Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber

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ABSTRACT

Biofertilizers have been used as sources to improve plant nutrients in sustainable agriculture. Experiments were conducted to evaluate the potential of phosphate solubilizing bacteria (PSB) *Bacillus megaterium* var. *phosphaticum* and potassium solubilizing bacteria (KSB) *Bacillus mucilaginosus* inoculated in nutrient limited soil planted with pepper and cucumber. Results showed that rock P and K applied either singly or in combination did not significantly enhance soil availability of P and K, indicating their unsuitability for direct application. PSB was a more potent P-solubilizer than KSB, and co-inoculation of PSB and KSB resulted in consistently higher P and K availability than in the control without bacterial inoculum and without rock material fertilizer. Integrated rock P with inoculation of PSB increased the availability of P and K in soil, the uptake of N, P and K by shoot and root, and the growth of pepper and cucumber. Similar but less pronounced results were obtained when rock K and KSB were added concomitantly. Combined together, rock materials and both bacterial strains consistently increased further mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer.

Keywords: pepper; cucumber; phosphate solubilizing bacteria; potassium solubilizing bacteria

Significant areas of cultivated soils in Korea and China are deficient of available P and K and have a low crop productivity (Xie 1998). The use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilizers, was suggested as a sustainable solution to improve plant nutrient and production (Vessey 2003). Phosphate and potassium are major essential macronutrients for plant growth and development and soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield. When phosphate is added into soils as a fertilizer in relatively soluble and plant available forms, it is easily converted into insoluble complexes with calcium carbonate, aluminum and iron oxides, and crystalline and amorphous aluminum silicate (Sample et al. 1980). Consequently, to achieve optimum crop yields, soluble phosphate fertilizers have to be applied at high rates which cause unmanageable excess of phosphate application and environmental and economic problems (Brady 1990). On the other hand, K deficiencies become problem because K decreases easily in soils due to crop uptake, runoff, leaching and soil erosion (Sheng and Huang 2002). Direct application of rock phosphate (rock P) and potassium (rock K) materials may be agronomically more useful and environmentally more feasible than soluble P and K (Rajan et al. 1996). Rock P and K materials are cheaper sources of P and K; however, most of them are not readily available to a plant because the minerals are released slowly and their use as fertilizer often causes insignificant yield increases of current crop (Zapata and Roy 2004). PSB have been used to improve rock P value because they convert insoluble rock P into soluble forms available for plant growth (Nahas et al. 1990, Bojinova et al. 1997). This conversion is through acidification, chelation and exchange reactions (Gerke 1992) and produces, in the periplasm, strong organic acids (Alexander 1977), which have become indicators for routine isolation and selection procedures of PSB (e.g. Illmer et al. 1995). *Bacillus megaterium* var.

phosphaticum is known for its ability to solubilize rock P material (Schilling et al. 1998). On the other hand, KSB are able to solubilize rock K mineral powder, such as micas, illite and orthoclases, also through production and excretion of organic acids (Friedrich et al. 1991, Ullman et al. 1996). It was shown that KSB, such as *Bacillus mucilaginosus*, increased K availability in soils and increased mineral content in plant (Sheng et al. 2002). An integrated application of rock P and K materials with co-inoculation of bacteria that solubilize them might provide 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 pepper and cucumber. The aim of the study was to evaluate the potential of the direct application of rock P and K materials and co-inoculation with PSB and KSB for the improvement of P and K uptake and growth of pepper and cucumber plants grown under limited P and K soil conditions in a greenhouse.

MATERIAL AND METHODS

The effects of P- and K-solubilizing bacteria on pepper (*Capsicum annum* L.) and cucumber (*Cucumis sativus* L.) growth were examined in a greenhouse experiment using Aquepts Series, Typic Endoaquepts (USDA, Inceptisols) soil collected from Chinju, Kyungnam province, Korea. The chemical properties of the soil were: pH (1:5 w/v water) 5.4, organic matter 18 g/kg, total nitrogen 2.1 g/kg, available P 9.1 mg/kg, and available K 52.4 mg/kg (1M NH_4-OAc). We used two strains of plant growth promoting rhizobacteria (PGPR), i.e. PSB and KSB. *Bacillus megaterium* var. *phosphaticum*, selected for PSB, was isolated from 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 strain was identified by Dr. H. Han (Molecular Biology Laboratory, Department of Biology, Sunchon National University, Korea) and was proven for its capability to solubilize many phosphatic compounds, such as aluminum phosphate and rock phosphate (Sundara et al. 2002). In the case of KSB, we used *Bacillus mucilaginosus* (KCTC3870, Korean Collection of Type Cultures).

PSB and KSB were cultured in Tryptone Yeast (TY) medium (Vincent 1970) and sucrose-minimal salts medium (Sheng et al. 2002), 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 × g for 15 min at 4°C and washed with sterilized tap water. The pelleted cells were resuspended with sterilized tap water and then the cells were adjusted to about 10^8 cells/ml, based on optical density OD_{620} = 0.08 (Bhuvaneswari et al. 1980), and 1 ml of inoculum was applied into each seedling.

Experiment for studying the effect of the bacterial strain on plant growth, P and K uptake of pepper and cucumber was conducted in pots (17 cm diameter and 15 cm deep) layered with plastic bags and containing 2.0 kg of sterilized soil. The soil was sterilized by dry autoclaving at 20 psi for 120 min. The experiment was 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 soil], rock K [illite powder (total K 3.9%, 106 μm mesh), 3 g/kg soil], rock (P + K), rock P + PSB, rock K + KSB, and rock (P + K)+(P + K)SB. Rock materials and inoculants were mixed thoroughly with the soil in a plastic pot to ensure the solubilization effect of rock nutrient by PGPRs. Seeds of pepper and cucumber were surface-sterilized in 2% sodium hypochlorite for 3 min and then rinsed 5 times with distilled water (Bhuvaneswari et al. 1980). The seeds were germinated and grown in sterilized vermiculite in trays. At 20 days after sowing for pepper and 15 days for cucumber, one seedling was transplanted into each pot. At three days after transplanting, depending on the treatment, the healthy seedling was inoculated with 1 ml of inoculum containing around 10^8 cells. The temperature in the greenhouse was maintained at 30 ± 2°C with a relative humidity of 65% and a 16 hrs 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) at harvest time, just before harvesting the plants.

To analyze mineral elements, soils were sampled for chemical analyses before the experiments and after harvesting the plants. At harvest, following root removal, soils in the whole pot were mixed and a sample was drawn. Soil samples were air-dried and sieved (2 mm screen) and analyzed for the following: pH (1:5 water extraction), organic matter content (Wakley and Black method; Allison 1965), available P content (Lancast; RDA 1988) and contents of exchangeable or available K^+ (1M NH_4-OAc pH 7, AA, Shimazu 660; Richards and Bates 1989). Shoot and root tissues were separated after harvesting and air-dried at 70°C for 5 days. Dried soils were grounded and then digested in H_2SO_4 for determination of total nitrogen (Kjeldahl method) or in a ternary solution (HNO_3:H_2SO_4:$HClO_4$ = 10:1:4 with volume) for the determination of P and K.

All data were analyzed statistically by analysis of variance using CoStat software (CoHort Software, Monterey, USA). The experiment was structured following a randomized complete block design (RCBD) with four replications. Means comparisons were conducted using an ANOVA protected the least significant difference (*LSD*) test, with the ANOVA confidence levels of 0.95. Data were presented with their standard deviations (*SD*).

RESULTS AND DISCUSSION

Large areas of cultivated land in Korea and China are deficient in phosphorus and potassium nutrient; therefore, in our experiment we have included soil with addition of rock P and K and their solubilizing bacteria to increase the available P and K in this soil. Available P and K in the soils 30 days following the planting were presented in Table 1. Addition of rock materials into the soil did not significantly increase available P or K, and single inoculation of PSB or KSB resulted in a higher mineral availability in which PSB strain was a more potent solubilizer for rock P than KSB strain. Co-inoculation with PSB and KSB or combined PSB inoculation and rock P material significantly increased the availability of P and K. When applied all together, PSB, KSB and rock P and K resulted in the highest availability of P and K in soils, and caused an increase of about 36% for P and 31% for K as compared to untreated control (without bacterial inoculum and without rock material fertilizer). The results also showed that there was a relatively higher availability of P and K in soils planted with pepper than with cucumber.

Soil inoculation with PSB or KSB significantly increased N, P and K uptake in pepper and cucumber plants, especially when the respective rock P or rock K were added (Tables 2 and 3). Further significant increases in N, P and K uptakes from the inoculated soils were generally found in the all-integrated treatment, i.e. soil co-inoculated with both bacteria and fertilized with rock P and K. This combined treatment increased N, P and K uptake in shoot (21, 31 and 33%, respectively, for pepper and 29, 41 and 29% for cucumber) and in root (16, 33 and 26% for pepper; 29, 34 and 50% for cucumber). Application of insoluble rock fertilizers in the sterilized soil did not increase mineral content in pepper and cucumber. This might occur because these rock materials are solubilized slowly only by

Table 1. Effects of PSB and KSB strains on available P and K (mg/kg) in soil planted with pepper and cucumber, 30 days after planting; the values are the mean of four replications (± *SD*)

Treatments	Pepper		Cucumber	
	P	K	P	K
Control	8.0 ± 0.47	41.2 ± 2.08	7.8 ± 0.80	38.3 ± 1.45
R-P	8.5 ± 0.49	42.0 ± 2.14	8.4 ± 0.67	39.2 ± 2.92
R-K	8.3 ± 0.32	43.3 ± 2.27	8.2 ± 0.70	41.6 ± 1.39
R-(P + K)	8.7 ± 0.40	45.1 ± 2.50	8.8 ± 0.28	41.5 ± 1.82
PSB	9.4 ± 1.08	45.5 ± 2.51	9.3 ± 0.40	41.4 ± 2.70
KSB	8.5 ± 0.69	47.3 ± 2.38	8.5 ± 0.30	44.7 ± 2.72
(P + K)SB	9.8 ± 0.48	49.9 ± 5.28	9.7 ± 0.64	46.8 ± 2.21
R-P + PSB	10.1 ± 0.80	47.3 ± 3.82	9.8 ± 0.93	43.8 ± 2.71
R-K + KSB	9.0 ± 0.50	49.5 ± 1.99	8.9 ± 0.50	46.4 ± 2.71
R-(P + K) + (P + K)SB	10.9 ± 1.24	54.2 ± 3.19	10.6 ± 0.58	50.1 ± 1.70
$LSD_{0.05}$	1.03	4.22	0.91	3.35

R-P = rock phosphate; R-P = rock K (illite); PSP = phosphate solubilizing bacteria; KSB = potassium solubilizing bacteria; *LSD* = least significant difference

Table 2. Effects of PSB and KSB strains on shoot and root nutrient uptake (mg/plant) of pepper; the values are the mean of four replications (± *SD*)

Treatments	Shoot			Root		
	N	P	K	N	P	K
Control	13.6 ± 0.74	1.61 ± 1.61	19.2 ± 1.69	3.69 ± 0.18	0.46 ± 0.04	5.92 ± 0.25
R-P	14.3 ± 0.80	1.72 ± 0.08	19.9 ± 1.74	3.76 ± 0.23	0.48 ± 0.06	6.19 ± 0.33
R-K	14.1 ± 0.70	1.68 ± 0.09	20.3 ± 2.29	3.71 ± 0.34	0.47 ± 0.05	6.17 ± 0.30
R-(P + K)	14.2 ± 0.67	1.75 ± 0.05	20.9 ± 2.16	3.87 ± 0.16	0.50 ± 0.05	6.68 ± 0.34
PSB	14.5 ± 0.64	1.88 ± 0.12	21.1 ± 1.40	3.90 ± 0.41	0.50 ± 0.05	6.48 ± 0.36
KSB	14.5 ± 0.79	1.75 ± 0.12	21.7 ± 2.19	3.82 ± 0.45	0.49 ± 0.03	6.68 ± 0.29
(P + K)SB	14.7 ± 0.57	1.99 ± 0.23	23.4 ± 2.31	3.92 ± 0.28	0.54 ± 0.06	6.95 ± 0.62
R-P + PSB	15.0 ± 0.56	1.91 ± 0.09	21.7 ± 1.43	3.95 ± 0.26	0.53 ± 0.06	6.70 ± 0.76
R-K + KSB	14.8 ± 0.66	1.84 ± 0.13	22.4 ± 1.59	3.90 ± 0.18	0.51 ± 0.05	6.93 ± 0.32
R-(P + K) + (P + K)SB	16.5 ± 0.83	2.11 ± 0.08	25.7 ± 2.35	4.27 ± 0.31	0.61 ± 0.06	7.46 ± 0.29
$LSD_{0.05}$	1.01	0.18	2.81	0.43	0.07	0.60

Explanations see Table 1

plant root system in sterilized soil and therefore not generally available to plants (Table 1). It is interesting that jointly bacterial inoculation and rock material fertilizer application also increased N uptake by plants (Table 3).

Single or double application of rock P and rock K did not significantly improve plant growth (shoot and root dry weight) and photosynthesis in pepper and cucumber plants (Table 4). Double inoculation of PSB and KSB increased shoot and root dry weight; PSB inoculation increased shoot dry weight, whereas KSB did not. Although combined PSB inoculation with application of rock P consistently increased shoot and root dry weight as compared to control, a treatment which joints together both bacteria and mineral rocks, further

Table 3. Effects of PSB and KSB strains on shoot and root nutrient uptake (mg/plant) of cucumber; the values are the mean of four replications (± *SD*)

Treatments	Shoot			Root		
	N	P	K	N	P	K
Control	16.9 ± 0.94	1.66 ± 0.08	22.8 ± 1.91	3.55 ± 0.12	0.44 ± 0.04	8.67 ± 0.33
R-P	17.3 ± 1.15	1.70 ± 0.08	23.1 ± 2.27	3.60 ± 0.28	0.46 ± 0.02	8.84 ± 0.56
R-K	17.2 ± 0.82	1.70 ± 0.07	24.1 ± 1.55	3.64 ± 0.27	0.46 ± 0.04	9.12 ± 0.93
R-(P + K)	18.9 ± 0.76	1.80 ± 0.07	24.6 ± 1.54	3.87 ± 0.22	0.48 ± 0.06	9.86 ± 0.94
PSB	18.0 ± 1.14	1.85 ± 0.11	24.0 ± 2.69	3.75 ± 0.18	0.49 ± 0.04	9.05 ± 0.26
KSB	17.8 ± 1.06	1.77 ± 0.08	25.5 ± 0.90	3.70 ± 0.31	0.47 ± 0.05	9.97 ± 0.85
(P + K)SB	19.6 ± 1.27	1.93 ± 0.07	26.6 ± 1.63	4.01 ± 0.31	0.51 ± 0.04	10.50 ± 1.11
R-P + PSB	18.5 ± 0.67	1.96 ± 0.11	24.9 ± 0.54	3.95 ± 0.30	0.53 ± 0.05	9.52 ± 0.48
R-K + KSB	18.2 ± 0.62	1.86 ± 0.05	26.4 ± 1.53	3.96 ± 0.30	0.49 ± 0.02	10.48 ± 1.33
R-(P + K) + (P + K)SB	21.8 ± 0.76	2.34 ± 0.11	29.4 ± 0.90	4.59 ± 0.31	0.59 ± 0.07	13.05 ± 1.37
$LSD_{0.05}$	1.36	0.12	2.41	0.39	0.065	1.30

Explanations see Table 1

Table 4. Effects of PSB and KSB strains on dry weight (g/plant) and photosynthetic rate (μmole/cm²/s) of pepper and cucumber; the values are the mean of four replications (± *SD*)

Treatments	Pepper			Cucumber		
	dryweight		photosynthetic rate	dry weight		photosynthetic rate
	shoot	root		shoot	root	
Control	0.670 ± 0.033	0.268 ± 0.023	18.2 ± 0.50	0.757 ± 0.039	0.291 ± 0.016	11.1 ± 0.59
R-P	0.692 ± 0.035	0.288 ± 0.016	18.7 ± 0.67	0.810 ± 0.046	0.307 ± 0.020	11.3 ± 0.61
R-K	0.685 ± 0.037	0.283 ± 0.019	18.5 ± 0.47	0.805 ± 0.042	0.302 ± 0.023	11.5 ± 0.54
R-(P + K)	0.700 ± 0.037	0.289 ± 0.021	18.8 ± 1.13	0.781 ± 0.044	0.317 ± 0.007	11.6 ± 0.49
PSB	0.762 ± 0.035	0.294 ± 0.027	19.4 ± 0.48	0.845 ± 0.043	0.314 ± 0.021	12.1 ± 0.57
KSB	0.723 ± 0.033	0.286 ± 0.013	18.9 ± 0.22	0.819 ± 0.040	0.310 ± 0.023	11.6 ± 0.79
(P + K)SB	0.786 ± 0.055	0.300 ± 0.016	19.7 ± 0.87	0.832 ± 0.069	0.338 ± 0.018	12.1 ± 0.70
R-P + PSB	0.790 ± 0.039	0.310 ± 0.030	20.5 ± 1.02	0.871 ± 0.044	0.339 ± 0.024	12.5 ± 0.32
R-K + KSB	0.765 ± 0.042	0.295 ± 0.024	19.2 ± 0.40	0.828 ± 0.038	0.317 ± 0.014	12.0 ± 12.0
R-(P + K) + (P + K)SB	0.846 ± 0.039	0.345 ± 0.021	21.8 ± 0.54	0.920 ± 0.047	0.369 ± 0.011	12.9 ± 0.74
$LSD_{0.05}$	0.056	0.031	0.99	0.066	0.027	0.085

Explanations see Table 1

increased plant growth: 26% in shoot and 29% in root dry weight for pepper and 22% in shoot and 27% in root dry weight for cucumber plant over the controls during 30 days following planting. Photosynthesis responses of pepper and cucumber to the treatments demonstrated similar trends to shoot dry weight responses (Table 4). The integrated treatment of co-inoculation with two strains and application of insoluble rock materials significantly increased leaf photosynthesis 20% in pepper and 16% in cucumber plants over the controls.

Increasing the bioavailability of P and K in soils with inoculation of PGPR or with combined inoculation and rock materials, which may lead to increased P uptake and plant growth, was reported by many researchers (e.g. Omer 1998, Wahid and Mehana 2000, Lin et al. 2002, Şahin et al. 2004). Omer (1998) reported that the application of rock P to nonsterilized soil with bacteria inoculation increased P uptake and shoot and total dry mass of wheat plant. Our one-year field experiment using the same PSB strain demonstrated results similar to these greenhouse experiments: increased P availability in the soils and increased dry mater of lettuce, especially when rock P was added (data not shown). This indicates that the bacterial strains can compete with existing natural bacteria, which is understandable as they are isolated from similar soil conditions in Korea. Although such a competition with indigenous less-effective rhizobia was studied in rhizobial inoculant (Sessitch et al. 2002, Lopez-Gracia et al. 2002, Loh and Stacey 2003), competition in PGPR inoculants was not intensively explored probably because some PGPR inoculants are selected for increasing plant systemic acquired resistance or for suppression of pathogenic bacteria (Zehnden et al. 2001, Anith et al. 2004). Bacteria inoculation, which can improve P and K availability in soils by producing organic acids and other chemicals, stimulated growth and mineral uptake of plants (Alexander 1977, Park et al. 2003). Combined all together, our results with results from others suggest that integration of bacteria inoculation with PSB and KSB and rock P and K materials amendment can improve crop mineral nutrients in nutrient-deficient soils.

Our experiment demonstrated the advantage of co-inoculation of two *Bacillus* species, which have been reported as PSB and KSB strains, on mineral uptake and growth of pepper and cucumber. Synergistic effects of combined inoculation of PGPRs have also been reported in various crops, for examples potatoes (Kundu and Gaur 1980), rice (Tiwari et al. 1989) and sugar beet and barley (Çakmakçi et al. 1999). In our case, co-inoculation of PSB and KSB strains synergistically solubilized rock P and K which were added into the soil and make them much more available for uptake by plant roots. Higher N, P and K uptake may subsequently promote the plant growth. Growth enhancement by *Bacillus* may be also associated to its ability to produce hormone, especially IAA (Sheng and Huang 2001), and siderophore (Ito 1993, Hu and Boyer 1996). 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 legume, is phyllogenically closer to *Bacillus* than to other rhizobial genera (Zakhia and Lajudie 2001, Şahin et al. 2004). Therefore, *Bacillus* strain used in this study might have the capacity to fix atmospheric nitrogen. It is also known that P availability in soils is important for the uptake of N from soils and its utilization in plant (Kim et al. 2003). Hence, higher available P due to the solubilization with inoculated PSB might cause an enhancement of N uptake. In short, results from all these and other experiments suggest that co-inoculation of PGPR with different beneficial properties should be the future trends of bio-fertilizer application for sustainable crop production.

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