Plant Growth Promoting Activities of Spore-Forming and Vegetative Cells of Salt-Tolerant Rhizobacteria under Salinity Condition

Tiptida Kidtook[1], Jindarat Ekprasert[2], and Nuntavun Riddech[2*]

[1]*Graduate School of Microbiology Program, Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*

[2]*Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*

ARTICLE INFO

Received: 29 Oct 2021
Received in revised: 18 Jan 2022
Accepted: 23 Jan 2022
Published online: 17 Feb 2022
DOI: 10.32526/ennrj/20/202100211

Keywords:
Spore forming rhizobacteria/
Vegetative cell rhizobacteria/
Salinity condition

*** Corresponding author:**
E-mail: nunrid@kku.ac.th

ABSTRACT

Plant growth promoting rhizobacteria are able to enhance plant growth. This study isolated spore-forming rhizobacteria from vegetable rhizosphere soil samples. These isolates were tested for their abilities to promote plant growth and to be used in bio-fertilizers. Thirty-nine isolates with different characteristics were obtained. Three isolates, TYS1.1, TYS3.3, and TYS3.5, showed multifunctional activities on nitrogen fixation and potassium solubilization. They were tested for IAA production in liquid medium supplemented with tryptophan and NaCl, with the vegetative cells of isolate TYS3.5 showing the highest IAA production. The colonization of the three isolates on okra roots was checked by spread plate technique and scanning electron microscope. It was found that rhizobacteria could colonize plant roots with a concentration of 8.19 log CFU/g in the presence of 50 mM NaCl solution. Bio-fertilizer was produced by immobilizing the mixture of three isolates on carriers. The viable cells were enumerated during the storage at room temperature for 60 days. The results showed that the highest number of survival cells in the form of vegetative and spore-forming cells were obtained when using rice husk ash and vermiculite as a carrier. The concentration of viable cells was in a range of 8.14-8.44 log CFU/g. These isolates were *Bacillus* sp. according to the 16S rDNA sequencing analysis.

1. INTRODUCTION

Saline soil is an unfertile soil. In saline soil, salt ions accumulate and affect the growth and productivity of plants, especially in the dry season. Moreover, the imbalance of nutrient-uptake by plants in saline soil might be caused by salt ions. Where agriculture is undertaken, it is therefore necessary to improve the quality of saline soils. Chemical fertilizer is commonly utilized, yet its continuous use over prolonged periods can lead to negative effects on the soil, the wider environment and the organisms present. Consequently, the availability of an ecofriendly alternative such as a bio fertilizer would be beneficial

Plant growth promoting rhizobacteria (PGPR) are genetically diverse microorganisms found in soil. They are an important factor in the decomposition and turnover of minerals and also promote plant growth and provide protection. There are many types of soil microorganisms, including bacteria, fungi and actinomycetes which are classified as PGPR. PGPRs have the ability to convert unavailable forms of soil nutrients into available forms for plant growth. Soil microbes have naturally versatile functional relationships. In the environment, interaction between microbes show both positive and negative effects. Thus, to apply and also to keep a long shelf-life of microbial products in the natural soils, it is necessary to immobilize microbes on supporting material such as organic or inorganic carriers.

The production of microbial inoculants as bio-fertilizers requires development. The first step of the simple production process is the propagation of microbial cells in a culture broth. Next is the immobilization of microorganisms on sterile carrier materials and the last step is the incubation of immobilized products at room temperature for 1-2 weeks. Many types of carriers can be used for bio-fertilizer production. For instance, organic carriers

Citation: Kidtook T, Ekprasert J, Riddech N. Plant growth promoting activities of spore-forming and vegetative cells of salt-tolerant rhizobacteria under salinity condition. Environ. Nat. Resour. J. 2022;20(3):257-265. (https://doi.org/10.32526/ennrj/20/202100211)

such as peat, bagasse and rice husk ash, and inorganics carriers such as alginate and vermiculite are normally used as carrier materials. To reduce the cost of bio-fertilizer and to realize zero waste for agricultural sectors, the residues or waste from agro-industries could be used as carriers. The microbial inoculant can be both in the form of vegetative cells and endospores. However, the use of microbial vegetative cells in bio-fertilizer is less recommended due to both their intolerance to soil abiotic and biotic stresses and their short shelf-life. Thus, to enhance microbial survival rates under stress environments, utilizing endospores is most preferable.

Bacillus spp. is classified as a plant growth promoting bacteria (PGPB). It has the ability to promote plant growth through its nitrogen fixation activity, phosphate and potassium solubilization activities and also phytohormone production, such as IAA and gibberellic acid (Shen et al., 2016). The role of *Bacillus* in the ecosystem is as a decomposer of organic matter in soil, which can become nutrients for plant growth. *Bacillus* is characterized as an aerobic gram-positive bacterium. It is spore-forming and so able to tolerate stress conditions. Therefore, *Bacillus* can be found in heat, cold, drought, nutrient-lacking conditions, and in saline soil. Under suitable conditions for growth, *Bacillus* endospores can turn into vegetative forms of bacterial cells. Moreover, it has the ability to survive and proliferate in various environments (Lyngwi and Joshi, 2014). A previous report found that *Bacillus* alleviates the harmful effects of salt stress and enhances the growth of peanuts grown in sodium chloride-adjusted soils with various plant growth-promoting microbial properties such as phosphate solubilization, ammonia, IAA and siderophore production (Goswami et al., 2014). For this reason, *Bacillus* sp. can be utilized as a bio-fertilizer and applied to cultivation areas with stress conditions. The aim of this study was to compare the abilities of vegetative cells and endospores of rhizobacteria for use as a bio-fertilizer to promote plant growth. The results obtained from this study can be used as a model to produce bio-fertilizers suitable for plant cultivation in saline soil.

2. METHODOLOGY

2.1 Screening of spore-forming microorganisms from rhizosphere soils

Rhizosphere soil samples of vegetable plants (*Brassica rapa* L., *Apium graveolens* L., *Lactuca sativa* L., *Brassica alboglabra*, and *Coriandrum sativum* L.) were collected from agricultural fields in Khon Kaen Province. Rhizobacteria were isolated from soil samples using spread plate technique according to a modified method of Bal and Adhya (2012). Five grams of soil was suspended in 45 mL of distilled water and then incubated in a water bath at 80°C for 12 min (Watterson et al., 2014) to stimulate spore germination. After that, the flasks were immediately transferred to incubate in a water bath at room temperature. Soil suspensions were diluted to 10^{-4}-10^{-6} and then spread on the Nutrient Agar (NA) and Tryptone Yeast Extract (TYE) agar media in order to screen for spore forming colonies (Verma et al., 2013). Plates were incubated at 30°C for 24-48 h. Single colonies were subsequently subcultured onto fresh media for purification. Cultures of pure isolates were stored in 20% glycerol and kept at -20°C for PGPR activity test and biofertilizer production.

2.2 Gram Staining and Endospore staining

Colonies of the isolates were gram-stained for visualization under a microscope and their Gram types classified. Gram-positive bacterial isolates were stained with malachite green solution in order to visualize spore formation under a microscope. Spore-forming bacteria were selected for use in further experiments.

2.3 Preparation of vegetative cells and endospores

To cultivate vegetative cells, bacterial isolates were cultured in nutrient broth (NB) and incubated at 30°C with shaking at 150 rpm for 18 h. Then, cell suspensions were centrifuged at 7,000 rpm for 20 min at 4°C to remove supernatants. Cell pellets were washed twice with 0.85% NaCl. Bacterial cells were dissolved in a 0.85% NaCl solution to obtain a cell density of 10^8 CFU/mL.

Endospores were prepared by using the modified method of Omer (2010). Bacterial isolates were cultured in TYE medium and incubated with shaking at 150 rpm at 30°C for 48 h. Then culture broth was boiled at 80°C for 12 min, afterward the endospores were harvested by centrifugation at 7,000 rpm for 20 min and subsequently washed twice with 0.85% NaCl solution. Concentrations of endospores were adjusted to 10^8 CFU/mL. The number of endospores was determined by counting on agar plate and the formation of spores confirmed using a spore staining technique.

2.4 Determination of plant growth promoting abilities of the vegetative cells and spore-forming cells of the selected bacterial isolates

2.4.1 Screening for nitrogen fixation activity

To determine the nitrogen fixation activity, vegetative and spore-forming cell suspensions were diluted to 10^{-1}-10^{-6} and spread onto a nitrogen free culture medium (Ashby's agar). Plates were incubated at 30°C for seven days. Colonies grown on Ashby's agar were indicative of positive nitrogen fixing activity. This was also confirmed by the inoculation of bacterial suspension into 10 mL Ashby's broth, and incubation by shaking at 150 rpm, at 30°C, for three days. The uninoculated media served as a control. Microbial growth in the nitrogen-free culture broth (Kumar et al., 2014) indicates a positive activity.

2.4.2 Phosphate solubilization activity

Phosphate solubilization activity of the bacterial isolates was evaluated using the National Botanical Research Institute's Phosphate growth medium (NBRIP) according to the modified method of Roslan et al. (2020). Bacterial cultures were point-inoculated onto NBRIP agar supplemented with tricalcium phosphate as an inorganic phosphate source. Plates were then incubated at 30°C for seven days. Clear zones around the colonies indicated the ability of bacteria to solubilize the inorganic phosphates.

2.4.3 Potassium solubilization activity

Potassium solubilization activity (Sun et al., 2020) was determined by point inoculation of the bacterial isolates onto the Aleksandrov agar medium containing potassium aluminum silicate as a source of inorganic potassium. Plates were then incubated at 30°C for seven days. Clear zones around the colonies showed the inorganic potassium-solubilizing ability.

2.5 IAA production

IAA-producing activity of the bacterial isolates was tested using the modified method of Ozdal et al. (2017). One mL of vegetative cells and spore forming cells of bacteria was inoculated into 10 mL of Tryptic soy broth (TSB) supplemented with tryptophan 1,000 mg/L and NaCl at a concentration of 0, 50, and 100 mM. Bacterial culture was incubated at 30°C with shaking at 150 rpm for three days. Two mL of the suspension was centrifuged at 10,000 rpm for 2 min. Supernatants were mixed with 2 mL of Salkowski reagent and then incubated in the dark for 25 min. A pink color appearing in the solution is an indicator of positive IAA production. The amount of IAA produced was determined by measuring absorbance at a wavelength of 530 nm using a spectrophotometer and then compared to an IAA standard curve.

2.6 Determination of root colonization of rhizobacteria under salinity condition using a conventional method and a scanning electron microscope (SEM)

According to the conventional method, seeds were grown on 6% water agar supplemented with 0, 50, and 100 mM of sodium chloride solution under a sterile condition for 7-15 days at room temperature. Root colonization was checked using a spread plate technique on tryptic soy agar.

For the determination of root colonization by SEM (Gajbhiye et al., 2019) the initial concentration of vegetative cells was 10^8 CFU/mL. Seeds were surface-sterilized by using 70% ethanol for 5 min and soaking with 3% (v/v) sodium hypochlorite for 5 min. Then, they were rinsed five times with sterile water, and soaked in rhizobacterial suspension for 30 min. Seeds were transferred into Hoagland's solution containing 0, 50, and 100 mM of sodium chloride solution under a sterile condition. Seeds were then incubated at room temperature for 7-15 days. Root samples were rinsed with 0.1 M phosphate buffered saline (PBS) pH 7.0 and then fixed with 2.5% glutaraldehyde, the samples were stored at 4°C for 2 h. The root samples, sized 3-5 mm, were soaked in PBS solution for 10 min three times. Then, the roots were soaked with different concentrations of ethanol (50, 60, 70, 80, and 90%) for 15 min of each concentration and finally soaked with absolute ethanol for 30 min at 4°C. Root samples were dried by critical point dryer (CPD). These samples were gold-coated prior to observation under SEM.

2.7 Bio-fertilizer production in the form of vegetative cells and spore-forming cells

In this study, bio-fertilizer was produced by immobilizing the vegetative cells and spore-forming cells on the carriers such as rice husk ash, bagasse, vermiculite and sodium alginate. The carrier materials (rice husk ash, bagasse and vermiculite) were prepared by autoclave sterilization. The humidity of carrier materials was then adjusted to be in the range 40-50%. Bacterial cells were inoculated at a concentration of 10% into each carrier material, mixed with 15% of starch and 2% PEG to make the granular bio-fertilizer, and then incubated at 50°C for 24 h.

The immobilization of bacteria on sodium alginate was performed by the method of Bashan et al. (2002). One hundred mg of skimmed milk powder was dissolved in hot water at 90°C and 1.6 g of sodium alginate (NaAlg) was added before sterilization. The solution was left to cool down at room temperature. Then, 10% (v/v) of a bacterial suspension was inoculated into the solution of sodium alginate under a sterilized condition. The mixture of rhizobacterial and immobilization solution was transferred into a 10 mL syringe and added dropwise into the 0.1 M $CaCl_2$ solution with stirring. After 30 min, alginate granules were separated from 0.1 M $CaCl_2$ solution and washed twice with 0.85% NaCl solution under a sterilized condition. Surviving vegetative and spore-forming cells of microorganisms immobilized on the carriers as bio-fertilizers were studied for the eight treatments: T1: alginate mixed with vegetative cells, T2: alginate mixed with spores, T3: bagasse mixed with vegetative cells, T4: bagasse mixed with spores, T5: rice husk ash mixed with vegetative cells, T6: rice husk ash mixed with spores, T7: vermiculite mixed with vegetative cells, T8: vermiculite mixed with spores. Survival of bacterial cells and spore-forming cells was determined for 60 days at 30°C (sampled at day 0, 15, 30, 45, and 60) by spread plate technique. The pH and electrical conductivity (EC) values were measured.

2.8 Statistical analysis

The significant differences among the data were analyzed using ANOVA and the Least Significant Difference (LSD) by using the Statistic 10 program ($p<0.5$).

3. RESULTS AND DISCUSSION

3.1 Isolation of rhizobacteria and determination of plant growth promoting activities in vegetative and spore-forming cells

The spore-forming rhizobacteria was isolated from rhizosphere soil samples of five types of vegetable. Soil samples were pre-treated by heating at 80°C in a water bath for 12 min, then spread onto tryptone yeast extract (TYE) and nutrient agar (NA) for culturing microbes, and incubated at 30°C. For thirty-nine isolates of rhizobacteria with different characteristics, it was found that 12 isolates were able to produce IAA and three isolates (TYS1.1, TYS3.3, and TYS3.5) showed the highest potential for IAA production. Nitrogen fixation, phosphate solubilization and potassium solubilization were also determined for these isolates. The results revealed that isolates TYS1.1, TYS3.3, and TYS3.5 exhibited nitrogen fixation and potassium solubilization activities (data shown in Table 1); all three isolates were *Bacillus* sp. according to the 16S rDNA sequencing analysis.

Table 1. Characterization of rhizobacterial isolates for nitrogen fixation, phosphate and potassium solubilization and Gram types

Isolates	Nitrogen fixing activity		Phosphate solubilization		Potassium solubilization		Gram stain	
	Vegetative cells	Spores	Vegetative cells	Spores	Vegetative cells	Spores	Gram positive	Endospores
TYS1.1	+	+	–	–	+	+	+	+
TYS3.3	+	+	–	–	+	+	+	+
TYS3.5	+	+	–	–	+	+	+	+

+ or – indicate positive and negative results, respectively

PGPRs have great potential to promote plant growth (Bashan et al., 2014). In this experiment, we isolated rhizobacteria from rice rhizosphere soil and used these bacteria to produce bio-fertilizers. *Bacillus* spp. is in a genus of bacteria that have properties for enhancing plant growth, such as phytohormones production (IAA and gibberellic acids), inorganic nutrient solubilization and nitrogen fixation (Gharib et al., 2015). In addition, *Bacillus* spp. are able to form endospores when grown under stress conditions, resulting in tolerance to heat and unfertile soils (Bressuire et al., 2018). The exhibition of endospore formation ability was reported by Pesce et al. (2014), which applied *Bacillus* spp. to environments and found that they were able to survive under stress environments. Our results were similar to Kumar et al. (2012), who screened 7 rhizobacterial isolates belonging to the genus *Bacillus* from bean rhizosphere samples. Their study found isolates that had IAA producing activity, siderophore production, organic acid production, ACC deaminase activities, and the dissolution of inorganic phosphate and potassium.

3.3 IAA production under different levels of salinity conditions

Auxin can promote plant growth through several mechanisms such as stimulating root elongation of primary or lateral roots, cell

enlargement, cell division, and root germination (Spaepen et al. 2007).

Three isolates of rhizobacteria (TYS1.1, TYS3.3, and TYS3.5) in the form of vegetative cells and spore-forming cells were tested for IAA production activity under various salinity conditions (0, 50, and 100 mM NaCl solution). It was found that at salinity concentrations greater than 100 mM of NaCl, three rhizobacterial isolates were able to produce IAA after 72 h of incubation, especially in the form of vegetative cells. The range of IAA production by vegetative cells of three TYS1.1, TYS3.3, and TYS3.5 were 69.78, 62.18, and 84.78 µg/mL, respectively. In the case of spore-forming cells, IAA was produced in a range of 23.17, 25.85, and 28.70 µg/mL, respectively, as shown in Figure 1. On the other hand, Rojas-solis et al. (2020) found that the amount of IAA produced from *Bacillus* sp. E25 was reduced from 31.18 µg/mL (control condition, without NaCl solution) to 20.51 and 20.46 µg/mL in the presence of NaCl solutions at concentrations of 100 and 200 mM, respectively. Moreover, an increase of salinity level in the culture broth of *Bacillus* sp. CR71 did not show a significant effect on IAA production. This is similar to the experiment of Ansari et al. (2019) which found that IAA production of *Bacillus pumilus* FAB10 was 105.8, 87.8, 73.3, 60.4, and 51.2 µg/mL, respectively, in the presence of NaCl concentrations of 0, 75, 125, 250, and 500 mM.

3.4 Root colonization

In this experiment, okra was selected for study because it is a popular vegetable for consumers in Thailand. Okra contains high levels of vitamins and minerals which are healthy for humans (Oyelade et al., 2003). Normally, okra can grow in every season and can tolerate moderate salinity. However, when it is cultivated in saline soil, the germination and the productivity of okra is reduced (Khan et al., 2001).

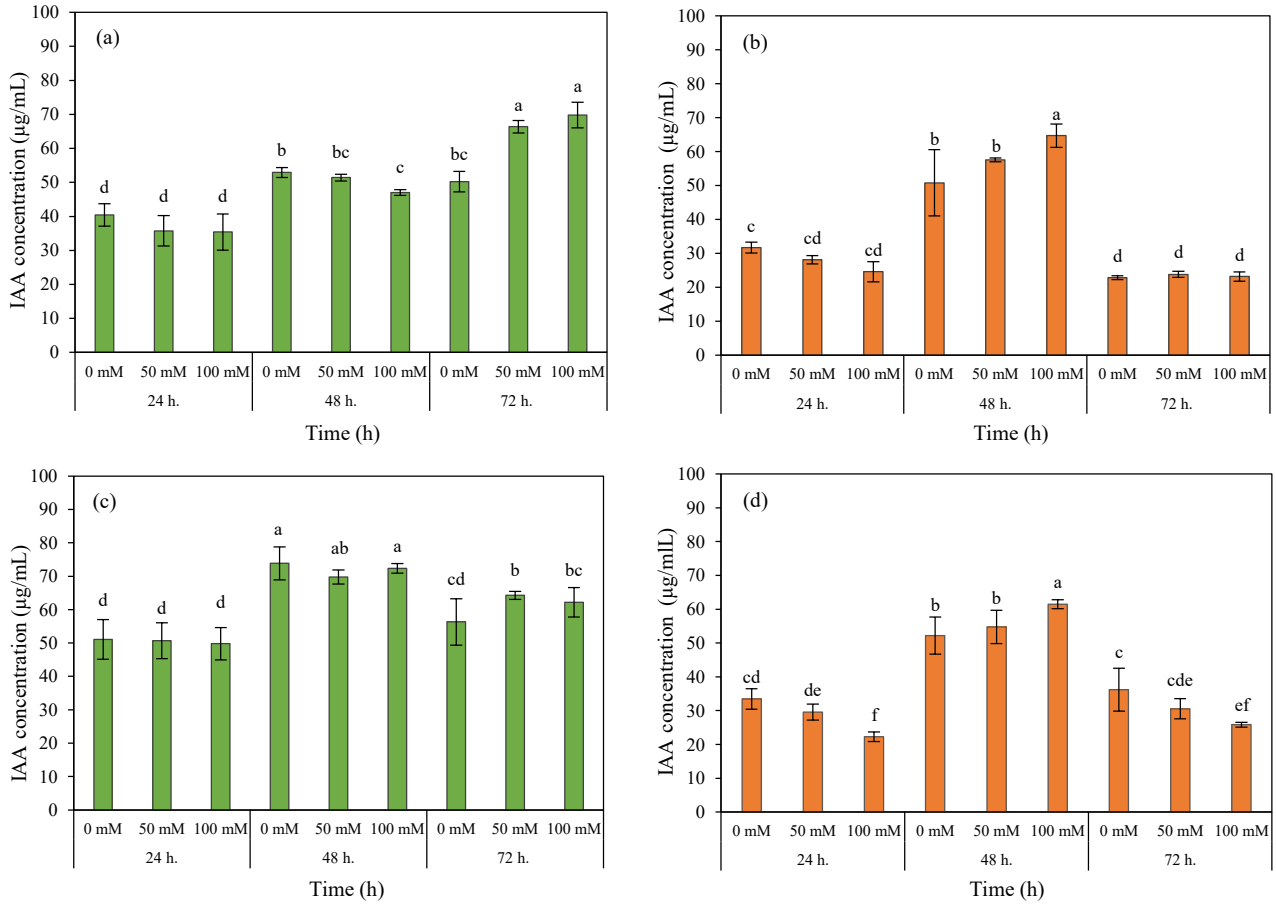

Figure 1. The IAA (indole-3-acetic acid) content produced by the isolate TYS1.1 including vegetative cells (a) and spore-forming cells (b), isolate TYS3.3; vegetative cells (c) and spore-forming cells (d), isolate TYS3.5; vegetative cells (e) and spore-forming cells (f) under different concentrations of NaCl solution (mM)

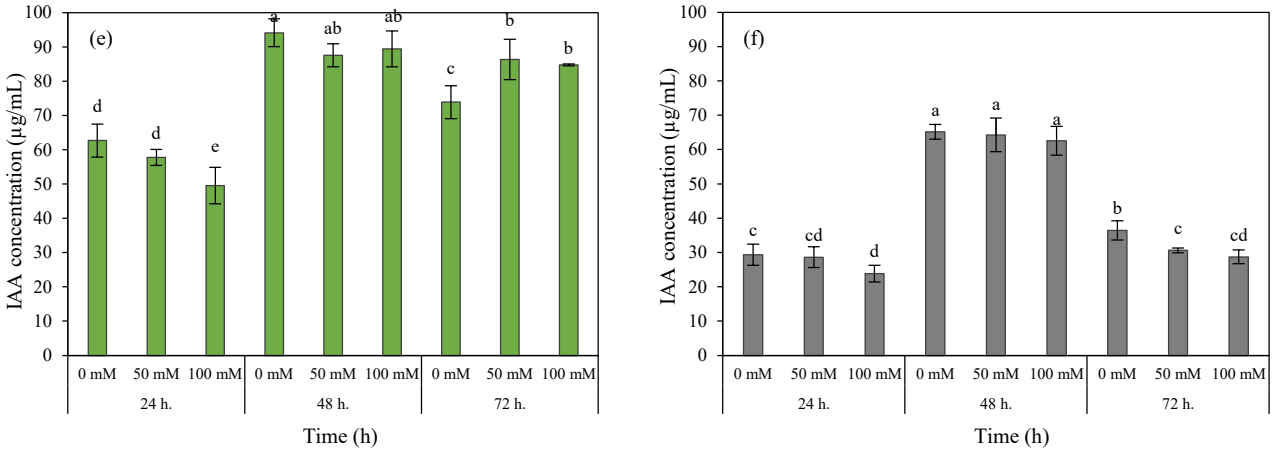

Figure 1. The IAA (indole-3-acetic acid) content produced by the isolate TYS1.1 including vegetative cells (a) and spore-forming cells (b), isolate TYS3.3; vegetative cells (c) and spore-forming cells (d), isolate TYS3.5; vegetative cells (e) and spore-forming cells (f) under different concentrations of NaCl solution (mM) (cont.)

Microbial colonization on okra roots under varying salinity conditions was studied using scanning electron microscopy (SEM). The results showed that the three isolates (TYS1.1, TYS3.3, and TYS3.5) of rhizobacteria lived on the surface of okra roots (Figure 2). Root colonization was quantified by counting the microorganisms that adhered around the roots. It was found that at the salinity concentrations of 0, 50, 100 mM, the amount of bacteria were 10^8 CFU/g, 10^8 CFU/g and 10^7 CFU/g, respectively (Figure 3). This result was similar to the report of Sathya et al. (2016) showing that motility-related features of the bacterium *Bacillus amyloliquefaciens* VB7 was found on the roots of chili seeds when visualized on a scanning electron microscopy (SEM). The results revealed that the bacterial cells had adhesion around the roots at a concentration of 3.8×10^5 CFU/g.

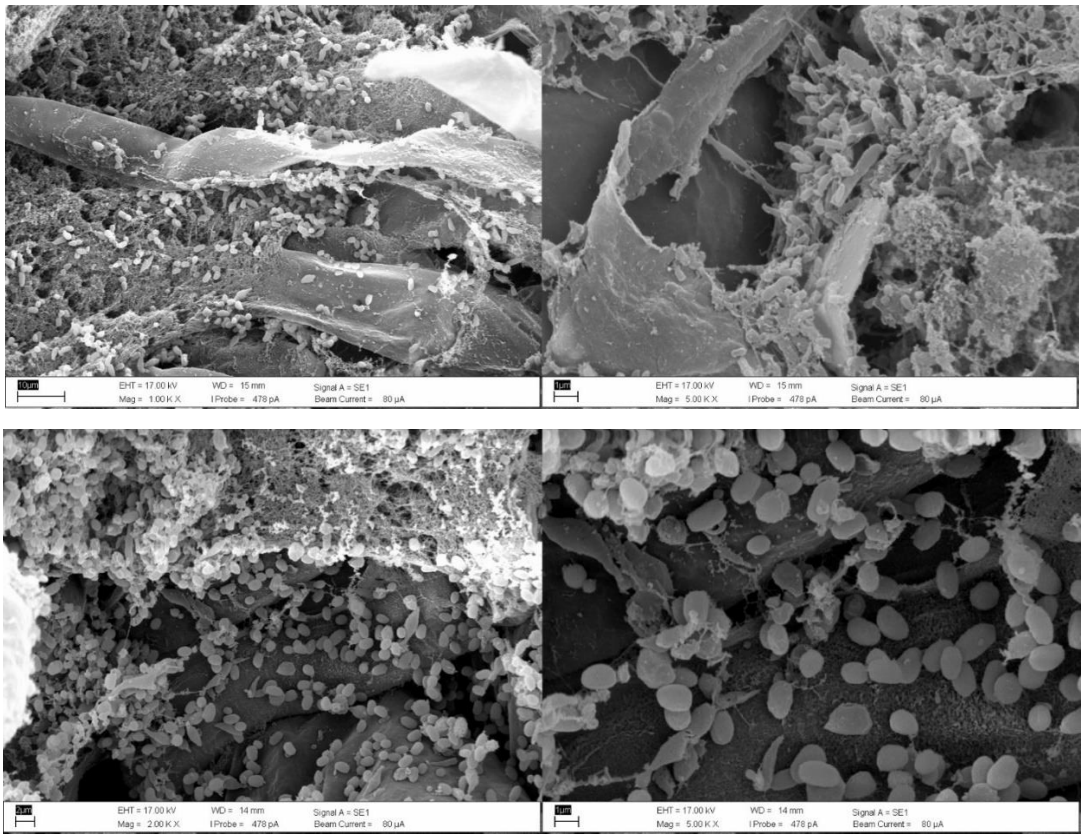

Figure 2. The colonization of rhizobacteria on the root surface of okra under various salinity conditions, on: 0 mM NaCl solution, center: 50 mM NaCl solution, and lower:100 mM NaCl solution

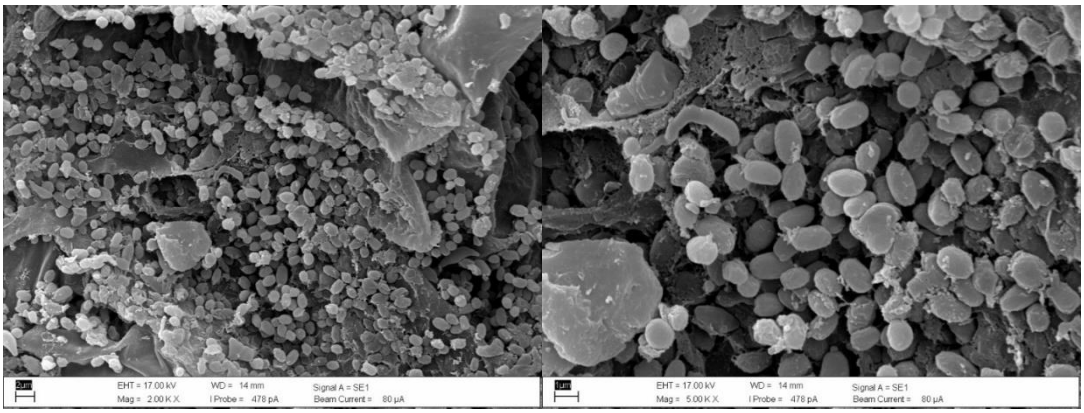

Figure 2. The colonization of rhizobacteria on the root surface of okra under various salinity conditions, on: 0 mM NaCl solution, center: 50 mM NaCl solution, and lower:100 mM NaCl solution (cont.)

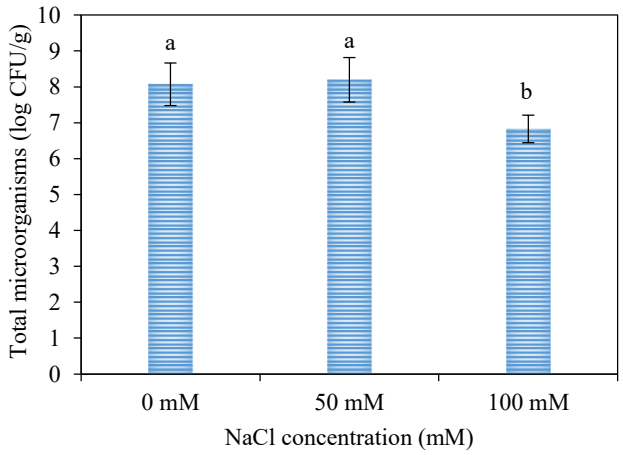

Figure 3. The viable cells of total microorganisms on the root surface determined by a conventional method (spread plate technique)

3.5 Bio-fertilizer production from vegetative cells and spore-forming cells

Surviving vegetative cells and spore-forming cells in the bio-fertilizer product, which was stored at room temperature for 60 days, were counted. The mixture of rhizobacteria was immobilized on four kinds of carrier materials (alginate, bagasse, rice husk ash, and vermiculite). The greatest amount of both forms of microbial cells was found in two carrier materials, rice husk ash and vermiculite, (T5: rice husk ash+vegetative cell, T6: rice husk ash+spores, T7: vermiculite+vegetative cell, T8 vermiculite+spores). The survival numbers in treatments T5-T8 were consistent on the carriers in all sampling days (15, 30, 45, and 60 days) as shown in Figure 4.

The pH and EC of the bio-fertilizer did not change during storage at room temperature for 60 days. The pH and EC values for the alginate carrier were in a range of 6.37-7.85 and 1.79-4.17 dS/m, respectively. In the case of the bagasse carrier, the pH was at 3.58-4.69, and EC was at 0.40-0.60 dS/m. Rice husk ash had a pH in a range of 7.71-8.73, and EC of 0.69-12.47 dS/m. The pH and EC of vermiculites were 8.34-9.27 and 0.31-0.55 dS/m, respectively. The neutral pH and slight salinity conditions in sodium alginate carrier made it the best carrier for immobilizing rhizobacterial cells and growing plants. Our results showed a high rate of surviving cells in rice husk ash and vermiculite. This might be due to the porous structures of both these carriers, causing the microbes to adhere to and live within these materials.

One of the major concerns when applying microbial inoculants to saline soil in the form of vegetative cells is the survivability of the microbial cells under salinity stresses. Abiotic stress might suppress the growth of bacterial cells in inappropriate environments. Therefore, the use of spore-forming rhizobacteria that tolerate salinity could be an alternative option for applying microbial inoculants into saline soil. These microbes are able to solubilize nutrients in saline soil, increasing their availability for plant uptake and growth. Moreover, this method provides a long shelf-life in natural fields under stress conditions when compared with vegetative cells (El-Sayed et al., 2014).

The quality of the carrier materials is an important factor for supporting rhizobacterial cells. Desirable properties of carrier materials are being rich in nutrients, structurally porous, and non-toxic. The quality of the carrier material is an important factor for supporting rhizobacterial cells. Enumeration of the total microorganisms immobilized on four types of carrier materials, alginate, bagasse, rice husk ash and vermiculite, was determined after 60 days of biofertilizer production. The highest numbers of total

microorganisms (in the form of vegetative and spore-forming cells) were found in rice husk ash and vermiculite. It showed a consistent survival rate. Both kinds of carriers are agricultural materials popularly used in cultivation. Rice husk ash has a porous structure allowing good air-circulation and high-water absorption ability (Ogbo and Odo., 2011), while vermiculite is a natural biological stimulant for plant growth which can maintain soil moisture content and has high porosity (Marinova et al., 2012). These carrier materials have promising physical, chemical and biological properties for use in soils and support bacterial growth, especially in stress conditions. This bio-fertilizer should be tested and developed as an alternative product for use in both fertile and unfertile soils under stress and non-stress condition.

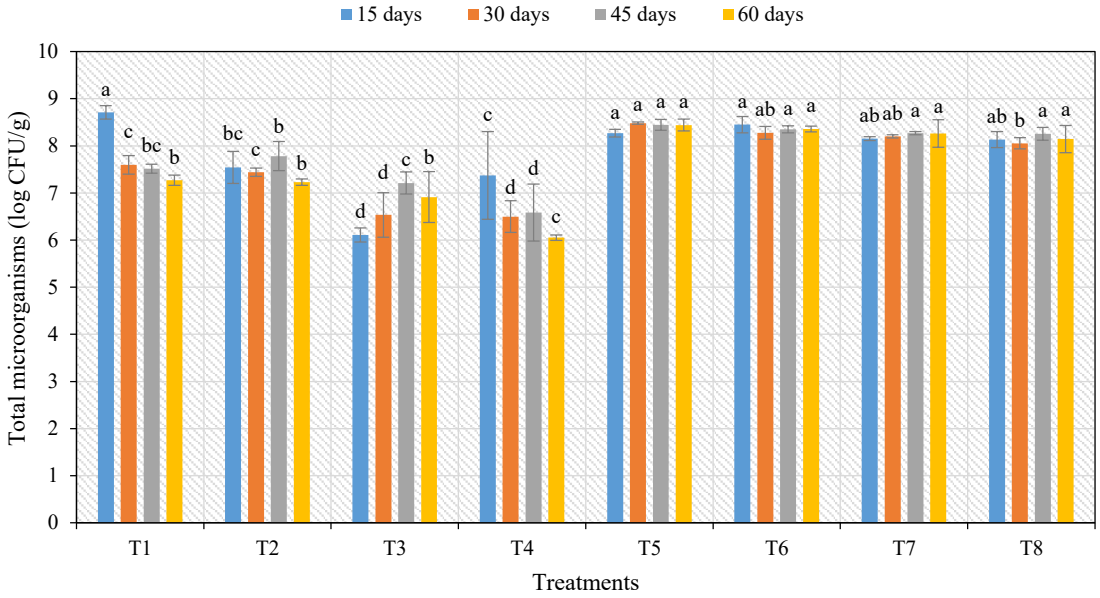

Figure 4. The survival of vegetative and spore-forming cells of microorganisms in bio-fertilizers for 8 treatments were as followed T1: alginate+vegetative cells, T2: alginate+spores, T3: bagasse+vegetative cells, T4: bagasse+spores, T5: rice husk ash+vegetative cells, T6: rice husk ash+spores, T7: vermiculite+vegetative cells, T8: vermiculite+spores

4. CONCLUSION

PGPRs demonstrated great potential on the promotion of plant growth in both forms (vegetative and spore forming cells). They showed similar abilities on the promotion of plant growth under salinity conditions such as fixed the nitrogen, solubilized phosphate and potassium on the agar plate. Moreover, both forms of rhizobacterial cells were able to produce IAA product. However, our data showed evidence that to maintain the survival rate of rhizobacterial cells under stress conditions, immobilization of rhizobacterial cells on a carrier is recommended. The microbial inoculum must be prepared on supporting material before application to the soil. In this study, rice husk ash and vermiculite were suitable carriers for protecting both forms of bacterial cells from salinity stress. These two carriers are widely found in Thailand and would increase in importance and value due to their role in bio-fertilizer production in the agro-industry.

ACKNOWLEDGEMENTS

This project was supported by the research capability enhancement program through graduate student scholarship in the year 2018, Faculty of Science, Khon Kaen University. Finally, thanks to the members of Microbial Fertilizer Laboratory, Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand for assisting the laboratory works. We would like to thank Mr. Matthew Savage, Head of English (Upper Level), Khonkaen Wittayayon EP School, Khon Kaen Province for proofing on English writing of this manuscript.

REFERENCES

Ansari FA, Ahmad I, Pichtel J. Growth stimulation and alleviation of salinity stress to wheat by the biofilm forming *Bacillus pumilus* strain FAB10. Applied Soil Ecology 2019;143:45-54.

Bal HB, Adhya TK. Diversity of plant growth promoting rhizobacteria (PGPR) in rice soil of Odisha. Plant Science Research 2012;34:27-33.

Bashan Y, de-Bashan LE. Prabhu SR, Hernandez JP. Advance in plant growth-promoting bacterial inoculants technology:

Formulations and practical perspectives (1998-2013). Plant and Soil 2014;378:1-33.

Bashan Y, Hernandez JP, Leyva LA, Bacillio M. Alginate microbeads as inoculant carriers for plant growth promoting bacteria. Biology and Fertility of Soils 2002;35:359-68.

Bressuire IC, Broussolle V, Carlin F. Sporulation environment influences spore properties in *Bacillus*: Evidence and insights on underlying molecular and physiological mechanisms. FEMS Microbiology Reviews 2018;42(5):614-26.

El-Sayed WS, Akhkha A, El-Naggar MY, Elbadry M. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Frontiers in Microbiology 2014;5:Article No. 651.

Gharib AA, Shahen MM, Ragab AA. Influence of Rhizobium inoculation combined with *Azotobacter chrococcum* and *Bacillus megaterium* var phosphaticum on growth nodulation, yield and quality of two snap bean (*Phasealus vulgaris* L.) cultivers. Annals of Agricultural Sciences 2015;53(2):249-61.

Gajbhiye T, Pandey SK, Lee SS, Kim KH. Size fractionated phytomonitoring of airborne particulate matter (PM) and speciation of PM bound toxic metals pollution through *Calotropis procera* in an urban environment. Ecological Indicators 2019;104:32-40.

Goswami D, Dhandhukia P, Patel P, Thakker JN. Screening of PGPR from saline desert of Kutch: Growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. Microbiological Research 2014;169:66-75.

Khan AA, McNeilly T, Azhar FM. Stress tolerance in crop plants. International Journal of Agriculture and Biology 2001; 3(2):250-5.

Kumar A, Maurya BR, Raghuwanshi R. Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). Biocatalysis and Agricultural Biotechnology 2014;3(4):121-8.

Kumar P, Dubey RC, Maheshwari DK. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiological Research 2012;167(8):493-9.

Lyngwi NA, Joshi SR. Economically important *Bacillus* and related genera: A mini review. Biology of Useful Plants and Microbes 2014;3:33-43.

Marinova SV, Toncheva R, Zlatareva E, Pchelarova H. Characteristics of vermiculite and its influence on the yield of lettuce in greenhouse experiments. Proceedings of the 5th Water Observation and information System for Decision Support Conference; 2012 May 27- Jun 2; Ohrid, Republic of Macedonia, Bulgaria; 2012.

Ogbo FC, Odo MO. Potential of rice husk and cassava peel as carriers for bio-fertilizer production. Nigerian Journal of Biotechnology 2011;23:1-4.

Omer AM. Bioformulations of *Bacillus* spores for using as biofertilizer. Life Science Journal 2010;7(4):124-31.

Oyelade OJ, Ade-Omowaye BIO, Adeomi VF. Influence of variety on protein, fat contents and some physical characteristics of okra seeds. Journal of Food Engineering 2003;57(2):111-4.

Ozdal M, Ozdal OG, Sezen A, Algur OF, Kurbanoglu EB. Continuous production of indole-3-acetic acid by immobilized cells of *Arthrobacter agilis*. 3 Biotech. 2017;7(1):Article No. 23.

Pesce G, Rusciano G, Sasso A, Isticato R, Sirec T, Ricca E, et al. Surface charge and hydrodynamic coefficient measurements of *Bacillus subtilis* spore by optical tweezers. Colloids and Surfaces B: Biointerfaces 2014;116:568-75.

Rojas-Solis D, Vences-Guzman MA, Sohlenkamp C, Santoyo G. Antifungal and plant growth-promoting *Bacillus* under saline stress modify their membrane composition. Journal of Soil Science and Plant Nutrition 2020;20(3):1549-59.

Roslan MAM, Zulkifli NN, Sobri ZM, Zuan ATK, Cheak SC, Abdul Rahman NA. Seed biopriming with P- and K-solubilizing *Enterobacter hormaechei* sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. Public Library of Science One 2020;15(7):e0232860.

Sathya S, Nakkeeran S, Lakshmi S. Effect of biopriming on populations of *Bacillus amyloliquefaciens* VB7 in chilli seeds. The Bioscan: An International Quarterly Journal of Life Sciences 2016;11(2):907-10.

Shen H, He X, Liu Y, Chen Y, Tang J, Guo T, et al. A complex inoculant of N_2-fixing, P- and K-solubilizing bacteria from a purple soil improves the growth of kiwifruit (*Actinidia chinensis*) plantlets. Front Microbiol 2016;7:Article No. 841.

Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. Federation of European Microbiological Societies 2007;31(4):425-48.

Sun F, Ou Q, Wang N, xuan Guo Z, Ou Y, Li N, et al. Isolation and identification of potassium-solubilizing bacteria from *Mikania micrantha* rhizospheric soil and their effect on *M. micrantha* plants. Global Ecology and Conservation 2020;23:e01141.

Verma N, Singh NA, Kumar N, Raghu HV. Screening of different media for sporulation of *Bacillus megaterium*. International Journal of Microbiology Research 2013;1(4):68-73.

Watterson MJ, Kent DJ, Boor KJ, Wiedmann M, Martin NH. Evaluation of dairy powder products implicates thermophilic sporeformers as the primary organisms of interest. Journal of Dairy Science 2014;97(4):2487-97.