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Screening of Efficient Halotolerant Phosphate Solubilizing Bacterium and its Effect on Promoting Plant Growth under Saline Conditions

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Abstract: Eighty-four halotolerant bacterial strains were tested to screen for the efficient halotolerant phosphate solubilizing bacterium (PSB). They were selected mainly on the basis of the solubilizing ability of the insoluble phosphate in the modified Pikovskaya broth under various salt concentrations and temperature. A *Bacillus megaterium* A12ag was selected as the efficient halotolerant PSB because it demonstrated the highest soluble phosphate solubilization activity under saline conditions. The tomato (*Licopersicon esculentum* Mill. cv. Seeda) seeds which were inoculated with the bacterium significantly increased the germination percentage and germination index, especially at NaCl concentration between 30 to 90 mM and increased the seedling dry weight at NaCl up to 120 mM. The results suggested that the halotolerant PSB may be used to alleviate the effects of salts and provide great potential for use as biofertilizer in the arid and salt affected areas.

Key words: Phosphate solubilizing bacteria · Halotolerant bacteria · Plant growth-promoting bacteria · Bacillus

INTRODUCTION

Saline soil is distributed throughout the world especially in the arid and semiarid regions where agriculture performs under irrigation [1]. The phosphorus deficiency frequently compounds the problems of saline soil of the tropics [2]. High salinity affects plant growth through (i) the osmotic effects; (ii) toxicity of salt ions; and (iii) the changes in the physical and chemical properties of soil [3]. It also suppresses the phosphorus uptake by plant roots and reduces the available phosphorus by sorption processes and low solubility of the Ca-P minerals [4]. Since phosphorus is a critical nutrient limiting plant growth [5], the adverse effects on plant growth in saline soil are multiplied. Consequently, the yields and profits of agricultural productions in saline soil are reduced drastically [3]. In this respect, the use of chemical fertilizers is the most common approach to improve soil fertility. However, the available phosphorus in the chemical fertilizers is rapidly fixed to the unavailable forms [6] especially in saline soil and accounts for low phosphorus use efficiency. Phosphate solubilizing bacteria (PSB) are known to improve the solubilization of fixed soil phosphorus by the production of organic acids and acid phosphatases and result in increased of crop yields [7]. The PSB-based biofertilizers appear more effective choice than the chemical fertilizers in maintaining the high available phosphorus and are also friendly to the environment. The accomplishments of PSB in promoting plant growth were documented elsewhere [8-13].

Key factors for the successful applications of microbial inoculants are their abilities to survive, outcompete with the often well adapted native microflora and colonize in the rhizosphere [14]. In addition, the stressful environmental conditions appear to influence the growth and phosphate solubilizing activity of PSB [15,16]. An influence of high salinity on the phosphate solubilizing activity of PSB has been reported elsewhere [17,18]. Basically, the salinity stress causes less effect on halotolerant bacteria since they have adapted during evolution (genotypic and/or phenotypic adaptation) to tolerate and optimally grow in hypersaline environments [18]. The study of Johri *et al.* [17] confirmed the performance of bacterial strains, which were isolated from alkaline soils containing salt 2% (w/v), in the solubilization of insoluble phosphates at high salt and high pH. However, little information is available on the use of halotolerant PSB to enhance crop yields under saline condition. To reach this goal, the present study was designed (i) to screen for the efficient halotolerant phosphate solubilizing bacterium which can solubilize the insoluble phosphate in the wide ranges of salinity and temperature; and (ii) to evaluate its effects on tomato plants grown *in vitro* and under saline conditions.

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MATERIALS AND METHODS

Microorganism and Culture Media: Eighty-four halotolerant bacterial strains, isolated from saline soil in Thailand, were used in this study. They were identified using phenotypic characterization, chemical analysis and genotypic characterization from the previous work of Chookietwattana [19]. For inoculum preparation, bacterial strains were individually cultured at 30°C for 24 h on halobacteria medium which contained the following ingredients per liter: $MgSO_4 \cdot 7H_2O$, 10.0 g; casein hydrolysate 5.0 g; KCl, 5.0 g; disodium citrate 3.0 g; KNO_3 1.0 g; yeast extract 1.0 g; $CaCl_2 \cdot 6H_2O$, 0.2 g; NaCl 0.6 M; and agar 15.0 g. Bacterial cells were scraped from the plates and diluted to obtain 10^8 CFU ml^{-1} by adjusting their optical density at 600 nm. to approximately 1.0 using phosphate buffer (0.1 M, pH 7.0).

The modified Pikovskaya (MPVK) medium was used for screening of efficient halotolerant PSB experiment. This medium was first described by Pikovskaya [20] and was modified by Son *et al.* [16]. The medium contains (per liter): 1.0% (w v^{-1}) glucose, 0.05% (w v^{-1}) $(NH_4)_2SO_4$, 0.02% (w v^{-1}) NaCl, 0.02% (w v^{-1}) KCl, 0.01% (w v^{-1}) $CaCl_2 \cdot 2H_2O$, 0.01% (w v^{-1}) $MgSO_4 \cdot 7H_2O$, 0.05% (w v^{-1}) $MnSO_4 \cdot 7H_2O$, 0.05% (w v^{-1}) $FeSO_4 \cdot 7H_2O$ and 0.05% (w v^{-1}) yeast extract in distilled water (pH 7.5). NaCl and agar were supplemented as required. The tricalcium phosphate ($Ca_3(PO_4)_2$) at 0.5% (w v^{-1}) was added as a source of an insoluble phosphate. It was autoclaved separately from the other ingredients then aseptically mixed together.

Screening of Efficient Halotolerant PSB: The screening procedure comprised the three consecutive screening steps. Each step was performed with three replicates. For a primary screening step, eighty-four halotolerant bacterial strains were screened for their phosphate solubilizing ability by point inoculating them on modified Pikovskaya (MPVK) agar medium containing 0.6 M NaCl. The plates were incubated at 30°C for 5 d. Only the bacterial strains that formed the clear haloes around their colonies were selected for using in the next screening step. In a secondary screening step, the quantitative analysis of phosphate solubilization was carried out with Erlenmeyer flasks (250 ml) containing 150 ml of MPVK broth inoculated with the bacterial strains (1% inoculum with 10^8 CFU ml^{-1}). The autoclaved and uninoculated medium served as the negative controls. The flasks were incubated for 5 d at 30°C with shaking at 200 rpm. The cultures were harvested by centrifugation at 17,418 rpm for 30 min. The soluble phosphate in the culture supernatant was determined colorimetrically by using the method of APHA *et al.* [21]. A tertiary screening step was carried out to quantify the soluble phosphate from the phosphate solubilizing ability of bacterial strains under various salt concentrations (0.2, 0.4, 0.8, 1.2 and 1.6 M NaCl) at 30°C and under various temperatures (30, 35 and 45°C) at 0.6 M NaCl.

Germination and Seedling Growth Assessment: Tomato (*Licopersicon esculentum* Mill. cv. Seeda) seeds were surface disinfected by immersion in 70% ethanol for 1 min, followed by 15 min in 0.9% (w w^{-1}) sodium hypochlorite. They were then washed three times with sterile distilled water. An inoculum of the efficient halotolerant PSB was prepared. The disinfected seeds were immersed in either the bacterial inoculum (10^8 CFU ml^{-1}) or phosphate buffer (0.1 M, pH 7.0) for one hour and were used as inoculated and uninoculated treatment, respectively. Germination assays were performed according to International Seed Testing Association [22]. Four replicates of 25 seeds were germinated in sterilized Petri dishes (9.0 cm diameter) containing two sheets of filter papers moistened initially with 4 ml of 0.5% (w v^{-1}) sterilized tricalcium phosphate solution supplemented with NaCl at 0, 30, 60, 90 and 120 mM. The Petri dishes were then placed under artificial light which provided light intensity at 2,000 lux for 16 h daily and at a temperature of 28±2°C. Germination was observed daily. The germination percentage and germination index were calculated. Two weeks after germination, the root length of the seedlings was measured and then dry weight of the seedlings was determined by drying at 70°C for 3 d.

Bacterial Root Colonization Assessment: The survival and ability of the inoculated bacteria to colonize plant roots were assessed by the visualization of plant roots under the light and scanning electron microscopy (SEM). For SEM visualization, root pieces of interest were dissected about 1 cm from the whole roots and fixed in 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer pH 7.2 at 4°C for overnight. The fixed specimens were washed with phosphate buffer three times, each for 15 min. The specimens were further fixed in 1% osmic acid for 2 h at room temperature and rinsed with distilled water for three times, each for 15 min. The samples were frozen with liquid nitrogen for 2 min and then dehydrated using the freeze dryer. After that, the samples were gold coated and examined under a scanning electron microscope (JEOL, JSM-6460LV).

Statistical Analysis: Means of soluble phosphate (mg l^{-1}) solubilized by halotolerant PSB were statistically compared using LSD values ($P < 0.05$). The experimental design for the study of germination and seedling growth assessment was two factors factorial arranged in a randomized completely block design; with four replications and 25 seeds per replicate. One way analysis of variance (ANOVA) was made to determine any significant differences between the groups at $P < 0.05$. All statistical analyses were performed by using the Statistix (NH Analytical Software, USA.).

RESULTS AND DISCUSSION

Screening of Efficient Halotolerant PSB: In the primary screening step, of the eighty-four halotolerant bacterial strains, seventeen bacterial strains were able to produce a clear zone on the MPVK agar plate in the presence of 0.6 M NaCl at 30°C. They were then tested for their ability to solubilize the insoluble phosphate in the MPVK broth containing 0.6 M NaCl in the secondary screening step. This screening step was conducted for obtaining more reliable results since the screening technique based on the visible clear zone on the agar plate was not an infallible technique [23]. In addition, it was also used for selecting the strains which have high phosphate solubilizing activity and also for reducing the time-consuming work and chemical costs to perform a tertiary screening. Out of seventeen halotolerant PSB strains, 2 bacterial strains, namely: *B. megaterium* AIIy; and *B. megaterium* A12ag, showed the highest soluble phosphate at the first and second rank (data not presented), hence they were chosen for a further screening step. The species of PSB selected in this screening step was the same as that studied by Arora and Gaur (1979) [24] since bacteria in the genus *Bacillus* are reported as one of the most powerful PSB [25, 7, 26]. Increased crop yields by inoculation of *Bacillus* spp. either through single inoculation or co-inoculation with the other plant growth-promoting bacteria, were reported by several researchers with several plant species [11, 27, 28, 12] Furthermore, among species in the genus *Bacillus*, *B. megaterium* is one of the most well known PSB which was successful in enhancing crop yields in the Soviet Union and India [29].

The tertiary screening step was performed in order to screen for the best halotolerant phosphate solubilizing bacterium which had the highest phosphate solubilizing activity at a wide range of salt and temperature levels. Comparative studies on the influence of salt (NaCl) and temperature on phosphate solubilizing activity of *B. megaterium* AIIy and *B. megaterium* A12ag revealed that both of them were able to solubilize the insoluble phosphate at all levels of NaCl and temperatures studied (Table 1 and 2). These results infer that they had been well adapted to the salt stress conditions. It seemed, therefore, that the strains isolated from saline soil have the genetic potential to solubilize the insoluble phosphate at high salt and high temperature. The insoluble phosphate solubilization of both strains were significantly enhanced with increasing amounts of NaCl from 0.2 to 0.4 M, but decreased when NaCl was equal to or above 0.8 M. These results could be due to the influence of salt on their growth. In this respect, although they are halotolerant bacteria but their adaptations are limited in some salinity ranges which results from their ancestry. This is not a surprise result since halotolerants microorganisms have limited degrees of salt tolerances [30,31]. The P solubilization of both strains was highest in MPVK broth containing NaCl at 0.4 M (approximately 40 dS m^{-1}) and decreased at or above 0.8 M NaCl. These results imply that they could have a high potential to serve as PSB inoculants for increasing available P in saline soil. The reasons for this consideration are that the soil salinity recommended for plantation of most crops should not be higher than 80 mM. The salinity above 0.3 M is vital for plant growth even in many halophytes. In addition, there are also very few opportunities for the microbial inoculants to face with such extreme salinity. However, among both strains, *B. megaterium* A12ag released the highest amount of soluble phosphate than *B. megaterium* AIIy at every level of salt concentrations.

In studying the influence of temperature on phosphate solubilizing activity, the insoluble phosphate solubilization of both strains was significantly increased when temperature was increased from 30°C to 35°C, but decreased when temperature rose to 45°C. These results might be due to the influence of temperature on their growth which subsequently affects the phosphate solubilization. The results agree with the study of Nautiyal *et al.* [18] in which the phosphate solubilization was markedly reduced at 45°C. Overall, *B. megaterium* A12ag was less affected by fluctuations of salt and temperature than *B. megaterium* AIIy. According to the results obtained, these two strains were different genetically. *B. megaterium* A12ag proved to be the most efficient halotolerant PSB in which it performed the highest phosphate solubilizing activity and maintained the high activity in the wide ranges of salinity and temperature. Therefore, *B. megaterium* A12ag was

Table 1: The soluble phosphate (mg l^{-1}) solubilized by two strains of *B. megaterium* in the MPVK broth containing tricalcium phosphate at 30°C and various NaCl concentrations.

	NaCl (M)				
Culture	0.2	0.4	0.8	1.2	1.6
B. megaterium AIIy	119.8±1.4^{Ab}	121.2±3.2^{Ab}	97.2±6.3^{Bb}	55.8±1.4^{Ca}	47.8±1.3^{Ca}
B. megaterium A12ag	137.9±1.8^{Aa}	141.5±2.7^{Aa}	113.1±2.3^{Ba}	63.9±2.3^{Ca}	52.6±1.1^{Da}

Each value represents mean ± SD of three replicates per treatment.

^{ABC} Values with the same letter within rows indicate no significant differences with $P \geq 0.05$.

^{abc} Values with the same letter within columns indicate no significant difference with $P \geq 0.05$.

Table 2: The soluble phosphate (mg l^{-1}) solubilized by two strains of *B. megaterium* in the MPVK broth containing tricalcium phosphate and 0.6 M NaCl at various temperatures.

	Temperature (°C)		
Culture	30	35	45
B. megaterium AIIy	124.4±0.8^{Ab}	124.2±0.7^{Ab}	106.4±1.0^{Bb}
B. megaterium A12ag	140.8±1.1^{Aa}	148.5±1.8^{Aa}	119.3±0.8^{Ba}

Each value represents mean ± SD of three replicates per treatment.

^{ABC} Values with the same letter within rows indicate no significant difference with $P \geq 0.05$.

^{abc} Values with the same letter within columns indicate no significant difference with $P \geq 0.05$.

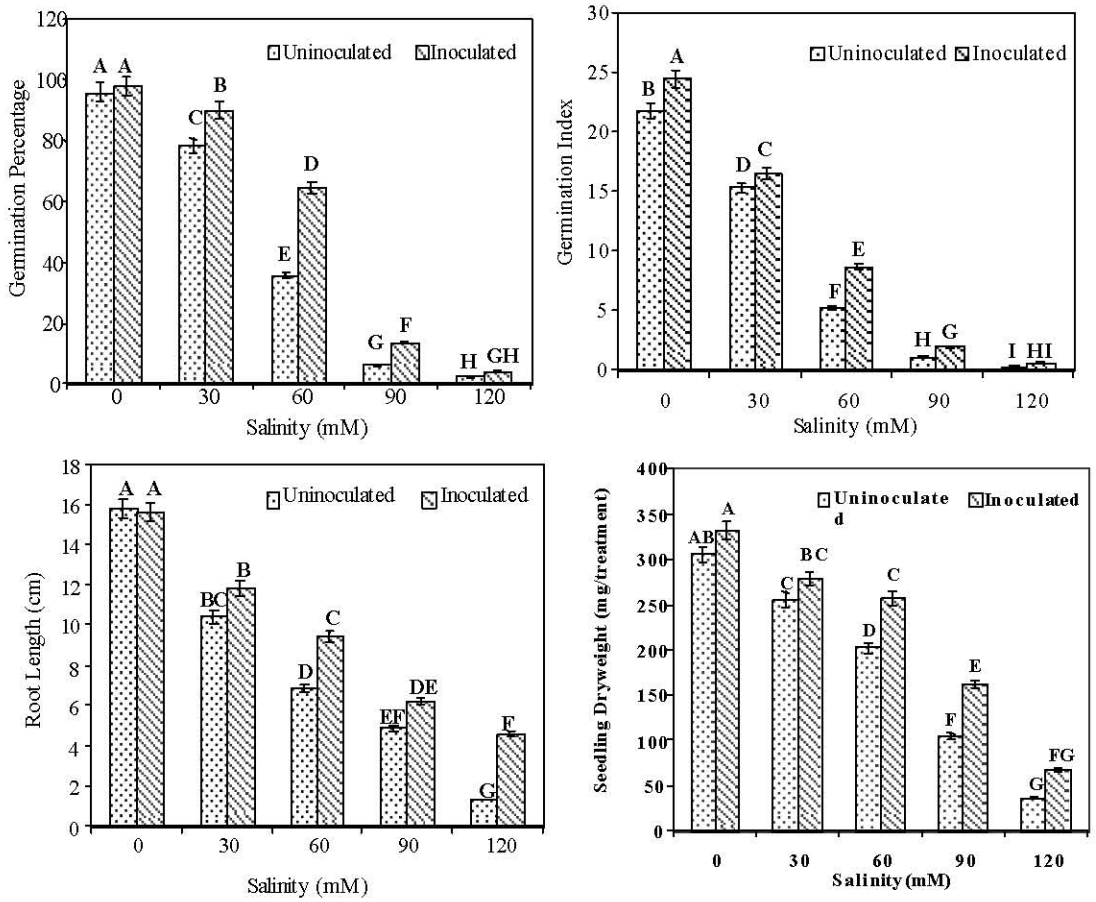

Fig. 1: Effect of halotolerant PSB *B. megaterium* A12ag on tomato plants grown under diverse salt concentrations and with additional insoluble phosphate. Each value represents mean ± SD of four replicates per treatment. ABC Values with the same letter above bar indicate no significant difference with P≥ 0.05.

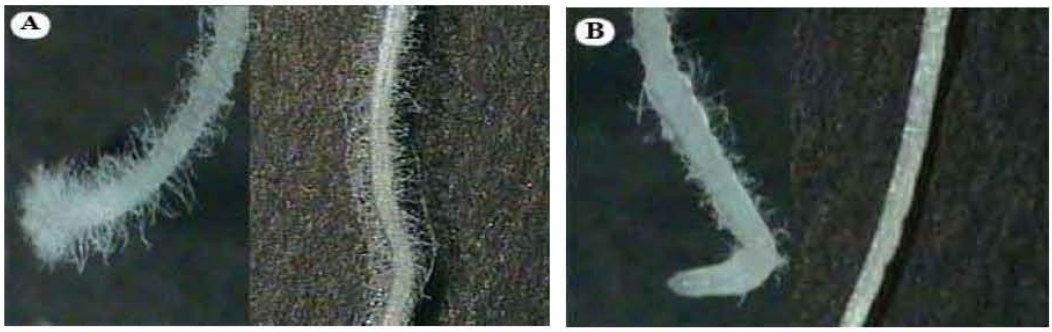

Fig. 2: External structure of tomato root: (A) uninoculated, (B) inoculated.

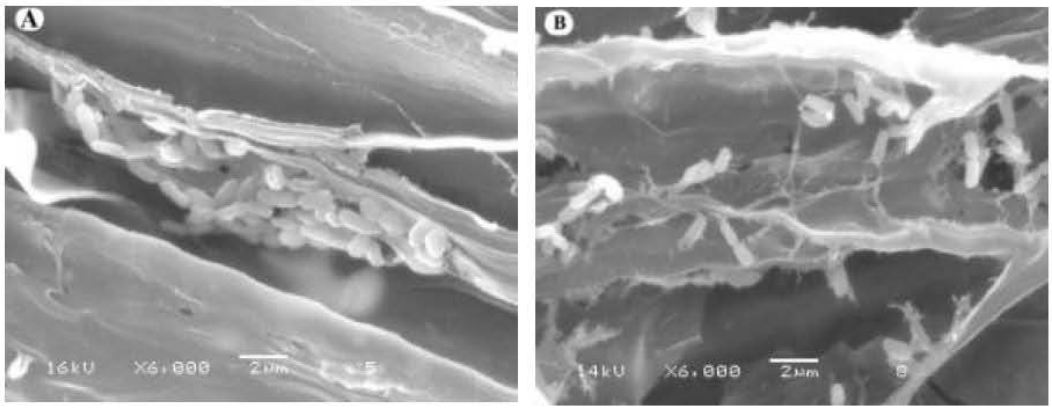

Fig. 3: SEM micrographs of tomato roots in which their seeds were inoculated with *B. megaterium* A12ag. Colonization of inoculated bacteria was observed on the surface of tomato roots under: (A) 30 mM NaCl at 14 d of incubation; and (B) 120 mM NaCl at 14 d of incubation.

selected as the efficient halotolerant phosphate solubilizing bacterium. It was then tested for the potential in promoting the growth of tomato which grew under saline conditions.

Effect of Efficient Halotolerant PSB on Promoting Tomato Growth under Saline Conditions: The rationale of this study is based on the hypothesis that an inoculation of the efficient halotolerant PSB, the *B. megaterium* A12ag, could solubilize the insoluble phosphate and provides sufficient available P for plants grown under saline conditions which consequently results in enhancement of plant growth. To study the effects of the selected bacterium on promoting tomato growth under saline conditions, the suspension of *B. megaterium* A12ag was used to inoculate tomato seeds prior to plant under saline conditions (0, 30, 60, 90 and 120 mM NaCl) and supplemented with 0.5% (w v^{-1}) of insoluble phosphate. The germination percentage, germination index, root length and dry weight of the seedlings inoculated with *B. megaterium* A12ag were compared with those uninoculated seedlings. In order to clearly determine the effects of salt stress and bacterial inoculation, uninoculated treatment was also conducted at all NaCl concentrations along with the inoculated treatment. The study on salt influencing tomato growth found that the germination percentage, germination index and seedling dry weight of both the inoculated and uninoculated treatment were significantly reduced as salinity increased (Figure 1). The reduction of tomato seed germination was mainly due to the delayed-actions of salt on seed germination by interfering with the uptake of essential nutrients, the direct toxicity effects of salt ions and the prevention of seed water uptake in the first phase of germination [32, 33]. In addition, salinity also caused changes in growth morphology and physiology of plant roots [34]. In the respect of salinity on P uptake, salinity inhibits P uptake by plant roots, P translocation from root to shoot and retranslocation of P from old to young leaves [35].

Observing the effect of the efficient halotolerant PSB on tomato plants grown under saline conditions and supplemented with insoluble phosphate reveals that the tomato seeds inoculated with *B. megaterium* A12ag

showed trends in enhancing germination percentage, germination index, root length and seedling dry weight over the uninoculated treatment. A significant increase in germination percentage and root length was found at NaCl between 30 to 120 mM. The effect of halotolerant PSB on germination percentage and root length was less marked at 0 mM NaCl. These results could be due to the fact that tomato is a moderate salt sensitive plant which can tolerate an EC_e (electrical conductivity of the saturated soil extracted) of about 2.5 dS m^{-1} (~ 25 mM NaCl) [36]. However, a significant increase in the germination index and seedling dry weight of the inoculated treatments was evidenced at NaCl between 0-120 mM. Moreover, the inoculated treatment had a 28% increase in the germination percentage over the uninoculated treatment at NaCl 60 mM. A substantial increase in germination index, root length and seedling dry weight of the inoculated treatments over theirs uninoculated treatments was also observed at NaCl 60 mM. An enhancement of tomato growth under saline conditions might be a result of increasing available phosphorus from microbial activity which was then taken up by plants for the emergence and development of the seedlings since only the insoluble phosphate was applied as a source of nutrients for tomato seeds. These results were agreed with the study of Awad *et al.* [37]. The results obtained could infer an ability of the selected halotolerant PSB in providing available phosphorus for plants grown in saline soil.

The visual observation of tomato roots indicated that roots of tomato seedlings inoculated by halotolerant PSB had less root hair length and density than the uninoculated treatment (Figure 2). These results may be due to the higher level of available phosphorus in the inoculated treatment than the uninoculated treatment according to the activity of halotolerant PSB. The reasons for these results are that low P concentration in the root medium of uninoculated treatment led to the development and elongation of root hair to ensure a sufficient uptake of P when available P in soil limits growth [38, 39]. Bates and Lynch [40] also reported that root hair development strongly responded to the change in local phosphate concentration which is controlled by a large number of genes [41].

In order to ascertain the colonization of halotolerant PSB on the plant roots, roots of inoculated treatment were observed under the light microscope and SEM. The SEM observations (Figure 3) confirmed *in vitro* binding of *B. megaterium* A12ag to tomato root surface and root hairs. They could colonize tomato roots in NaCl ranging from 0-120 mM.

CONCLUSION

The present study revealed that the selected salt-tolerant PSB, *B. megaterium* A12ag, can solubilize phosphate in the presence of high salt and high temperature. The bacterium showed a significant promoting effect on the germination and growth of the tomato seedlings grown *in vitro* and under saline conditions. This study can support the useful information for the production of effective PSB-based biofertilizer for saline soil. However, saline soil is much more complex than those *in vitro*. Therefore, further study on the activity of halotolerant PSB in combination with the environmental factor of saline soil is suggested to obtain the great potential for using the bacterium for future application as biofertilizer.

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REFERENCES

1. Pessarakli, M. and I. Szabolcs, 2000. Soil salinity and sodicity as particular plant/crop stress factors. In Handbook of Plant and Crop Stress, Ed. Pessarakli, M. New York: Marcel Dekker, pp: 1-15.
2. Landon, J.R., 1991. Booker Tropical Soil Manual. Essex: Longman.
3. Keren, R., 2000. Salinity. In Handbook of Soil Science, Ed. Sumner, M.E. Boca Raton: CRC Press, pp: G3-G25.
4. Grattan, S.R. and C.M. Grieve, 1999. Mineral nutrient acquisition and response by plants grown in saline environments. In Handbook of Plant and Crop Stress, Ed. Pessarakli, M. New York: Marcel Dekker, pp: 203-213.
5. Sharpley, A., 2000. Phosphorus availability. In Handbook of Soil Science, Ed. Sumner, M.E. Boca Raton: CRC Press, pp: D18-D30.

6. Yadav, K.S. and K.R. Dadarwal, 1997. Phosphate solubilization and mobilization through soil microorganisms. In Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production, Ed. Dadarwal, K.R. Jodhpur: Scientific Publishers, pp: 293-308.
7. Rodríguez, H. and R. Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17(4-5): 319-339.
8. Datta, M., S. Banik and R.K. Gupta, 1982. Studies on the efficacy of a phytohormone producing phosphate solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. Plant and Soil, 69: 365-383.
9. Kim, K.Y., D. Jordan and G.A. McDonald, 1998. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fert. Soils, 26: 79-87.
10. Wahid, O.A. and T.A. Mehana, 2000. Impact of phosphate-solubilizing fungi on the yield and phosphorus-uptake by wheat and faba bean plants. Microbiol. Res., 155(3): 221-7.
11. Rojas, A., G. Holguin, B.R. Glick and Y. Bashan, 2001. Synergism between *Phyllobacterium* sp. (N_2-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiol. Ecol., 35(2): 181-187.
12. Egamberdiyeva, D. and G. Höflich, 2004. Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. J. Arid Environ. 56: 293-301.
13. Mehrvarz, S., M.R. Chaichi and H.A. Alikhani, 2008. Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of Barely (*Hordeum vulgare* L.). American-Eurasian J. Agric. and Environ. Sci., 3(6): 822-828.
14. Bashan, Y., 1998. Inoculant of plant growth-promoting bacteria. Biotechnol Adv., 16(4): 729-770.
15. Yahya, A.I. and S.K. Al-Azawi, 1989. Occurrence of phosphate-solubilizing bacteria in some Iraqi soils. Plant and Soil, 117: 135-141.
16. Son, H.J., G.T. Park, M.S. Cha and M.S. Heo, 2006. Solubilization of insoluble inorganic phosphates by a novel salt- and pH- tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizophere. Biores. Technol., 97(2): 204-210.
17. Johri, J.K., S. Surange and C.S. Nautiyal, 1999. Occurrence of salt, pH and temperature tolerant phosphate solubilizing bacteria in alkaline soils. Curr. Microbiol., 39: 89-93.
18. Nautiyal, C.S., S. Bhadauria, P. Kumar, H. Lal, R. Mondal and D. Verma, 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol. Lett., 182: 291-296.
19. Chookietwattana, K., 2003. Diversity of halophilic bacteria in saline soil at Nong Bo Reservoir, Mahasarakham Province, Thailand, Ph.D. Thesis, Suranaree University of Technology, Nakhon Ratchasima, Thailand.
20. Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya, 17: 362-370.
21. APHA, A.W.W.A. and WEF, 1995. Standard Methods for the Examination of Water and Wastewater. 19th edition. Baltimore: United Book Press.
22. International Seed Testing Association, 2005. International Rules for Seed Testing. Bassersdorf: International Seed Testing Association (ISTA).
23. Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett., 170: 265-270.
24. Arora, D. and C. Gaur, 1979. Microbial solubilization of different inorganic phosphates. Indian J. Exp. Biol., 17: 1258-1261.
25. Illmer, P. and F. Schinner, 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol. Bioch., 24: 389-395.
26. Ray, R.C. and S. Edison, 2005. Microbial biotechnology in agriculture and aquaculture- An overview. In Microbial Biotechnology in Agriculture and Aquaculture Volume I, Ed. Ray, R.C. Enfield: Science Publishers, pp: 3-20.
27. Sundara, B., V. Natarajan and K. Hari, 2002. Influence of phosphate solubilizing bacteria on the changed in soil available phosphorus and sugarcane and sugar yields. Field Crop Res., 77: 43-49.
28. Ghosh, S., J.N. Penterman, R.D. Little, R. Chavez and B.R. Glick, 2003. Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica campestris*. Plant Physiol. Bioch., 41: 277-281.
29. Gaur, A.C., 1990. Phosphate Solubilizing Microorganisms as Biofertilizers. New Delhi: Omega Scientific Publishers.

30. Larsen, R., 1986. Halophilic and halotolerant microorganisms-an overview and historical perspective. FEMS Microbiol. Rev., 39: 3-7.
31. Kushner, D.J., 1993. Growth and nutrition of halophilic bacteria. In The Biology of Halophilic Bacteria, Eds. Vreeland, R.H. and L. I. Hochstein, Boca Raton: CRC Press, pp: 87-89.
32. Ayers, A.D., 1952. Seed germination is affected by soil moisture and salinity. Agron. J., 44: 82-84.
33. Flowers T.J. and S.A. Flowers, 2005. Why does salinity pose such a difficult problem for plant breeders?. Agr. Water Manage., 78: 15-24.
34. Cuartero, J. and R. Fernández-Muñoz, 1999. Tomato and salinity. Scientia Horticulturae, 78: 83-125.
35. Martínez, V., N. Bernstein and A. Läuchli, 1996. Salt-induced inhibition of phosphorus transport in lettuce plants. Physiol. Plantarum, 97: 118-122.
36. Maas, E.V., 1990. Agricultural Salinity Assessment and Management. New York: American Society of Civil Engineers.
37. Awad, A.S., D.G. Edwards and L.C. Campbell, 1990. Phosphorus enhancement of salt tolerance of tomato. Crop Sci., 30: 123-128.
38. Foeshe, D. and A. Jungk, 1983. Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. Plant and Soil, 74: 359-368.
39. Lambers, H., M.W. Shane and M.D. Cramer, 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Annals. Bot., 98: 693-713.
40. Bates, T.R. and J.P. Lynch, 2001. Root hairs confer a competitive advantage under low phosphorus availability. Plant and Soil, 236: 243-250.
41. Herrera-Estrella, L., J. Lopez-Bucio and C. Grierson, 2001. Molecular characterization of the effects of phosphorus on the development of root hairs in Arabidopsis. In Plant Nutrition - Food Security and Sustainability of Agro-ecosystems Through Basic and Applied Research, Eds. W.J. Horst *et al.* Dordrecht: Kluwer Academic Publishers, pp: 20-21.