Effects of *Bacillus* FS-3 on growth of tomato (*Lycopersicon esculentum* L.) plants and availability of phosphorus in soil

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ABSTRACT

The effects of phosphate solubilizing bacterium (*Bacillus* FS-3) application on phosphorus contents of tomato (*Lycopersicon esculentum* L.) plant, growing performance and phosphorus forms in soil were evaluated under greenhouse condition. Five different phosphorus fertilizer treatments (normal superphosphate, triple superphosphate, di-ammonium phosphate, phosphoric acid, and rock phosphate) with and without bacterium (*Bacillus* FS-3) were applied in pots as 344 kg P/ha. Basal fertilizers were applied to all the pots as 180 kg N/ha (NH_4NO_3 33% N), 100 kg K/ha (K_2SO_4 50% K_2O). The results obtained showed that phosphorus availability from soil increased with phosphate solubilizing bacterium (PSB) application. The amount of plant available form of soil phosphorus fraction (resin-Pi + $NaHCO_3$-Pi + $NaHCO_3$-Po + NaOH-Pi + NaOH-Po) increased with PSB application. In all fertilizer types, bacteria application converted approximately 20% of less available phosphorus into labile forms. Statistically significant differences were obtained in shoot and root dry weight of tomato plants treated with PSB application. In all of the fertilizers, plant shoot and root weight and P uptake were greater with PSB applications than without PSB. The highest shoot-root dry weight and P uptake of plant were determined in triple superphosphate (TSP) with PSB application treatment. The data in the present study suggest that the application of PSB (FS-3) may increase the availability of soluble phosphate by dissolving the inorganic forms of phosphate and that bacterial strain tested in this study has a potential to be used as a bio-fertilizer in sustainable and organic agriculture.

Keywords: phosphorus availability; phosphorus solubilizing bacterium; rock phosphate; tomato

Phosphorus is one of the major essential macronutrients for biological growth and development. Soils generally contain substantial reserves of total phosphorus; however most of it remains relatively inert, and only less than 10% of soil phosphorus enter the plant-animal cycle (Kucey et al. 1989). Phosphorus deficiency is widespread and phosphorus fertilizers are almost universally required to maintain crop production because when it is added to soil in the form of phosphatic fertilizers, only a small part of phosphorus is utilized by plants and the rest is converted into insoluble fixed forms (Rodriguez and Fraga 1999). When added to soils the soluble phosphates react with the constituents of soil and form compounds that are less soluble, depending upon the soil. Thus, in acid soils, the reaction products are aluminium and iron phosphates; in the predominantly calcareous soils, the reaction products are calcium phosphates. Different phosphatic fertilizers yield different reaction products. The formation of these reaction products depends on soil environment and the types of fertilizer material added (Sundara et al. 2002).

It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Phosphate solubilizing microorganisms render these insoluble phosphates into soluble form. This process not only compensates for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil. Microbial solubilization of rock phosphate (RP), especially low grade, and its use in agriculture

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is receiving a great attention. Therefore, their use as biofertilizers or control agents in agriculture has been a focus of research for a number of years. A large number of heterotrophic and autotrophic microorganisms including bacteria, fungi, and cyanobacteria, are reported to solubilize insoluble phosphate forms, e.g. hydroxyapatite, tricalcium phosphate, and rock phosphate (Roychoudhury and Kaushik 1989). Such activities are often demonstrated in agricultural soils where crop production was augmented considerably (Bhattacharya et al. 1986).

Phosphorus solubilizing bacteria in general have been found effective in solubilizing inorganic phosphorus in the soils. The solubilization effect is generally due to the production of organic acids such as citric, glutamic, succinic, lactic, oxalic, malic, fumaric and tartaric acid and it has been observed in the liquid medium. The action of organic acids has been attributed to their chelating property. As phosphorus solubilizing microorganisms render more phosphates into solution than is required for their growth and metabolism, the surplus could be absorbed by plants (Sundara et al. 2002).

Phosphorus fertilizers are expensive for growers, particularly in developing countries. They are either imported or manufactured using raw material. Due to the increase in their cost in the recent decade, there has been a trend towards the discontinuation of phosphorus fertilizer application or reduction in the amounts applied in developing countries.

The purpose of this study was to examine the effect of added phosphorus solubilizing bacterium on the solubilization of different phosphorus fertilizers and rock phosphate, used in generally sustainable agriculture, and its interaction with soil phosphorus form and tomato P uptake.

MATERIAL AND METHODS

Phosphorus solubilizing bacterium (PSB) strain

The bacterial strain *Bacillus* (FS-3) was originally isolated from pepper plants at Atatürk University, Erzurum, Turkey (Şahin et al. 2004). The *Bacillus* (FS-3) used in the present study was originally selected for its phosphate solubilizing capacity (Mehta and Nautiyal 2001) under laboratory conditions (Şahin et al. 2004). The bacterial strain was maintained for long-term storage in nutrient broth with 15% glycerol at –80°C for further tests. For this experiment, pure cultures were grown in nutrient broth (NB) at 28°C and diluted to a final concentration of 10^9 cfu/ml in sterile distilled water containing 0.025% Tween 20.

Soil sampling and laboratory analysis techniques

The soil was sampled from the Ap horizon in Erzurum province (39°55'N, 41°61'E), Turkey, dried indoors until it could be crumbled to pass through a 4-mm sieve for pots experiment and 2-mm sieve for analyses of physicochemical properties. The soil is classified as Ustorthents according to USDA soil taxonomy (Soil Survey Staff 1992).

The loam (33.2% sand, 38.4% silt, and 28.4% clay) soil had 2.4% $CaCO_3$, 14.2 mg/kg P, 256 mg/kg K, 7.25 pH (H_2O) and 1.63 dS/m electrical conductivity. Initially, the soil had 1.3 mg/kg resin-Pi, 2.6 mg/kg $NaHCO_3$-Pi, 78.7 mg/kg NaOH-Pi, 85.4 mg/kg H_2SO_4-Pi, 4.4 mg/kg $NaHCO_3$-Po, 100.8 mg/kg NaOH-Po, 138.3 mg/kg residual-P and 500.6 mg/kg total-P.

Soils samples were collected in each pot, air-dried and sieved (2-mm sieve) for soil analysis. Particle size analysis was performed by the pipette method, after the pre-treatment with 35% H_2O_2 and 1.0M HCl to remove organic matter and carbonates, respectively (Gee and Bauder 1986). Soil pH was determined in 2:1 water-soil suspension by pH-meter (McLean 1982).

Sequential phosphorus extraction: sub-samples were passed through a 0.15-mm sieve and subjected to the sequential P fractionation method of Hedley et al. (1982a), modified by Araujo et al. (1993) (Table 1). The sequential extraction procedure of Hedley and co-authors fractionates soil P in various inorganic and organic pools, with a decreasing availability to plants (Hedley et al. 1982a, b) as follows: resin-Pi and $NaHCO_3$-Pi and Po are considered the most labile; the NaOH extracted fractions have a lower lability, including P associated with Al and Fe oxides; the H_2SO_4-Pi fraction includes apatite and some other recalcitrant Ca phosphates; the residual-P is the most resistant fraction and may contain inorganic and organic P.

Plants were harvested by cutting the shoots from the soil surface and washed with deionized water. Plant roots were separated from the soil and washed with water until free of soil and then washed three times with deionized water. Plant shoot and root weights were determined.

Table 1. Flow chart of the sequential P fractionation procedure and significance of phosphorus fractions (modified from Hedley et al. 1982a)

Chemical procedure	Forms of P extracted	Geochemically significant	Ecological significant
Soil + resin + water – shake for 16 h centrifuge resin + 0.5 mol/l HC1 – shake for 1 h	resin Pi	non-occluded; adsorbed on surface of crystalline compounds	plant available, direct exchangeable with soil solution; rapid turnover
Soil + 0.5 mol/l $NaHCO_3$ – shake for 16 h centrifuge aliquot	$NaHCO_3$-Pi	non-occluded; adsorbed on surface of crystalline compounds and soil colloids	easy plant available; rapid turnover
Aliquot – H_2SO_4 + $K_2S_2O_8$ digestion	$NaHCO_3$-Pt		
Total P minus inorganic P	$NaHCO_3$-Po	non-occluded; adsorbed on soil colloids	easy mineralisable, plant available P; rapid turnover
Soil + 0.1 mol/l NaOH – shake for 16 h centrifuge aliquot	NaOH-Pi	non-occluded; chemi-adsorbed to amorphous and crystalline Al and Fe and associated with humic compounds	lesser plant available; slow turnover
Aliquot – H_2SO_4 + $K_2S_2O_8$ digestion	NaOH-Pt		
Total P minus inorganic P	NaOH-Po	non-occluded; associated to humic compounds and chemi-adsorbed to Fe and Al compounds	no directly plant available; slow turnover
Soil + mol/l H_2SO_4 – shaking for 16 h centrifuge	H_2SO_4-Pi		
Soil + H_2SO_4 + H_2O_2 – digestion at 360°C	residual-P	occluded; Pi and Po compounds highly resistant	no directly plant available; probably slow turnover

The mature leaves and plant roots were sampled, oven-dried at 68°C for 48 hour and ground. Subsamples of ground plant materials were analyzed. Phosphorus was determined after wet digestion at dried and ground sub-samples in a H_2SO_4-Se-salisilic acid mixture, in the diluted digests; P was measured spectrophotometrically by the indophenol-blue method and after reaction with ascorbic acid (AOAC 1990).

Pot experiment

Pots were sterilized with 20% sodium hypochlorite solution, filled with 3.0 kg soil and tomato (*Lycopersicon esculentum* L.) seedlings were planted in pots. Five different phosphorus fertilizer treatments with and without *Bacillus* (FS-3) [(1) control (T_1 = no inoculation and fertilizer), (2) normal superphosphate (T_2 = NSP), (3) triple superphosphate (T_3 = TSP), (4) di-ammonium phosphate (T_4 = DAP), (5) phosphoric acid (T_5 = PA), (6) rock phosphate (T_6 = RP), (7) bacterium alone (T_7 = FS-3), (8) T_8 = NSP+FS-3, (9) T_9 = TSP+FS-3, (10) T_{10} = DAP + FS-3, (11) T_{11} = PA+FS-3 and (12) T_{12} = RP+FS-3] were applied in pots as 344 kg P/ha. Basal fertilizer was also applied to all of the pots as 180 kg/ha N (NH_4NO_3 33% N), 100 kg K/ha (K_2SO_4 50% K_2O). There were twelve treatments, and eight replicates of each treatment, giving a total of 96 pots. Pots were arranged on a bench in the greenhouse according to a randomized complete block design. Plants were grown in a greenhouse under a day/night cycle of 15/9 h natural light,

25/16°C and 55% relative humidity during the experimental period. After phosphorus fertilizer application, bacterial suspension (5×10^9 cfu/ml) was applied into the pot. The treatment was repeated 5 times with a 7-day interval. The pots were watered to 70% water-holding capacity and were maintained at this moisture content by watering to the constant weight every 2–3 days. Sterile water was slowly added over the topsoil in each pot. At the end of the experimental period (60 days), the plants were harvested, measured, and analyzed. Soil samples were taken from the plant rhizosphere area of each pot after harvest.

Statistical analyses

All data in the present study were subjected to the two-way analysis of variance (ANOVA). The Duncan's multiple range tests were performed for mean comparisons using SAS statistical software (SAS 1982).

RESULTS AND DISCUSSION

Phosphorus fractionation of soil

Total P content. Phosphate content of soil in all treatments is presented in Tables 2 and 3. The results showed that there were statistically significant differences in total P concentrations between treatments with/without phosphate solubilizing bacterium (PSB) application. Average soil P contents in treatments without PSB (FS-3) application were in the range from 385.3 to 410.9 mg/kg, and were higher than those (380.2–404.8 mg/kg) of the treatments with PSB inoculation. The treatments with PSB resulted in statistically significant differences in total P concentrations and significant decrease in the soil total P content due to microbial solubilization of inorganic and organic phosphorus forms in soil. This result showed that PSB applications render a part of insoluble phosphate form into soluble forms for plant utilization. In terms of P fertilizer sources, there were not statistically significant differences in total P concentration between fertilizer forms.

The labile and plant less available P. In each treatment with/without PSB application, the labile P pool in the soil, as expressed by the sum of resin-Pi and $NaHCO_3$-Pi and Po, and moderately labile fractions (NaOH-Pi + NaOH-Po) are given in Table 3. The amount of plant available form of soil phosphorus fractions (resin-Pi + $NaHCO_3$-Pi + $NaHCO_3$-Po + NaOH-Pi + NaOH-Po) increased with PSB application and was also statistically significant. The rates changed by around 20% for all fertilizer types. For instance, in the NSP type fertilizer, while the amount of labile P fraction was 176.9 mg/kg (Pi + $NaHCO_3$-Pi + $NaHCO_3$-Po + NaOH-Pi + NaOH-Po, 1.7 + 3.9 + 2.8 + 74.3 + 94.2,

Table 2. ANOVA tests for P-fractions of the experimental soil

S.V.	*df*	Resin-Pi	$NaHCO_3$-Pi	NaOH-Pi	H_2SO_4-Pi	$NaHCO_3$-Po	NaOH-Po
Phosphorus fertilizer sources (PFS)	5	67.81*	94.10**	2.91 ns	3.81 ns	4.92 ns	1442.79**
Phosphorus solubilizing bacteria application (PSBA)	1	63.22*	76.32**	2644.30*	1128.21*	19.61*	26.62*
(PFS) × (PSBA)	5	18.12*	12.42*	3.24 ns	5.44 ns	1.23 ns	14.13*
Error	24						

S.V.	*df*	residual-P	total P	pH of soil	plant shoot	plant root	P content of plant
Phosphorus fertilizer sources (PFS)	5	4.8 ns	2486.0*	770.51*	83.35*	20.04*	13.76*
Phosphorus solubilizing bacteria application (PSBA)	1	1238.72*	2331.1*	2813.69**	62.69*	22.64*	13.63*
(PFS) × (PSBA)	5	2.14 ns	1119.41*	151.79*	7.35*	3.44*	0.56 ns
Error	24						

**$P < 0.01$ significant, *$P < 0.05$ significant, ns – non-significant

Table 3. Mean values for P-fractions, pH of experimental soil and, shoot-root weight and phosphorus content of tomato plant

Treatment		Inorganic fractions (mg/kg)				Organic fractions (mg/kg)	
		resin-Pi	$NaHCO_3$-Pi	NaOH-Pi	H_2SO_4-Pi	$NaHCO_3$-Po	NaOH-Po
Without PSB application	T_1 no P	0.4 ± 0.1b[2]	0.8 ± 0.2b	72.4 ± 4.4	84.4 ± 5.8	2.6 ± 0.7	98.2 ± 6.8a**
	T_2 NSP	1.7 ± 0.4a	3.9 ± 1.4a*	74.3 ± 4.6	85.1 ± 6.3 ns	2.8 ± 0.9	94.2 ± 6.7ab
	T_3 TSP	1.6 ± 0.3a	3.8 ± 1.2a	73.6 ± 4.2	83.2 ± 6.2	2.9 ± 1.0 ns	95.5 ± 69ab
	T_4 DAP	1.7 ± 0.4a	3.6 ± 1.1a	74.8 ± 4.8	84.4 ± 5.7	2.8 ± 0.7	97.4 ± 7.1a
	T_5 PA	1.8 ± 0.5a*	3.7 ± 1.0a	72.2 ± 4.6	79.3 ± 6.4	2.7 ± 0.4	90.2 ± 6.4b
	T_6 RP	0.8 ± 0.1a[2]	1.6 ± 0.6b	75.3 ± 4.5 ns	83.7 ± 6.1	2.7 ± 0.8	94.7 ± 6.8ab
	mean[1]	1.33 B*	2.9 B**	73.76 A*	83.35 A*	2.75 A**	95.03 A*
With PSB application	T_7 no P	0.8 ± 0.1b	1.0 ± 0.3b	58.5 ± 4.2 ns	83.2 ± 6.7 ns	0.4 ± 0.1	82.3 ± 8.2a**
	T_8 NSP	2.4 ± 0.8a*	5.2 ± 1.5a*	56.4 ± 3.7	82.4 ± 6.2	0.8 ± 0.1 ns	81.1 ± 7.6ab
	T_9 TSP	2.3 ± 0.7a	4.9 ± 1.4a	55.2 ± 3.5	81.3 ± 6.7	0.6 ± 0.1	80.1 ± 7.5b
	T_{10} DAP	2.2 ± 0.7a	4.8 ± 1.6a	57.6 ± 3.8	82.6 ± 6.4	0.5 ± 0.1	81.6 ± 6.4ab
	T_{11} PA	1.9 ± 0.6a	4.5 ± 1.7a	53.1 ± 3.6	74.6 ± 5.9	0.4 ± 0.1	81.7 ± 5.9ab
	T_{12} RP	1.7 ± 0.5a	4.3 ± 1.2a	55.7 ± 3.7	75.2 ± 5.2	0.5 ± 0.1	80.4 ± 6.2b
	mean	2.88 A**	4.95 A**	56.08 B*	79.88 B**	0.53 B**	81.2 B*
Treatment		residual-P (mg/kg)	total P (mg/kg)	pH of soil (2:1 W/S)	shoot weight (g/pot)	root weight (g/pot)	P content of plant (g 100 g/pot dw)
Without PSB application	T_1 no P	135.7 ± 10.2	385.3 ± 14.9c	7.14 ± 0.4a*	18.13 ± 1.2d	1.29 ± 0.07c	0.33 ± 0.01c
	T_2 NSP	136.8 ± 11.4 ns	400.4 ± 15.3b	6.81 ± 0.3b	23.12 ± 0.9b	1.82 ± 0.06a	0.55 ± 0.02a
	T_3 TSP	135.4 ± 9.10	410.9 ± 16.5ab	6.40 ± 0.6b	23.78 ± 1.3b	1.78 ± 0.05a	0.62 ± 0.01a**
	T_4 DAP	133.2 ± 9.8	415.7 ± 17.2a*	6.90 ± 0.2ab	25.56 ± 1.2a*	1.90 ± 0.06a*	0.55 ± 0.02a
	T_5 PA	130.6 ± 10.7	406.3 ± 16.3b	5.75 ± 0.5c	20.43 ± 1.0c	1.25 ± 0.04c	0.42 ± 0.01b
	T_6 RP	134.8 ± 11.1	410.2 ± 1616.7ab	7.05 ± 0.3a	20.32 ± 0.9c	1.59 ± 0.05b	0.45 ± 0.02b
	mean	134.41 A*	404.80 A*	6.68 A*	21.89 B*	1.61 B*	0.49 B*
With PSB application	T_7 no P	129.2 ± 10.8	380.2 ± 17.2b	7.07 ± 0.4a*	18.68 ± 1.3c	1.90 ± 0.02a	0.37 ± 0.01d
	T_8 NSP	130.8 ± 11.7	388.2 ± 14.3b	5.75 ± 0.5b	28.03 ± 1.4a	1.99 ± 0.03a	0.78 ± 0.02a
	T_9 TSP	131.4 ± 13.2 ns	401.5 ± 17.7a	5.15 ± 0.2b	27.56 ± 1.2a	2.03 ± 0.03a*	0.83 ± 0.03a**
	T_{10} DAP	130.2 ± 10.5	402.3 ± 16.9a	5.92 ± 0.5b	29.09 ± 1.0a*	1.93 ± 0.04a	0.80 ± 0.03a
	T_{11} PA	128.4 ± 8.4	400.0 ± 15.0a	4.05 ± 0.4c	20.42 ± 1.4c	1.28 ± 0.05b	0.48 ± 0.01c
	T_{12} RP	131.6 ± 9.8	404.8 ± 14.8a*	5.10 ± 0.2b	24.78 ± 1.0b	1.81 ± 0.02a	0.68 ± 0.01b
	mean	130.26 B*	396.16 B*	5.50 B**	24.76 A*	1.82 A*	0.65 A*

Symbols are explained in Table 2; Pi – inorganic phosphorus, Po – organic phosphorus; values are the means of ten soil samples from each pot

[1]means in columns followed by capital letter – evaluation of PSB application; [2]means in columns followed by small letter – evaluation of different phosphorus fertilizer sources

The means with different letters $^{**}P < 0.01$ significant, $^{*}P < 0.05$ significant, ns – non-significant

respectively), it decreased to 145.9 mg/kg with PSB application (Table 3). The results showed that PSB application was statistically significant and the labile P pool increased with PSB application, which enhanced plant phosphorus uptake. In the control group P content of plant increased from 0.33 g 100 g/pot to 0.37 g 100 g/pot with PSB application. Depending upon fertilizer type, PSB application increased plant-P with different rates. For instance, the effectiveness of PSB application on P uptake was 51% in RP, but it was only 14% in PA, as compared to the treatment without PSB.

Residual P fraction. PSB application converted 134.41 mg/kg residual-P into 130.26 mg/kg residual P. High quantities of H_2SO_4-Pi are thought to be a necessary condition for sustainable, continuous productivity (Crews 1996). Then, phosphorus availability would be mostly governed by P-Ca dissolution rate, when soil organic matter bound P was not sufficient to sustain crop requirements. This may be particularly important in the semi-arid areas, where low amounts of organic matter and low input agriculture prevail. This also contrasts with acid soils from humid tropics, where H_2SO_4-Pi is generally exhausted due to intense weathering and P release from Fe/Al-oxides is insufficient to support a continuous production (Tiessen et al. 1992).

The data showed that the increase of labile P level in the soil is the result of PSB-solubilization of moderately, less available and residual P content of organic and inorganic fraction in the soil (Table 3).

pH value of soil

There were statistically significant differences in soil pH degree between soils treated with PSB and those without the treatment. Fertilizer sources also affect soil pH decrease. The highest pH decrease in soil was obtained at T_5 treatment (with phosphate solubilizing bacterium). PSB applications decreased soil pH with by 0.42% (control), 15.44% (NSP), 19.53% (TSP), 14.20% (DAP), 28.94% (PA) and 27.14% (RP) as compared to the samples without PSB application (Table 3). Rodriguez and Fraga (1999) found a clear correlation between the decrease in pH value and bacterial P mobilization.

Plant response

The effect of fertilizers with/without PSB treatments on dry weight of shoot and root of tomato plants suggested that there were significant differences between different fertilizer treatments (Table 3). The plant shoot and root weights were generally greater in the PSB application than without PSB application in case of all fertilizer treatments. The highest shoot dry weight (29.1 g/pot) was determined in T_{10} (DAP and PSB application) treatments. PSB applications to soil increased the shoot weight of plants with the rates in the range of 3.03% (control), 21.87% (NSP), 15.89% (TSP), 16.36% (DAP), 0.10% (PA) and 21.94% (RP) as compared to the samples without PSB application treatments (Table 3).

The highest root weight (2.0 g/pot) was determined in T_9 (TSP and PSB application) treatments. PSB applications to plants growth media increased the root weight of plants by 3.4% (control), 9.0% (NSP), 14.0% (TSP), 1.5% (DAP), 2.4% (PA) and 13.8% (RP) as compared to the samples without PSB application (Table 3). Our data showed that a higher nutrient uptake with PSB application treatments significantly improved seedling growth. This result confirmed that the PSB application might effectively increase the root weight (Bertrand et al. 2001, Bashan et al. 2004). Similar findings were reported by Puente et al. (2004), who showed that the separate inoculation of cactus seedling with *Bacillus pumilus* and *Bacillus subtilis* changed several plant growth parameters.

On the other hand applications with/without PSB significantly affected P contents of plants. The highest P content was determined in T_9 (TSP and PSB treatment). PSB applications increased P contents of plant by 12.1% (control), 41.8% (NSP), 33.8% (TSP), 45.4% (DAP), 20.0% (PA) and 51.1% (RP) as compared to the samples without PSB application (Table 3). It can be caused by an organic acid production by plants and PSB in the rhizosphere, which stimulates the availability of P.

There are several reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P from soil after their inoculation of soil or plant seeds (Kucey et al. 1989). Hence, the findings in the present study were supported by a number of previous studies (Çakmakçı et al. 2001, Sundara et al. 2002, Shen et al. 2004, Turan et al. 2006).

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