Research Journal of Agriculture and Biological Sciences 1(2): 176-180, 2005

Phosphate and Potassium Solubilizing Bacteria Effect on Mineral Uptake, Soil Availability and Growth of Eggplant

[1]H.S. Han and [2,3]K.D. Lee

[1]Department of Biology, Sunchon National University, Suncheon, Jeonnam 540-742 Korea.
[2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Chinju. 660-701 Korea.
[3]Department of Plant Science, McGill University, Macdonald Campus, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, QC H9X 3V9 Canada.

Abstract: Experiments were conducted to evaluate the potential of phosphate solubilizing bacteria (PSB) *Bacillus megaterium* and potassium solubilizing bacteria (KSB) *Bacillus mucilaginosus* inoculated in nutrient limited soil planted with eggplant. Results showed that rock P and K materials either applied singly or in combination did not significantly enhance soil availability of P and K. PSB increased higher soil P availability than KSB, which was recommended as a K-solubilizer. Inoculation of these bacteria in conjunction with amendment of its respective rock P or K materials increased the availability of P and K in soil, enhanced N, P and K uptake, and promoted growth of eggplant.

Key words: Eggplant, phosphate solubilizing bacteria, potassium solubilizing bacteria

INTRODUCTION

The use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB), as biofertilizers has become of interest in Korea and China as significant areas of cultivated soils in these countries are deficient in soil available P and K[1]. P and K are major essential macronutrients for plant growth and development and hence they are commonly added as fertilizer to optimize yield. PSB have been used to convert insoluble rock P material into soluble forms available for plant growth[2,3]. This conversion is through acidification, chelation and exchange reactions[4] and produce, in the periplasm, strong organic acids[5], which have become indicators for routine isolation and selection procedures of PSB[6]. *Bacillus megaterium* is known for its ability to solubilize rock P material[7]. On the other hand, KSB are able to solubilize rock K mineral powder, such as micas, illite and orthoclases, through production and excretion of organic acids[8,9]. It has been shown that KSB, such as *Bacillus mucilaginosus,* increased K availability in soils and increased mineral content in the plant[10]. A combination of application of rock P and K materials with co-inoculation of bacteria that solubilize them might provide a faster and continuous supply of P and K for optimal plant growth. However little is known about the combined effects of rock materials and co-inoculation of PSB and KSB on mineral availability in soils, mineral content and growth of eggplant. The present work reported the synergistic effects of soil fertilization with rock P and K materials and co-inoculation with PSB and KSB on the improvement of P and K uptake and growth of eggplant grown under limited P and K soil under greenhouse conditions.

MATERIALS AND METHODS

Soil and bacteria materials: To determine the effects of P- and K-solubilizing bacteria (PSB and KSB) on eggplant (*Solanum torvum* L. NIVOT) growth in a greenhouse experiment, the soil used Typic Endoaquepts (USDA, Inceptisols). The chemical properties of the soil were as follows: pH (1:5 *w/v* water) 5.4, organic matter 18 g kg^{-1}, total nitrogen 1.4 g kg^{-1}, available P 9.1 mg kg^{-1} and available K 52.4 mg kg^{-1} (1 M NH_4-OAc). We used two strains of plant growth promoting rhizobacteria (PGPR) in these experiments. For PSB, *Bacillus megaterium* was isolated from a plastic film house area in Korea by using the PBY medium (1.0% polypeptone, 0.5% beef extract, 0.2% NaCl, 0.05% yeast extract). This PSB biovariety was identified by Dr. H. Han, Department of Biology, Sunchon National University, Korea, and has been proven for its solubilization activity on all phosphatic compounds, such

Corresponding Author: Kyung Dong Lee, Department of Plant Science, McGill University, Macdonald Campus, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, QC H9X 3V9 Canada.

as aluminum phosphate and rock phosphate[11]. For KSB, we used *Bacillus mucilaginosus* KCTC3870.

Bacterial culture and inoculant preparation: PSB and KSB were cultured in Tryptone Yeast (TY) medium[12] and sucrose-minimal salts medium[10], respectively, and incubated on an orbital shaker at 150 rpm for 48 h at 27°C. The cells in cultured bacterial broth were collected by centrifugation at 2,822 x g for 15 min at 4°C and washed with sterilized tap water. The pelleted cells was resuspended with sterilized tap water and then the cells were adjusted to about 10^8 cells mL^{-1}, based on an optical density OD_{620} = 0.08[13], and 1 mL of inoculums was applied to each seedling.

Cultivation conditions. Experiments were conducted in pots (17 cm diameter and 15 cm deep) layered with the plastic bags and containing 2.0 kg of sterilized soil for 2 hr at 130°C. Three experiments (phosphorus, potassium and a combination of both of them) were established with 10 treatments: without rock P and K materials or bacteria inoculation (control), PSB, KSB, (P+K)SB, rock P [rock phosphate powder (total P 15%, 106 µm mesh), 3 g kg^{-1} soil], rock K [Illite powder (total K 3.9%, 106 µm mesh), 3 g kg^{-1} soil], rock (P+K), rock P+PSB, rock K+KSB and rock (P+K)+(P+K)SB. Rock materials were mixed thoroughly with the soil in a plastic pot. Eggplant seeds were surface-sterilized in 2% sodium hypochlorite for 3 min and then rinsed 5 times with distilled water[13]. The seeds were germinated and grown in sterilized vermiculite in trays. After 20 days after sowing, one seedling was transplanted into each pot and inoculated with 1 mL of inoculum containing approximately 10^8 cells. The temperature in the greenhouse was maintained at 30 ± 2°C with a relative humidity of 65% and a 16 hr photoperiod created by using supplemental lighting from high-pressure sodium lamps. All plants were harvested 30 days after transplanting. The photosynthesis of plants was measured using a Li-Cor 6400 (Li-Cor Inc, Lincoln, Nebraska, USA) just before harvesting the plants. The experiment was structured following a randomized complete block design (RCBD) with four replications.

Mineral content: To analyze mineral elements, soil samples were collected before and after the experiment and air-dried for chemical analysis. Soil samples were sieved (2 mm screen) and analyzed for the following: pH (1:5 water extraction), organic matter content (Wakley and Black method[14]), available P content (Lancast[15]) and contents of exchangeable or available K^+ (1 M NH_4-OAc pH 7, AA, Shimazu 660)[16]. Shoot and root tissues were separated after harvesting and air-dried at 70°C for 5 days. Dried materials were ground and then digested in H_2SO_4 for the determination of total nitrogen (Kjeldahl method[15]) or in a ternary solution (HNO_3:H_2SO_4:$HClO_4$ = 10:1:4 with volume) for the determination of P and K.

Statistical analysis: All data were analyzed statistically by an analysis of variance using CoStat software (CoHort Software, Monterey, USA). Two fertilizer materials and PGPR strains and its co-inoculation treatments were compared using a randomized complete block model with four replications of each treatment. Mean comparisons were conducted using an ANOVA utilizing the least significant difference (LSD) (P<0.05) test.

RESULTS AND DISCUSSIONS

Available P and K in the soil: Available P and K in the soil 30 days following planting were presented in Table 1. Addition of rock material and single bacteria (PSB or KSB) into the soil only caused slight non-significant increases in available P or K, respectively. The PSB strain was a more potent solubilizer for rock P and K materials than the KSB strain. When each strain was inoculated in conjunction with the addition of its respective rock material into the soil, there was even a higher available P and K. Co-inoculation with both PSB and KSB caused a similar P and K availability to that resulting from a combined PSB inoculation and rock P material supplementation treatment. Applied together, mix-inoculation and rock P and K materials, resulted in the highest availability of P and K in the soil, namely an increase of about 25% for P and 15% for K as compared with the untreated control (without the bacterial inoculum

Table 1: Effects of PSB and KSB strains on available P and K in soil planted with eggplant, 30 days after planting

Treatment	P mg kg^{-1} soil	K mg kg^{-1} soil
Control	8.1	44.0
Rock P	8.7	44.4
Rock K	8.2	45.9
Rock (P+K)	8.8	46.7
PSB	9.0	45.2
KSB	8.6	48.3
(P+K)SB	9.8	49.8
Rock P+PSB	10.7	49.6
Rock K+KSB	9.8	51.1
Rock (P+K)+(P+K)SB	10.9	52.0
$LSD_{0.05}$	1.1	5.5

Table 2: Effects of PSB and KSB strains on shoot and root nutrient uptake of eggplant

Treatment	Shoot (mg plant^{-1})			Root (mg plant^{-1})		
	N	P	K	N	P	K
Control	16.4	5.04	30.7	5.36	0.86	8.92
Rock P	16.9	5.35	31.3	5.72	0.89	9.02
Rock K	16.8	5.09	32.0	5.43	0.83	9.54
Rock (P+K)	17.2	5.33	32.7	5.55	0.91	9.51
PSB	17.5	5.48	31.9	5.74	0.88	9.15
KSB	17.0	5.11	32.2	5.66	0.85	9.52
(P+K)SB	17.8	5.50	32.8	5.80	0.92	9.66
Rock P+PSB	18.1	6.07	33.0	5.91	0.96	9.76
Rock K+KSB	17.8	5.53	34.1	5.85	0.89	10.18
Rock (P+K)+(P+K)SB	18.7	6.16	34.9	5.94	0.98	10.83
$LSD_{0.05}$	1.5	0.9	2.3	0.6	0.06	1.0

Table 3: Effects of PSB and KSB strains on dry matter and photosynthesis of eggplant

Treatment	Plant height (cm)	Dry weight (g plant^{-1})			Photosynthetic rate (µmole cm^{-2} s^{-1})
		Shoot	Root	Total	
Control	18.9	1.33	0.60	1.93	12.7
Rock P	19.6	1.38	0.68	2.06	13.1
Rock K	19.5	1.30	0.61	1.91	12.9
Rock (P+K)	20.0	1.46	0.70	2.16	13.4
PSB	19.5	1.41	0.70	2.11	12.9
KSB	19.7	1.35	0.66	2.01	12.5
(P+K)SB	20.8	1.53	0.75	2.28	13.5
Rock P+PSB	21.5	1.68	0.72	2.40	13.9
Rock K+KSB	20.6	1.49	0.71	2.20	13.0
Rock (P+K)+(P+K)SB	21.9	1.69	0.78	2.47	14.2
$LSD_{0.05}$	2.8	0.14	0.11	0.25	0.8

and without the rock material fertilizer). Increasing the bioavailability of P and K in the soils with inoculation of PGPR or with combined inoculation and rock materials has been reported by many researchers [7,17], which may lead to increased P uptake and plant growth[18,19].

N, P and K uptake in plants: Results showed that rock P and K materials and soil inoculation with PSB or KSB did not significantly increase N, P and K uptake in eggplant (Table 2). Increase of N, P and K uptake generally occurred in the combined treatment, i.e. soil co-inoculated with both bacteria and fertilized with rock P and K materials. This combined treatment resulted in increases of N, P and K uptake in the shoot (14, 22 and 14%, respectively) and in the root (11, 14 and 21%). Application of insoluble rock fertilizers in the sterilized soil did not increase the mineral content in eggplant. This might be because these rock materials are solubilized so slowly in sterilized soil which results in P and K becoming generally not available to plants during the growing period. It is interesting to note that combined together bacterial inoculation and rock material fertilizer application also increased the N uptake by the plants. Increasing N uptake in our experiment with inoculation with *Bacillus* may be related to the fact that *Bradyrhizobium* sp., a genus which fixes atmospheric nitrogen in symbiosis with legumes, is phyllogenically closer to *Bacillus* than to other rhizobial genera[20]. Therefore, the *Bacillus* strain used in this study might have the capability to fix atmospheric nitrogen. Our results combined with the results from others suggest that the combined bacteria inoculation with PSB and KSB along with the rock P and K materials amendment can improve mineral nutrient uptake by crops grown under nutrient-deficient soils leading to plant growth.

Plant growth: The shoot and root dry weight in eggplant increased ($P<0.05$) in the combined fertilizer treatment with rock P or K material, in the inoculation with a single respective bacterial strain (PSB or KSB) treatment and in the combined bacteria plus rock material treatment, as compared with the control (Table 3). Application of rock P and K materials alone did not significantly improve plant height, dry weight or photosynthesis. Although inoculation with a single bacterial strain in combination with its respective phosphate source consistently increased shoot and root dry weight as compared with the control, the treatment which combined both bacteria and mineral rocks further increased shoot dry weight by 27% and root dry weight by 30% over the control 30 days following planting. Photosynthesic responses of eggplant demonstrated a similar trend to plant dry weight responses (Table 3). The combined treatment of the two strains co-inoculated with each other along with the applied insoluble rock material significantly increased leaf photosynthesis 12% over the control. In our case, co-inoculation of PSB and KSB strains synergistically solubilized the rock P and K materials which were added into the soil and made them more available to the plant. This led to the promotion of their uptake and plant growth. Growth enhancement by *Bacillus* may also relate to its ability to produce hormones, especially IAA[21]. In short, co-inoculation of PGPR with different beneficial properties may be the future trend for bio-fertilizer application to enable sustainable crop production.

In conclusion, co-inoculation of PSB and KSB in conjunction with direct the application of rock P and K materials into the soil increased N, P and K uptake, photosynthesis and the yield of eggplant grown on P and K limited soils.

REFERENCES

1. Xie, J.C., 1998. Present situation and prospects for the world's fertilizer use. Plant Nut. Fer. Sci., 4: 321-330.
2. Nahas, E., D.A. Banzatto and L.C. Assis, 1990. Fluorapatite solubilization by *Aapergillus niger* in vinasse medium. Soil Biol. Biochem., 22: 1097-1101.
3. Bojinova, D., R. Velkova, I. Grancharov and S. Zhelev, 1997. The bioconversion of Tunisian phosphorite using *Aspergillus niger*. Nutr. Cyc. Agroecosyst., 47: 227-232.
4. Gerke, L., 1992. Phosphate, aluminum and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Z. Pflanzenernahr. Bodenk., 155: 17-22.
5. Alexander, M., 1977. Introduction to soil microbiology. John Wiley and Sons Inc., New York, USA.
6. Illmer, P., A. Barbato and F. Schinner, 1995. Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganism. Soil Biol. Biochem., 27: 265-270.
7. Schilling, G., A. Gransee, A. Deubel, G. Lezovic and S. Ruppel, 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Z. Pflanzenernahr. Bodenkd., 161: 465-478.
8. Friedrich, S., N.P. Platonova, G.I. Karavaiko, E. Stichel and F. Glombitza, 1991, Chemical and microbiological solubilization of silicates. Acta Biotech., 11: 187-196.
9. Ullman, W.J., D.L. Kirchman and S.A. Welch, 1996. Laboratory evidence for microbially mediated silicate mineral dissolution in nature. Chemical Geology, 132: 11-17.
10. Sheng, X.F., L.Y. He and W.Y. Huang, 2002. The conditions of releasing potassium by a silicate-dissolving bacterial strain NBT. Agri. Sci. in China, 1: 662-666.
11. Sundara, B., V. Natarajan and K. Hari, 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. Field Crops Research, 77: 43-49.
12. Vincent, J.M., 1970. A Manual for the Practical Study of the Root-nodule Bacteria. Burgess and Son Ltd. Great Britain. pp. 45.
13. Bhuvaneawari, T.V., B.G. Turgeon and W.D. Bauer, 1980. Early events in the infection of soybean (*Glycine max* L. Merr.) by *Rhizobium japonicum* I. location of infectable root cells. Plant Physiol., 66: 1027-1031.
14. Allison, L.E., 1965. Organic carbon. In: Methods of Soil Analysis. Part II, Black C.A. (Eds.), Am. Soc. Agron. Inc. Publ., Madison, USA, pp. 1367-1376.
15. RDA (Rural Development Administration, Korea), 1988. Methods of Soil Chemical Analysis. National Institute of Agricultural Science and Technology, RDA, Suwon, Korea.
16. Richards, J.E. and T.E. Bates, 1989. Studies on the potassium-supplying capacities of southern Ontario soils. III Measurement of available K., Can. J. Soil Sci., 69: 597-610.

17. Lin, Q.M., Z.H. Rao, Y.X. Sun, J. Yao and L.J. Xing, 2002. Identification and practical application of silicate-dissolving bacteria. Agri. Sci. in China, 1: 81-85.
18. Omer, S.A., 1998. The role of rock-phosphate-solubilizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol., 14: 211-218.
19. 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: 221-227.
20. Zakhia, F. and P. de Lajudie, 2001. Taxonomy of rhizobia. Agronomie, 21: 569-576.
21. Sheng, X.F. and W.Y. Huang, 2001. Physiological characteristics of strain NBT of silicate bacterium. Acta Pedologica Sinica, 38: 569-574.