in Thailand and was chosen for this study as a model plant to investigate the effects of bio-fertilizer on improvement of growth in saline soil.

The use of bio-fertilizer is an alternative method to improve the quality of saline soils by enhancing nutrient rotation and the bio-degradation process using microorganisms. Plant growth promoting rhizobacteria (PGPR) is the group of many bacteria species. Two types of bacteria are in this group. The first group is the symbiotic type of microorganism, bacteria which can live in plant roots. The second type of microbe is the free-living forms which are found surrounding the root zone. Both types can stimulate the growth of plants and enhance crop yields [3]. These microorganisms have two mechanisms to stimulate the growth of plants. The first involves direct stimulation of growth by nitrogen fixation [4], phosphate solubilization, reduction of concentration of ethylene in plants [5] and the production of phytohormones, such as auxin, cytokinin, gibberellins [6]. The second mechanism is related to the ability of PGPR to protect plants from pathogen attack such as production of antibiotics for controlling plant disease [7] and the production of enzymes for degrading the cell wall of fungal pathogen [7].

The application of PGPR in Thailand has been utilized in both solid and liquid forms, which preserve the cells at the concentration of 10^8 cells per gram for at least 6 months. [8]. Cell survival rates depend on the chemical and biological properties of carriers. Thailand has large quantities of agricultural residues, such as rice straw and sugarcane leaves. After harvest, these residues are left in the field as fertilizers for soil maintenance and for promotion of plant growth. They are also suitable for composting. There is also rice husk ash and coconut coir, commonly used in crop cultivation. All of these materials are cheap and can be commonly found in agricultural areas. The objective of this study was to identify suitable agricultural residues to serve as carriers, supporting PGPR in the production of bio-fertilizers to promote the growth of tomatoes in saline soils.

2. MATERIAL AND METHODS

Three isolates of rhizobacteria were screened from rice and grass rhizosphere samples in the northeast of Thailand. These isolates were identified using 16S rRNA sequencing [9]. This indicated that they were similar to *Pseudomonas azotoformans* I2.1, *Enterobacter aerogenes* P8 and *Bacillus tequilensis* N15. Also, they possessed plant growth promoting properties such as nitrogen fixing, IAA producing, phosphate solubilizing

and antagonistic activity.

2.1 Testing of Plant Growth Promoting Activities in Various Saline Concentrations

2.1.1 Nitrogen fixing activity

One loop full of each rhizobacteria was inoculated on an agar plate that contained nitrogen free medium (Ashby's agar) supplemented with 0.2, 0.4, 0.8 and 1.0 M NaCl solutions and nitrogen free medium without NaCl as a control. All petri dishes were incubated at 30 °C for 3 days. The presence of colonies on the medium indicated the ability of bacteria to fix nitrogen and grow under salinity stress.

2.2.2 Phosphate solubilization activity

Three kinds of rhizobacteria were inoculated in the National Botanical Research Institute's phosphate growth medium (NBRIP) with different NaCl concentrations (0, 0.2, 0.4, 0.8 and 1.0 M), and supplemented with $Ca_3(PO_4)_2$, incubated for 2-3 days at 30 °C. The presence of a clearing halo zone around colonies is exhibited for positive phosphate solubilization

2.3.3 IAA producing activity

IAA production was determined using the modified method of Husen E. [10]. Rhizobacteria was grown in glycerol peptone broth with different NaCl concentrations (0, 0.2, 0.4, 0.8 and 1.0 M) and supplemented with tryptophan (500 μg l^{-1}), incubated for 2 days at 30 °C. The solution was centrifuged at 8000 rpm for 15 min and the supernatant was mixed with Salkowski's reagent and orthophosphoric acid. The test tubes were incubated in the dark for 25 min and the solution developed a pink color which was considered a positive result. IAA production was determined at 540 nm using a pure IAA standard graph. IAA was confirmed by using Thin layer chromatography method. Rhizobacteria was cultivated in nutrient broth containing 500 mg l^{-1} L-tryptophan, and incubated at 30 °C on a shaking incubator at 150 rpm for 3 days. IAA was extracted from the supernatants using ethyl acetate in the ratio 1: 2. TLC silica gel 60 F254 was used for detecting IAA production. Butanol-ethyl acetate - ethanol - water (ratio 3:5:1:1) was used as a solvent system. [11]. The extracted sample and standard IAA (10 mg/100 ml) were spotted on TLC plate. Chromatogram was developed with Salkowski's Reagent [12].

2.4.4 Cellulolytic activity

Three rhizobacteria strains were each inoculated on carboxymethyl cellullose agar (*CMC agar*) supplemented with different NaCl concentrations (0, 0.2, 0.4, 0.8 and 1.0 M) and incubated for 48 h at 30 °C. Later, Gram iodine reagent was added to each plate and after 20 min a halo was observed around the bacterial and the Hydrolysis Capacity (HC value) [13] was estimated.

2.5.5 ACC deaminase activity

Rhizobacteria were inoculated in Nutrient broth, incubated at 30 °C for 24 h and then centrifuged at 8000 rpm for 15 min. Bacterial cells were washed with sterilized distilled water three times. Suspension was inoculated by drop plate technique on Dworkin-Foster (DF) minimal salts medium [14] supplemented with different NaCl concentrations (0, 0.2, 0.4, 0.8 and 1.0 M) and supplemented with either ACC (1-aminocyclopropane-1-carboxylate) or $(NH_4)_2SO_4$ for positive control and DF medium without ACC or $(NH_4)_2SO_4$ for negative control. The samples were incubated at 30 °C for 2-3 days. The presence of a colony on the medium supplemented with ACC indicated growth and was considered a positive result.

2.2 Survival of Rhizobacteria in Carrier Materials

In this study, four types of agricultural residues were used as carriers: rice husk ash, rice straw, sugarcane leaves and coconut fiber. The residues were reduced to small pieces with a blender. Five g of carrier was placed into a plastic bag, covered with cotton plug and sterilized in an autoclave at 121 °C for 30 min (3 times). A moisture content of 50% was maintained by adding sterilized distilled water. To enumerate survival cell, the starter culture was prepared. Bacteria were grown overnight in nutrient broth (NB) at 30 °C and then aseptically collected by centrifugation at 8000 rpm for 15 min and re-suspended to get an inoculum density of 10^8 CFU/ml. Ten percentag (w/v) of the rhizobacteria mixture of 3 strains, in the ratio 1:1:1, (with the starter of each cell concentration 10^8 CFU/ml) were inoculated into carriers and incubated at 30 °C, 40 °C and 50 °C for 60 days. Every 15 days of incubation, the carriers were sampled to check rhizobacteria cell survival by spread plate on nutrient agar.

2.3 Application of Bio-fertilizers to Tomato Seedlings Grown in Saline and Normal Soils

The experiment was laid out in a Completely Randomized Design *(CRD*) with 12 treatments T1: (control : without carriers and without bacteria), T2: (chemical fertilizer formula 15-15-15), T3: (bacterial suspension), T4: (chemical fertilizer with rhizobacteria), T5: (rice husk ash), T6: (rice straw), T7: (sugarcane leaves), T8: (coconut fiber), T9: (rice husk ash with rhizobacteria), T10: (rice straw with rhizobacteria), T11: (sugarcane leaves with rhizobacteria), T12: (coconut fiber with rhizobacteria).

Natural saline soils and normal soils were placed into plastic pots (1.5 kg per each kind of soil). The pH, electrical conductivity (EC) and some physical soil properties were analysed before being used for the experiment.

Tomato (*Lycopersicon esculentum Mill*) type Plum Tomatoes of ®Chia tai, Thailand was cultivated on 104 well pots for 2 weeks, after which tomato seedlings were transplanted to the pots (1.5 kg of soil per pot).

For treatments involving the carriers (T5-T12), 5 g of carriers (contained rhizobacteria cell density of 10^9 CFU/g) per pot was added to the soil before transplantation. For T2 and T4, the chemical fertilizer (Nitrogen: Phosphorous: Potassium, ratio 15:15:15) was added into the soils according to the manufacturer's recommended ratio (50 Kg chemical fertilizer ha^{-1}). For T3 and T4, 5 ml of rhizobacteria solution with the cell density 10^9 CFU/ml was inoculated one day after the transplantation of tomato seedlings to the pots. Tomato plants were harvested at day 20 and 40 of cultivation. For plant biomass analysis, shoot and root dry weight were determined by oven drying at 80 °C for 2-3 days.

2.4 Analysis of Proline in Plants

The experiment was performed using the method described by Bates et al [15]. On the last day of harvesting, 0.5 g of fresh leaves sample of plant was taken and mixed with 10 ml of 3% sulfosalicylic acid solution and then ground and passed through filter paper. Two ml of filtrated sample, 2 ml of acid ninhydrin and 2 ml of glacial acid were mixed in the test tube and boiled at 100 °C for 1 h. The reaction was then stopped by immersing the tubes in cool water. Afterward, the reaction was supplemented with 4 ml of toluene with stirring. The absorbance of the toluene fraction was recorded at 520 nm. The proline content was determined by comparison with a standard curve.

2.5 Analysis of Chlorophyll in Plants

Fresh tomato leaves samples were cut into small pieces.(0.05 g) Five ml of 80% acetone was added to the leaves (0.05 g), before incubation in the dark for 48 h at room temperature. Chlorophyll content was determined by using spectrophotometer at 645 and 663 nm and then chlorophyll content was calculated according to the method of Arnon [16]

2.6 Electrical Conductivity (EC) and pH

For determination of EC, 5 g of soil on the day of plant harvest day was sampled. The soil sample was mixed with distilled water in the ratio 1:5. The measurement was performed according to the method of Houba et al. [17] using the EC meter (Mettler Toledo, model FiveEasy FE 30-1). One g of soil sample was mixed with distilled water in the ratio 1:2 and pH was recorded using a pH meter (OHAUS, model STARTER 2100).

2.7 Measurement of Total Enzyme Activity in soil sample

Total enzyme activity was determined by the fluorescein diacetate hydrolysis analysis (FDA). One g of soil sample on the day of plant harvest was mixed with 7.5 ml of 60 mM sodium phosphate buffer (pH 7.6) and 0.1 ml of fluorescein diacetate. The mixture was shaken at 150 rpm for 40 min on a rotary shaker. The chemical reaction was set up by adding 7.5 ml of chloroform and methanol (2:1), and the mixture was centrifuged at 8000 rpm for 10 min. The amount of hydrolyzed FDA was determined at 490 nm by using spectrophotometer (OPIZEN 3220 UV) and compared with the pattern of fluorescein standard curve [18].

2.8 Statistical Analysis

Statistical analysis was performed using the Statistics 8 program. Analysis of Variance (ANOVA) was performed for each dependent variable (plant biomass, soil enzymes, proline, and chlorophyll) versus the independent saline soil and normal soil. In addition, ANOVA was also performed to analyze the effects of microbial inoculation for each carrier. To detect the significant differences ($p < 0.05$) between means, the Tukey HSD test was performed.

3. Results

3.1 Properties of Plant Growth Promoting Rhizobacteria under Different NaCl Concentration

The properties of three species of PGPR are shown in Table 1. All three isolates showed positive results for nitrogen fixing and ACC degradation. Only *Enterobacter aerogenes* P8 was capable of phosphate solubilization and only *Bacillus tequilensis* N15 showed cellulolytic activity. *Pseudomonas azotoformans* I2.1 and *Bacillus tequilensis* N15 showed positive results on IAA production, and IAA product declined when the concentration of NaCl was increased.

In vitro results for nitrogen fixing, phosphate solubilization, cellulolytic activity and IAA production by rhizobacteria at different concentrations of NaCl (0, 0.2, 0.4, 0.8 and 1.0 M).

Thin layer chromatography showed pink colored spots at the Rf that related to the IAA standard (Rf=0.91), isolate N15 (Rf=0.90) and isolate I2.1 (Rf=0.90) as shown in Figure 1. In addition, two isolates grown in medium without tryptophan did not show pink colored spots. It was confirmed that two isolate of rhizobacteria were able to produce IAA.

Table 1. In vitro results for nitrogen fixing, phosphate solubilization, cellulolytic activity and IAA production by rhizobacteria at different concentrations of NaCl (0, 0.2, 0.4, 0.8 and 1.0 M).

	Nitrogen fixing					Tricalcium phosphate solubilization					Cellulolytic activity					ACC degradation					IAA production (mg ml^{-1})				
NaCl concentrations (mol L^{-1})	0	0.2	0.4	0.8	1.0	0	0.2	0.4	0.8	1.0	0	0.2	0.4	0.8	1.0	0	0.2	0.4	0.8	1.0	0	0.2	0.4	0.8	1.0
Pseudomonas azotoformans I2.1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	20.1±1.8	15.4±2.7	8.56±..3.5	3.22±1.4	1.25±3.2
Enterobacter aerogenes N15	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
Bacillus tequilensis P8	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	18.2±4.2	7.11±3.5	4.67±4.4	-	-

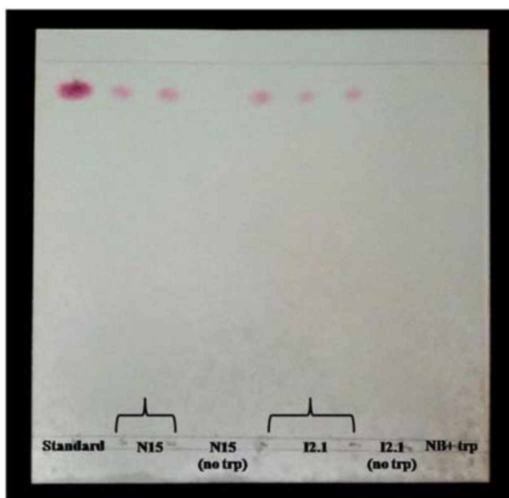

Figure 1. Thin layer chromatogram of rhizobacteria isolate N15 and I2.1 which presented IAA product and detected by using Salkowiski's reagent compared with standard, rhizobacteria grew on medium without tryptophan and nutrient broth containing tryptophan.

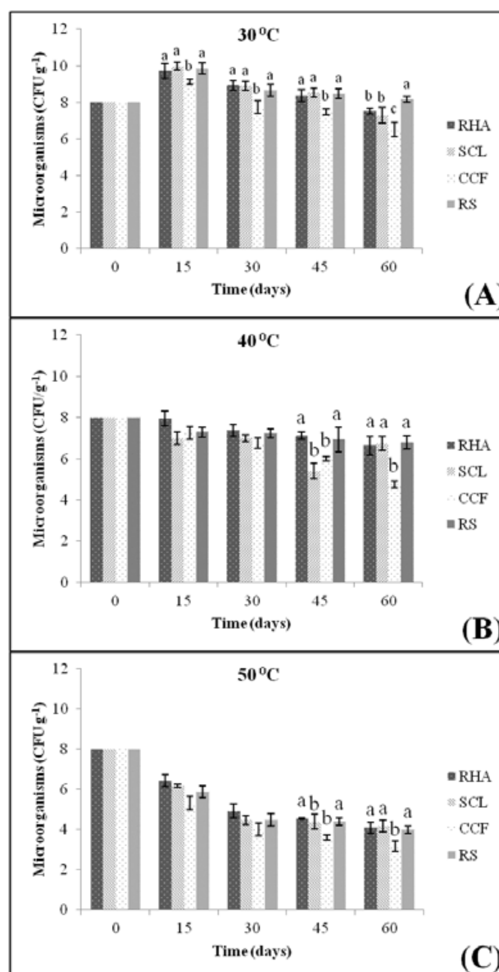

Figure 2. The number of survival cells of mixed rhizobacteria which were cultivated on 4 types of carriers at 30 (A), 40 (B) and 50 (C) °C for 15 to 60 days (RHA: rice husk ash, RS: rice straw, SC: sugarcane leaves and CCF: coconut fiber).

3.2 Survival of Rhizobacteria on the Carriers

The results show that the carriers most suitable for promoting growth and survival of rhizobacteria were rice husk ash followed by rice straw, and sugarcane leaves respectively. The initiating inoculums started at 10^8 CFU g^{-1}. The maximum number of surviving rhizobacteria cells was found (10^9-10^{10} CFU g^{-1}) on days 15 of incubation at 30 °C (Figure 2 A). The rhizobacteria cell cultured in nutrient broth was used as a control in this experiment. It was found that the number of surviving cells at 15 days of incubation was about 10^8 CFU/ml and after 30 days of incubation the l number decreased to 10^7 CFU/ml. This was not a higher amount than in the cells immobilized on carrier materials. Higher temperatures (40 °C and 50 °C) might inhibit the microbial activities and the growth of rhizobacteria cell. Thus, the amount of surviving cells of rhizobacteria in all types of carriers declined (Figure 2B and Figure 2C).

3.3 Plant Analysis

Biomass of plant

The growth of plant on normal and saline soils showed that shoot length, root length and biomass were significant different ($p < 0.01$) in the treatment of chemical fertilizer supplemented with rhizobacteria. The treatment which resulted in the highest growth of tomato plants in both types of soils was one with chemical fertilizer

supplemented with the rhizobacteria, 'chemical (B)' (Figure 3 and 4; Table 2). In normal soil, shoot length, root length and biomass in the 'chemical (B)' treatment were 56.33 cm, 33.00 cm and 4.46 g pot^{-1}, respectively while these parameters were 48.67 cm, 37.00 cm and 5.15 g pot^{-1}, respectively in saline soil (Table 2). In addition, testing of the effects of bio-fertilizer production on various carriers found that rice husk ash mixed with rhizobacteria treatment was the condition yielding the most positive effect on tomato growth .

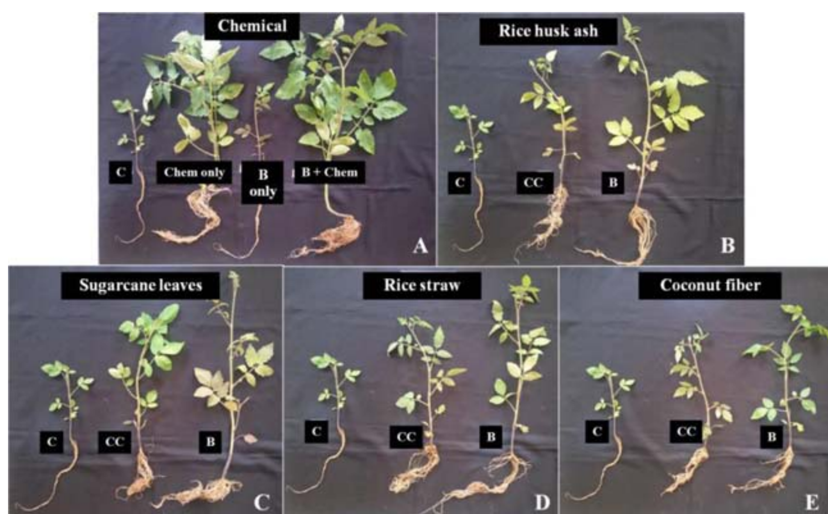

Figure 3. Growth of tomato in normal soil supplemented with chemical fertilizer (A), rice husk ash (B), sugarcane leaves (C), rice straw (D), and coconut fiber (E). The labels of plants in each figure represent the following treatments; C, control (without carrier and without rhizobacteria); CC, carrier without rhizobacteria; B, carrier with rhizobacteria; B only, rhizobacteria suspension only.

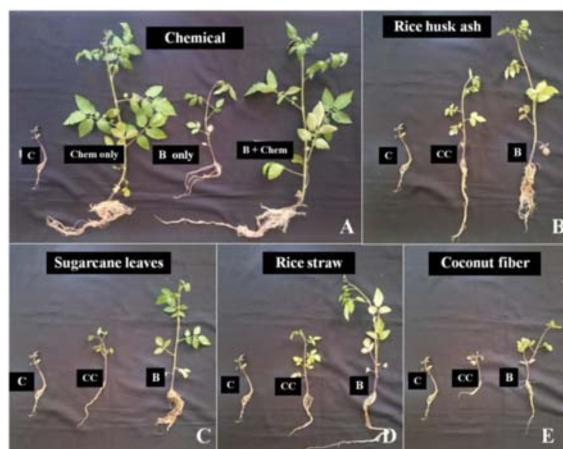

Figure 4. Growth of tomato in saline soil supplemented with chemical fertilizer (A), rice husk ash (B), sugarcane leaves (C), rice straw (D), and coconut fiber (E). The labels of plants in each figure represent the following treatments; C, control (without carrier and without rhizobacteria); CC, carrier without rhizobacteria; B, carrier with rhizobacteria; B only, rhizobacteria suspension only.

Table 2. Effect of bio-fertilizer produced mixing different carriers or chemical fertilizer with rhizobacteria on tomato growth (*Lycopersicon esculentum Mill.*) in normal and saline soil at 40 days of harvesting.

Treatments	Normal soil			Saline soil		
	Shoot length (cm)	Root length (cm)	Biomass (g pot^{-1})	Shoot length (cm)	Root length (cm)	Biomass (g pot^{-1})
T1: Control	11.67±0.6^{f}	15.67±1.2^{d}	0.28±0.1^{g}	7.00±1.0^{e}	12.67±3.8^{def}	0.16±0.6^{e}
T2: Chemical fertilizer	51.33±7.1^{ab}	31.00±3.6^{ab}	3.80±0.5^{b}	46.00±1.0^{a}	35.67±6.0^{ab}	3.85±0.3^{b}
T3: Rhizobacteria	20.67±1.4^{ef}	27.67±4.0^{abc}	0.86±0.1^{fg}	18.67±6.1^{bcd}	21.67±3.5^{cd}	0.56±0.5^{de}
T4: Chemical fertilizer (B)	56.33±6.5^{a}	33.00±3.5^{a}	4.46±0.2^{a}	48.67±2.3^{a}	37.00±6.6^{a}	5.15±0.2^{a}
T5: Rice husk ash	24.00±2.0^{de}	18.00±2.0^{d}	1.28±0.5^{ef}	20.00±5.1^{bcd}	21.67±2.1^{cd}	0.62±0.2^{de}
T6: Sugarcane leaves	25.33±0.6^{de}	21.00±4.0^{cd}	1.08±0.1^{ef}	11.33±1.7^{de}	20.00±3.6^{cde}	0.31±0.3^{e}
T7: Rice straw	26.00±1.0^{de}	24.00±3.0^{abcd}	0.96±0.7^{ef}	11.67±0.6^{de}	16.00±2.6^{def}	0.31±0.4^{e}
T8: Coconut fiber	20.67±0.6^{de}	24.33±3.8^{abcd}	0.72±1.0^{fg}	8.33±3.1^{e}	9.67±1.5^{f}	0.22±0.1^{e}
T9: Rice husk ash (B)	46.67±2.1^{b}	31.67±3.5^{ab}	2.70±0.4^{c}	25.00±1.2^{b}	26.67±2.6^{bc}	1.26±0.7^{c}
T10: Rice straw (B)	35.67±3.5^{c}	30.00±2.0^{abc}	1.94±0.4^{d}	22.67±1.5^{bc}	21.00±1.5^{cd}	0.97±0.4^{cd}
T11: Sugarcane leaves (B)	32.33±1.5^{cd}	29.33±1.2^{abc}	1.59±0.2^{de}	23.00±3.0^{bc}	10.67±0.6^{ef}	0.67±0.8^{de}
T:12 Coconut fiber (B)	26.67±0.6^{cde}	23.67±1.2^{bcd}	1.14±0.5^{ef}	15.33±2.5^{cde}	9.67±2.2^{f}	0.38±0.7^{e}
F-test	**	**	**	**	**	**
% CV	10.25	11.83	12.41	13.89	16.67	15.21

Control: without carrier and without rhizobacteria, **(B)** : supplemented with rhizobacteria
**significant at the level $p < 0.01$
Different letters in the column represent significant differences among treatments ($P < 0.05$).

3.4 Chlorophyll and Proline in Plant

The chlorophyll content in tomato leaves is shown in Figure 5A. The results revealed that T2 : saline soil supplemented with chemical fertilizer treatment and T4: normal soil supplemented with chemical fertilizer mixed with rhizobacteria treatment showed the highest chlorophyll content, which are significantly different ($p < 0.05$) from all other treatments.

Different letters represent significant differences among treatments ($p < 0.05$) and values with the same letters are not significantly different ($p > 0.05$)

Moreover, proline content in plant tissues was detected. Leaf tissues of tomato in control treatment (without supplemented rhizobacteria and without supplemented with carrier to normal and saline soil) accumulated significantly higher proline content ($p < 0.05$) than all other treatments. In addition, for each treatment the accumulation of proline content was higher in plants grown in saline soil than normal soil (Figure 5B).

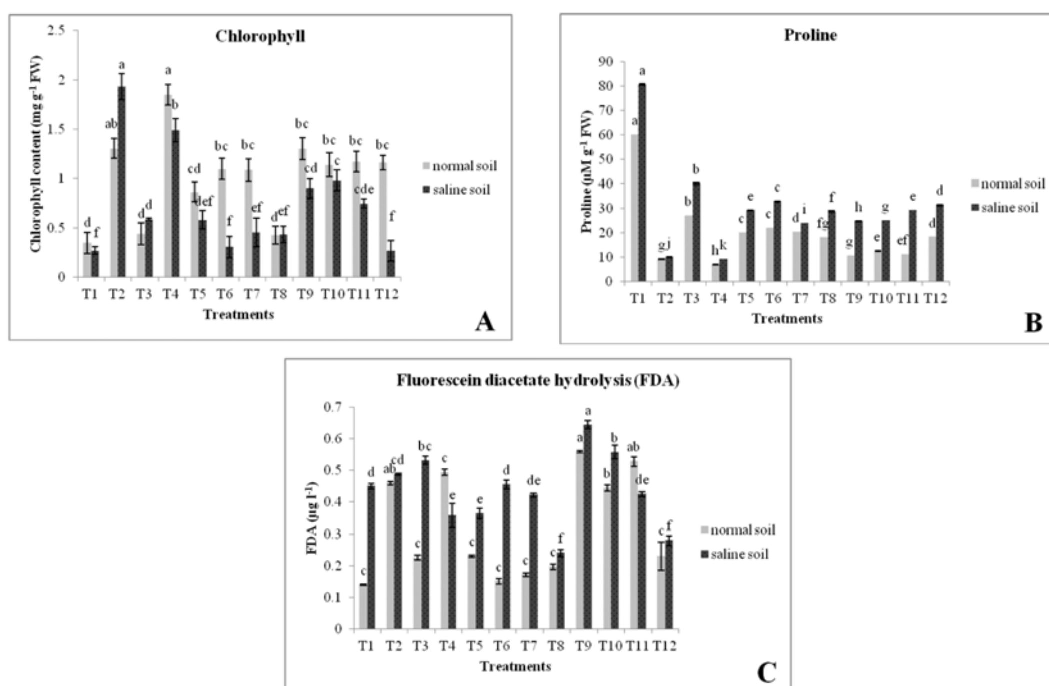

Figure 5. Chlorophyll content (A) and proline content (B) in tomato plants grown in normal and saline soils supplemented with chemical fertilizer or carriers with or without rhizobacteria and total enzyme activity in the soil samples collected on the final day of harvest determined by FDA hydrolysis test (C). **T1**: (Control : no carrier, no rhizobacteria), **T2**: (chemical fertilizer), **T3**: (rhizobacteria suspension), **T4**: (Chemical fertilizer with rhizobacteria), **T5**: (rice husk ash), **T6**: (rice straw), **T7**: (sugarcane leaves), **T8**: (coconut fiber), **T9**: (rice husk ash with rhizobacteria), **T10**: (rice straw with rhizobacteria), **T11**: (sugarcane leaves with rhizobacteria), **T12**: (coconut fiber with rhizobacteria).

3.5 Soil Analysis

3.5.1 EC and pH analysis

In this study two different soils (normal and saline soils) were used. Before being used for growing plants, the normal soil samples showed average EC values of 0.029 mS/m, pH value of 6. Total microorganisms in the normal soil included 10^7 CFU/ml of total bacteria, 10^4 CFU/ml of actinomyces and 10^5 CFU/ml of fungi. The saline soil samples showed an average EC of 2.15 mS/m, pH of 5 and total microorganisms in saline soil was 10^7 CFU/ml(bacteria) and 10^4 CFU/ml of fungi. On the harvesting day, when tomato plants were 40 days old, both soils were sampled to re-check EC and pH values. It was found that the EC values for both soils did not change but the pH of normal soil increased to 7 and that of saline soil increased to 6.

3.6 Soil Enzyme Activity

The total enzyme activity in soil samples on the day of harvest detected by fluorescein diacetate hydrolysis (FDA) method is shown in Figure 5C. The T9 (rice husk ash mixed with rhizobacteria) showed the highest FDA content in saline soil and normal soil which is significantly higher than the contents in all other treatments.

4. Discussion

Mostly, plant growth promoting rhizobacteria (PGPR) are in the genera *Pseudomonas, Azospirillum, Azotobacter, Bacillus*, *Burkholderia*, *Serratia*, and actinobacteria, especially species from the genus *Streptomyces*. In this experiment, rhizobacteria used were *Enterobacter aerogenes* P8, *Bacillus tequilensis* N15 and *Pseudomonas azotoformans* I2.1. The PGPR were able to enhance growth and yield of plants. These microorganisms have been used for fixing nitrogen gas to convert to ammonium and nitrate forms that plants can uptake for growth [3]. Moreover, the soil nutrients were changed to available forms by these microorganisms such as phosphate solubilizing microorganisms which can release organic acids to solubilize phosphorus or rock phosphate to become phosphate in available forms (HPO_4^{2} and $H_2PO_4^{-}$) [19]. Phosphate solubilizing bacteria was found in many genera such as *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Micrococcus, Streptomyces* and *Aereobacter*. For the phytohormones production of PGPR most work has focused on the production of auxin (indole-3-acetic acid or IAA) production which can activate cell elongation, cell division, and cell differentiation. PGPR which produce auxins are beneficial for root and shoot growth of plants. Moreover, PGPR can produce many enzymes such as hemicellulases, chitinases, amylases, cellulase and glucanases [20] for hydrolysis of cell wall, lignin, cellulose and hemicelluloses of plants. These enzymes have the role on degradation of organic matter and nutrients in soil. Plants produce 1-amino-cyslopropane-1-carboxylate (ACC) which is the ethylene precursor. High levels of ethylene in plants can inhibit seed germination and root elongation. However, PGPR is able to degrade ACC using 1-amino-cyslopropane-1-carboxylate (ACC) deaminase to ammonia and alphaketobutyrate resulting in growth promotion by inhibition or reduction of ethylene production in plants. [21]

The reason for supporting microbial cells on the carriers in bio-fertilizer products was to reserve and allow cells to multiply for a longer time. Kishore *et. al.* [22] presented that microorganisms could survive longer on peat carrier due to the ingredients in peat acting as a source of nutrient for the microorganism. However, in Thailand, peat is expensive and the supply is limited. Therefore, it is necessary to find suitable local materials to use in bio-fertilizer production. The carrier must be non-toxic, able to maintain moisture content and be rich in nutrients. The main components of rice straw and sugarcane leaves contain cellulose, hemicelluloses, lignin and subunits of cellulose (glucose) which is an important source of nutrients for microbes. Microorganisms can produce enzymes to degrade these substrates to glucose which is then consumed for growth and as an energy source. Degradation depends on pH and environmental factors such as temperature, moisture content and weather. The maximum number of surviving rhizobacteria cells was found (10^9-10^{10} CFU g^{-1}) on day 15 and incubated at 30 °C. Rice husk ash is the source of carbon and silica and the porous property structure is good for supporting microorganisms and retaining moisture. This means the plant can uptake silicon element via their root in a soluble form.[23] Moreover, using rice husk ask ash can improve soil the structure and increase soil aeration. The reports of Jackson et al. [24] presented that rice husk ash, vermiculite, peat, wheat bran, alginate and clay are good materials for use as a carrier. Microorganisms can multiply and survive in these carriers for long periods. We found that the best carrier for producing bio-fertilizer was rice husk ash. However,

the quality of cell adhesion depends on the physical and biological properties of the carrier material, which will promote the survival of the microorganisms.

Saline soil has high salt ion concentration which accumulates in soil, which has adverse effects on plant growth and development. This study was conducted to investigate the effects of salinity on tomato growth by comparing saline and normal soil. Tomato plant was used as a model in this experiment because it is moderately tolerant to salinity and is a commercially important vegetable crop for Thailand. In this present work, the treatment of chemical fertilizer supplemented with beneficial microorganisms showed the highest biomass production in both soil conditions. The biomass production in saline soil was 5.15 g pot^{-1} which was greater than in normal soil (4.46 g pot^{-1}). This was due to the salinity effects on tomato growth, similar to the results of Tank and Saraf [25] which showed that 2%NaCl had the negative effect on tomato plants by reducing growth. Moreover, the treatment of applying rice husk ash carrier supplemented with beneficial microorganisms (T9) was able to activate tomato plant growth better than applying rice husk ash carrier without microorganisms, and also better than using the other three types of carrier. Rice straw and sugarcane leaves are fresh agricultural materials which could produce both beneficial and toxic products after degradation by microbial activities. Coconut fiber, could improve the structural of soil by increasing porosity and enhancing air-flow, but it has fewer nutrients and was therefore less effective than rice husk ash for the growth of microorganisms or plant. Rice husk ash, on the other hand, contains silica components obtained from the burning process of rice husk. Elawad et al. in 1982 [26] found that at maturity stage of plants, silica compounds has the potential to increase plant height by stimulating cell growth. Moreover, the rice husk ash can also improve the structure of soil by increasing soil porosity and help to maintain soil moisture. Thus, using rice husk ash as carrier was effective for increasing soil microorganism population and hence enhancing plant growth. The better growth in rice husk ash was due to the properties of beneficial microorganism inoculants with multifunctional duties such as nitrogen fixation, phosphate solubilizing and synthesis of IAA. Moreover, in this experiment, rice husk ash presented the best qualities for supporting microorganism in the bio-fertilizer process and improved soil structure better than other carriers. In addition, the immobilization of bacteria in the carrier reveals that bacteria can survive longer when compared with using cell suspension directly into the soil. This is similar to the result of Shilev et al. [27] which identified the effects of IAA producing bacteria on plant growth and development under salinity stress.

Salinity has an adverse effect on plant growth and decreases crop yield. The accumulation of glycine and proline correlate with salinity, water stress, and other plant stresses [26]. Normally, proline accumulates in leaf and root tissues under salt stress. Aziz et al. [28] indicated that it plays an essential role in tolerance to these stresses and protects against the osmotic stress.

Hydrolysis of fluorescein esters has been used to determine microbial activity in environmental samples. This analysis method interprets protease, lipase, and esterase activities in the soil sample. In this study, all kinds of carriers supplemented with beneficial microorganisms showed the highest FDA value, which was greater than in carriers without microorganisms and two samples showed significant increasing of enzyme activity in both normal and saline soils. This might be due to the potential of beneficial

microorganisms (halotolelant rhizobacteria which used in this experiment) to enhance soil fertility. This is similar to the results of Sofia et al. [29] who studied FDA in cultivated sunflower soil, which found that the FDA was varied in soil irrigated with water and 1 and 2 g NaCl L^{-1}.

5. Conclusion

In this study, microorganisms immobilized on carriers promoted the growth of tomato in saline and normal soils. Using rice husk ash as a carrier for microorganism (T9) gave the best result according to biomass data The amount of microorganism and soil enzyme activity (FDA) were also highest in T9. We conclude that rice husk ash immobilized with microorganism production is suitable for treating plant as bio-fertilizer. Its utilization reduces the need for chemical fertilizers which can create a range of soil problems when used repeatedly for an extended period of time.

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