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Isolation of Phosphate Solubilizing Bacteria from the Rhizosphere of Faba Bean of Ethiopia and Their Abilities on Solubilizing Insoluble Phosphates

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ABSTRACT

Native phosphate solubilizing bacteria (PSB) were isolated from four areas (Ankober, Keyt, Mehalmeda and Molale) of Ethiopia to study their effect on releases of soluble phosphorus from insoluble P sources. The highest bacterial number was found at Keyt (2.6×10^3 g^{-1}soil) and the least at Molale (15 g^{-1}soil). Five efficient PSB were selected for further study based on their ability in forming a higher clear zone diameter than the other isolates. These isolates were identified based on phenotypic characters as *Pseudomonas* sp. Anb-105, Meh-008, Meh-101, Meh-303 and Meh-305. The phosphate solubilizing efficiency of these five isolates along with Jim-41 isolate from the National Soil Research Centre were studied using different P sources [Tricalcium Phosphate (TCP), Egyptian Rock Phosphate (ERP), Bikilal Rock Phosphate (BRP) and Old Bone meal (OB)] in an incubation study. The results revealed that all the PSB isolates significantly ($P\leq 0.01$) solubilized a higher amount of TCP, ERP and OB over the uninoculated control. The highest amount of solubilization was achieved for TCP with Meh-305 (39 mg per 50 ml) followed by ERP with Meh-101 (31 mg per 50ml) at pH 3.82 and 3, respectively. Although Meh-008 and Jim-41 isolates solubilized significant amount of BRP during the 20 days of incubation, the soluble P obtained was very small as compared to other P source tested.

Keywords: Ethiopia, Insoluble phosphorus, Phosphate solubilizing bacteria, Soluble phosphorus.

INTRODUCTION

Phosphorus is second only to nitrogen among mineral nutrients most commonly limiting the growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2 % of plant dry weight. Plants acquire P from soil solution as phosphate anions. In many soils, although phosphate is available in plenty, application of phosphatic fertilizers is a must to make up for the phosphorus lost due to the fixation of soluble phosphate by soil constituents and phosphate run off in P-loaded soil (Vikram and Hamzehzarghani, 2008). However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. As the results indicated, the amount available to plants is usually a small proportion of this total (Rdresh *et al*., 2004).

Phosphorus deficiency is the most important problem of Ethiopian soil and more than 70-75% of highland soils are characterized by phosphorus deficiency (Beyene, 1982). The deficiency is very severe in the acidic soils of the southern, southwestern and western regions. In these

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areas Al^{3+} and Fe^{3+} are totally incriminated with phosphorus fixation (Sertsu and Ali, 1983). Around 70% of Ethiopian vertisols have available phosphorus below 5 ppm, which is very low for supporting good plant growth and fixation in vertisols is related more to calcium, which is the predominant cation in all profiles than Al^{3+} and Fe^{3+} (Mamo *et al*. 1988).

The role of microorganisms in solubilizing inorganic phosphates in soil and making them available to plants is well known (Bhattacharya and Jain, 2000). They are called phosphate solubilizers (PSB) and they convert the insoluble phosphates into soluble forms by acidification, chelation, exchange reactions and production of gluconic acid (Chen *et al*., 2006). The use of PSB as inoculants for crop plants has received considerable attention over the years (Pandey *et al*., 2006). Inoculation of PSB has resulted in improving growth, yield and phosphorus uptake in several crops (Hameeda *et al*., 2006 a). It is believed that production of plant growth promoting substances by PSB may contribute to their stimulatory effect on plant growth (Hameeda *et al*., 2006b).

Phosphate solubilizing microorganisms isolated elsewhere have not been very consistent in their performance everywhere owing to their poor adaptability to the changing soil and agroclimatic conditions (Vikram *et al*., 2007). The aim of our investigations was to isolate the native phosphate solubilizing bacteria from some faba bean yield depleted and yield sustained areas of Northern Shewa region, Ethiopia to study their effect on solubilizing insoluble P into soluble P form.

MATERIALS AND METHODS

Sampling Site and Soil Sample Collection

Rhizosphere soil samples were collected from four study areas [Ankober and Keyt (yield sustained), Mehalmeda and Molale (yield depleted)] of Ethiopia between May and June 2007. The regions are found in Semien Shewa Zone with a classical 'Weina Dega' (subtropical) climate characterized by 1,500-2,300 altitude, dry sub humid highland forest vegetation, with six (long and short) rainy months, with 800-1,000 mm annual rainfall, 13-16.3°C average annual temperature and evapotranspiration of 110-125 cm. Soil samples were collected at a depth of 0-30 cm from ten randomly selected sites in each region. The physical and chemical properties of the soils are presented in Table 1. For microbial isolation and analysis they were preserved at 4°C.

Isolation of PSB from the Rhizosphere

The rhizosphere soil samples of various regions were collected and analysed for PSB population by the following method of Pikovskaya (1948) at the department of Biology, Addis Ababa University, Ethiopia. The bacterial colonies showing clear zone on Pikovskaya's agar medium were purified, identified, sub cultured and stored for further use. Each isolate is coded by three letters from the name of the sampling site- (Ankober (Anb), Keyt (Kyt), Mehalmeda (Meh), Molale (Mol). The population of phosphate solubilizing bacteria was determined following the method described in Ponmurugan and Gopi (2006). Based on the efficiency the following PSB isolates were used in the test (Anb-105, Meh-008, Meh-101, Meh-303, Meh-305 and Jim-41). The incubation study was carried out using the following treatments. Fifty ml of Pikovskay`s liquid medium without phosphorus was dispensed in a 250 ml Erlenmeyer flask and used as the control. Tricalcium phosphate (250 mg); Egyptian rock phosphate (300 mg); Bikilal rock phosphate, (1.2 gm) and Bone meal (200 mg) were added separately as a phosphorus source and used as different treatments. The above quantities gave the equivalent amount of phosphate ions. The flasks were sterilized at 121 °C, 15 Psi for 15 minutes. Each flask

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Table 1. Physico-chemical properties of the soil samples.

Site	Soil texture	pH	Organic matter %	Total nitrogen %	C/N	Available P (Olsen) (ppm)	Electrical conductivity (dS m^{-1})	Cation exchange capacity (CEC) (meq 100 g^{-1})	Exchangeable bases (meq 100 g^{-1})			
									Na	K	Ca	Mg
Ankober	Clay	5.9	1.155	0.155	8	18.5	0.099	20.68	0.26	0.20	11.6	6.00
Keyt	Clay	6.5	1.038	0.142	8	17.4	0.248	28.79	0.38	0.13	11.4	10.29
Mehalmeda	Clay	6.2	0.685	0.095	7	2.8	0.075	30.20	0.20	0.36	17.2	9.63
Molale	Clay	6.3	0.692	0.084	7	5	0.053	41.20	0.25	0.29	25.00	11.11

Table 2. Phosphate solubilizing bacteria colony forming unit (cfu) per gram of soil and average clear zone diameter around the colony from each sampling site.

S/N	Site	Clear zone diameter (mm)	CFU g^{-1} soil
1	Ankober	2.08	1.8×10^3
2	Keyt	1.25	2.6×10^3
3	MehalMeda	3.50	69
4	Molale	2.00	15

was inoculated with 0.1 ml of 24 hour active culture suspensions of each PSB isolate. The flask was kept on a rotary shaker for 10 hours per day until the day of sampling. Three replicate flasks were used for each PSB isolate, from which samples were collected at 5, 10, 15, and 20 days and analyzed for phosphorus and pH changes. Soluble phosphorus was quantitatively determined following the method cited in Subba Rao (1993). From each culture broth, insoluble materials were removed by filtering through whatman filter paper No.1 and the filtrate was centrifuged at 13,000 rpm for 15 minutes. Ten ml of the filtrate was then mixed with 2.5 ml of Barton's reagent. The volume was adjusted to 50 ml with distilled water. After 10 minutes, the resultant color was read in a Spectrophotometer at 430 nm. The quantitative data obtained were subjected to analysis of variance using SPSS 10.5 series statistical version computer software. Means were separated using LSD and Duncan's multiple range test at a 0.5 and 1% level of significance.

RESULTS

The phosphate solubilizing bacteria were present in all the soil samples (Ankober, Keyt, Mehalmeda, and Molale). However, they were found to differ both in number, as estimated by colony forming units per gram of soil, and efficiency as observed from clear zone diameter around their colonies. From these isolates, five efficient PSB were selected for further study and designated as Anb-105, Meh-008, Meh-101, Meh-303, Meh-305. These isolates formed a clear zone diameter of between 1 mm and 4.5 mm and the largest clear zone diameter of 4.5mm was found in Mehalmeda isolates of Meh-101, Meh-303, Meh-305 (Table 2). The isolates were further characterized on the basis of cultural, morphological and biochemical characters together with a reference isolate from Jimma (Jim-41) and were found to belong to the genus *Pseudomonas.*

The results of tricalcium phosphate solubilization by the selected isolates and the associated pH changes in the medium are shown in (Table 3). The result showed that PSB isolated from the Northern Shewa region were found to be more efficient in solubilizing tricalcium phosphate than the Jim-41. All the bacterial isolates solubilized significantly ($P \leq 0.01$) higher amounts of tricalcium phosphate over uninoculated control. The highest amount of solubilization was recorded for the bacterial isolate Meh-305 (39 mg) followed by Meh-303 (34 mg), Meh-101 (27 mg), Jim-41 (20.4 mg), Anb-105 (20 mg) and Meh-008 (19.5 mg) at 20 days of incubation time. An inverse correlation between the amount of soluble P and the reduction of pH in the inoculated medium ($r \geq -0.93$ and $R^2 \geq 0.95$) was found when compared with the uninoculated medium($r = 0.075$).

There was significant ($P \leq 0.01$) solubilization of Egyptian rock phosphate over the uninoculated control by the six isolates. The amount of soluble phosphorus and corresponding pH change of the medium is presented in Table 4. The solubilization increased steadily up to 20 days of incubation where the maximum amount of soluble phosphorus was released. The highest amount of soluble phosphorus was obtained with Meh-101, (31 mg) followed by Meh-305, (25 mg), Meh-303, (22.8 mg), Anb-105, (22.5 mg), Meh-008, (20.5 mg) and Jim-41, (19.5 mg). There was a drastic drop in the pH of the medium inoculated with all PSB isolates, whereas the pH change in the uninoculated flask throughout the 20 days was not significant ($P \geq 0.05$). The correlation analysis showed that there is an inverse relation between phosphorus release and decrease of pH ($R^2 \geq 0.96$) and ($r \geq -0.94$).

The results for the phosphorus solubilization efficiency of PSB isolates on Bikilal rock phosphate is shown in Table 5. The amount of phosphorus released by different isolates of PSB on the Bikilal rock

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Table 3. Tricalcium phosphate solubilizing efficiency of PSB isolates

Isolates	5 days		10 days		15 days		20 days	
	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})
Anb-105	5.6	6.4	5.49	9	5.16	13	4.98	20
Meh-008	6	5	5.80	9.8	4.45	13	4	19.5
Meh-101	5.8	9.9	5.40	19.8	5.0	23	4.6	27
Meh-303	5.4	11	5.29	17	5.0	22	4.4	34
Meh-305	5.4	13	5.20	19.5	5.0	25	3.82	39
Jim-41	5.1	7	5.0	10.5	4.4	14.9	4	20.4
Control	6.8	1.8	6.68	1.99	6.38	1.5	6.05	2
LSD [a](0.01)	0.975	0.219	0.806	0.715	1.00	0.139	1.00	0.47

[a] Least significant difference.

Table 4. Egyptian rock phosphate solubilizing efficiency of PSB isolates.

Isolates	5 days		10 days		15 days		20 days	
	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})
Anb-105	7	0.7	6.2	4.35	3.46	18.3	3.1	22.5
Meh-008	4.21	9.1	3.5	14	3.2	17.5	3	20.5
Meh-101	4.5	9.85	3.5	15.2	3.2	21.8	3	31
Meh-303	4.8	8.2	4.5	11.5	4	16	3.65	22.8
Meh-305	4.68	8.74	3.89	12.5	3.56	19	3.2	25
Jim-41	3.5	12.5	3.2	15.5	2.9	17.5	2.7	19.5
Control	7.5	0.6	7.4	0.6	7	0.96	6.88	1
LSD [a](0.01)	0.482	0.17	1.00	0.359	1.00	1.00	1.00	0.824

[a] Least significant difference.

phosphate as a function of time was not significant unlike the tricalcium phosphate and Egyptian rock phosphate. On the fifth day of inoculation, Meh-008 and Jim-41 isolates showed significant differences of soluble ($P \leq 0.05$) phosphorus as compared with other isolates and control. On the 10^{th} day, the isolates of Meh-008, Meh-305 and Jim-41 showed a significant difference ($P \leq 0.05$). On the 15^{th} and 20^{th} days after inoculation, all the isolates were found to solubilize significant amount of soluble phosphorus. Although the medium inoculated with PSB isolates showed a decrease in the pH, the amount of soluble phosphorus released in the medium was very low as compared to other phosphorus source tested ($r \geq -0.94$) and $R^2 \geq 0.99$).

The amount of soluble phosphorus released from the old bone meal and corresponding pH decrease of the medium by the PSB isolate is shown in (Table 6). There was a significant amount of P solubilization by the six isolates over the uninoculated control. Anb-105 was comparatively more efficient in solubilizing old bone meal than all of the rest of the PSB isolates, followed by Meh-008. The maximum solubilization was achieved at 20 days of incubation. Even though there was a drastic drop in the pH of the medium, which was inoculated with PSB isolates, there was no significant change ($P \geq 0.05$) in the pH of the control. There was inverse relationship between soluble P and decrease in pH ($r \geq -0.96$) and $R^2 \geq 0.98$).

DISCUSSION

The role of phosphate solubilizing bacteria (PSB) in solubilizing a fixed form of soil P and making it available is very well known. In vertisols, the problem of P fixation is usually greater due to the fact that the available P is fixed in the form of poorly soluble calcium mineral phosphates and is unavailable to plants (Vikram *et al*., 2007). Most of the Ethiopian soils are vertisols which are better for isolating native PSB, and this will be useful for increasing the agricultural productivity of both faba bean yield sustained (Ankober and Keyt) and yield depleted (Mehalmea and Molale) areas of Ethiopia. The population level is higher in the Ankober and Keyt areas as compared to the Mehalmeda and Molale areas. The colony-forming unit of the microbial population per gram of soil was about 10^9 for yield sustained areas and about 10^5 for the yield depleted' ones. The proportion of PSB is higher for the yield sustained Ankober and Keyt than the yield depleted Mehalmeda and Molale areas, which is about > 0.5% for the former and < 0.1 % for the latter. This finding is in line with the report of Pal (1998) who isolated phosphate solubilizing bacteria from the soil samples of forest, pasture, waste land, agricultural and horticultural land and observed potential phosphate solubilizing bacteria as varying between 32-60× 10^3 g^{-1} of soil. This variation might be attributed to many soil factors such as soil nutrients, pH, moisture content, organic matter and some enzyme activities (Ponmurugan and Gopi, 2006). The differences in population density of PSB in the yield sustained (Ankober and Keyt) and yield depleted areas (Mehalmeda and Molale) may be associated with the faba bean cropping history of the sampling site. Since the yield sustained regions have a continuous production of faba bean, their rhizosphere can be contributing to the continuous growth and development of microorganisms including the PSB. In this particular study, however, a higher population density of PSB was observed in the yield sustained Ankober and Keyt than the yield depleted areas of Mehalmeda and Molale, and more efficient PSB strains were isolated from yield-depleted areas of Mehalmeda. Out of all the bacterial isolate that showed a clean zone around the colony, five were selected based on having a clear zone diameter, including four of them isolated from Mehalmeda soil. All the isolates were Gram negative, straight, non spore formers, motile, non gas producing, in oxidative fermentative medium with

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Table 5. Bikilal rock phosphate solubilizing efficiency of PSB isolates.

Isolates	5 days		10 days		15 days		20 days	
	pH	Amount of P (mg 50 ml^{-1})	pH	mount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})
Anb-105	5.5	0.475	5.2	0.6	4.8	0.7	4.4	0.85
Meh-008	4	0.65	3.75	0.85	3.4	1	3.1	1.25
Meh-101	5	0.5	4.75	0.6	4.2	0.7	3.7	0.85
Meh-303	4.6	0.55	4.2	0.675	4	0.75	3.75	0.9
Meh-305	4.5	0.6	4	0.75	3.8	0.85	3.5	1
Jim-41	3.95	0.71	3.55	1.32	3.3	1.4	3	1.48
Control	6.8	0.4	6.8	0.4	6.68	0.45	6.68	0.47
LSD [a](0.01)	0.877	1.00	0.06	1.00	0.207	1.00	0.458	0.882

[a] Least significant difference.

Table 6. Old bone meal solubilizing efficiency of PSB isolates.

Isolates	5 days		10 days		15 days		20 days	
	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})	pH	Amount of P (mg 50 ml^{-1})
Anb-105	4.11	7.64	3.9	12.36	3.6	16.8	3.2	22.85
Meh-008	4.8	6.8	4.45	11.6	4.0	15.8	3.65	20.5
Meh-101	5.7	2.85	5.26	5.4	4.8	7.8	4.5	11.0
Meh-303	5.6	4.0	5.2	5.5	4.9	8.8	4.46	12.0
Meh-305	5.65	4.0	5.18	5.75	4.85	8.3	4.3	11.5
Jim-41	5.5	4.5	5.0	6.3	4.76	9.0	4.37	13.0
Control	7.0	0.68	6.8	1.0	6.5	1.2	6.5	1.5
LSD [a](0.01)	0.768	1.00	0.95	0.557	0.572	0.438	0.529	0.368

[a] Least significant difference.

glucose: open tube acid produced, tube sealed acid not produced, oxidase positive, catalase positive, indole negative, methyle red negative, voges proskauer negative. These characteristics render all the isolate as belonging to genus *Pseudomonas*. This finding is supported by an earlier report that says that most efficient and frequently encountered phosphate solubilizing bacteria belonging to the genus *Pseudomonas* or the genus *Bacillus* (Sundram, 1994). Haile *et al*. (1999) also isolated three phosphate solubilizing bacteria from Southern part of Ethiopia all of which are characterized as belonging to the genus *Pseudomonas*.

Evaluation of the efficiency of each isolate on solubilization of the different phosphorus sources showed that Meh-303 and Meh-305 are very effective on tricalcium phosphate followed by Meh-101. Meh-101 was found to be the most effective in the solubilization of Egyptian rock phosphate.

The solubilization efficiency of Meh-008 and Anb-105 was shown to be uniform on the different phosphorus sources. Not all the isolates were as effective on the Bikilal rock phosphate as they were on other phosphorus sources (Figure 1). All the five PSB isolates selected for the solubilization efficiency test solubilized a significantly greater amount of insoluble phosphate sources, except that of the Ethiopian rock phosphate (Bikilal rock phosphate), over the uninoculated control. Although the Jim-41 isolate and Meh-008 isolates were able to solubilize phosphorus from Bikilal rock phosphate throughout the 20 days as compared to the control. The amount of soluble phosphorus released was not comparable with the other phosphorus sources. The presence of a small amount of soluble phosphorus in the uninoculated control flask is believed to be due to the release of PO_4^{3-} during autoclaving. This finding is in agreement with that of Haile *et al.* (1999). There was a progressive increase in the solubilization of the tricalcium phosphate and Egyptian rock phosphate by tested PSB isolates during the 20 days of incubation. Bacteria isolated from rhizosphere soils are known to produce growth regulating substances and some of them are capable of dissolving phosphate. Some of the PSB are also able to produce vitamins towards the dissolution of

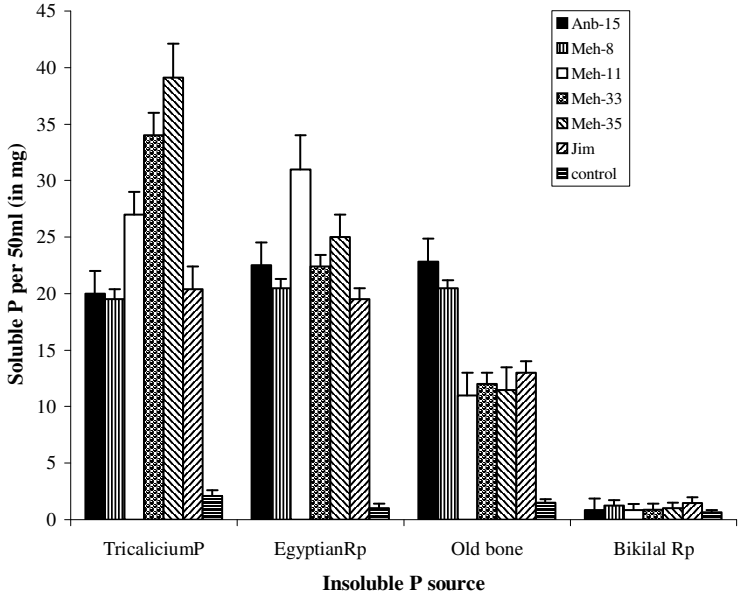

Figure 1. Solubilization efficiency of the native phosphate solubilizing bacteria (PSB) isolates.

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bicalcium phosphate and all the strains of phosphate bacteria were able to solubilize inorganic phosphate (Ponmurugan and Gopi, 2006). Increasing the bioavailability of P with the inoculation of PGPR or with a combination of inoculation and rock materials has been also reported by Han and Lee (2005).

There was also a prefect inverse relationship between release of soluble phosphorus and reduction in the pH of the medium ($r \geq -0.96$) for all substrates. In this investigation the highest soluble phosphorus was observed in tricalcium phosphate inoculated with Meh-305 and the corresponding pH decrease during 20 days of incubation was 3.82. Paul and Sundura Rao (1971) studied the relationship between tricalcium phosphate solubilization ability of 12 PSB isolates and the pH of the medium. The result revealed that there was a prefect inverse correlation with the amounts of tricalcium solubilized and the pH of the medium inoculated with PSB cultures. Among twelve PSBs tested, the highest amount of tricalcium phosphate solubilization was achieved by *Bacillus brevis* with the lowest pH of 4.4. Acid production and reduction of the pH of the medium is one of the mechanisms by which soluble phosphorus is released by PSB (Gaur, 1988).

The drop in pH of the inoculated medium containing Egyptian rock phosphate and Ethiopian rock phosphate (Bikilal rock phosphate) was more drastic than tricalcium phosphate. Among the different phosphorus sources tested, the lowest pH value was observed in Egyptian rock phosphate and Bikilal rock phosphate inoculated with Jim-41 isolate. This is supported by Haile *et al*. (1999) and this could be due to the high insolubility of rock phosphate compared to tricalcium phosphate. Thus, some PSB isolates when inoculated in a medium with rock phosphate released more acid than they did with tricalcium phosphate. It is also found that Meh-101, Meh-303 and Meh-305, which were most efficient in solubilizing tricalcium, and Egyptian rock phosphate were found to be poor in solubilization of old bone. Anb-105, Meh-008 and Jim-41 isolates were more effective in solubilizing old bone than other phosphorus sources. This may suggest that the mechanism employed by PSB isolates in solubilizing old bone is different from that of tricalcium phosphate and Egyptian rock phosphate. The solubilization of old bone is probably due to the production of enzymes such as acid phosphatases produced by PSB isolates. This could be substantiated by the findings of Gaur (1972), who reported that the solubilization of calcium phytate and lecithin by microorganisms was due to the production of enzymes such as phytase and lecithinase.

CONCLUSIONS

The phosphate solubilizing efficiency of five native isolates of Ethiopia revealed that all the PSB isolates significantly ($P \leq 0.01$) solubilized a higher amount of Tricalcium phosphate, Egyptian rock phosphate and bone meal.

The isolates can be used to solubilize insoluble phosphates, which is characteristic of Ethiopian highland soils due to phosphorus fixation.

REFERENCES

1. Beyene, D. 1982. Diagnosis of Phosphorus Deficiency in Ethiopian Soils. *Soil Sci. bull.,* **3**:15-22.
2. Bhattacharya, P. and Jain, R. K. 2000. Phosphorus Solubilizing Biofertilizers in the Whirl Pool of Rock Phosphate-challenges and Opportunities. *Fert. News.,* **45**: 45-52.
3. Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A. and Young, C. C. 2006. Phosphate Solubilizing Bacteria from Subtropical Soil and Their Tricalcium Phosphate Solubilizing Abilities. *Appl. Soil Ecol*., **34**:33-41.
4. Gaur, A. C. 1988. *Phosphorus Solubilizing Biofertilizer*. Division of Microbiology, Indian Agricultural Research Institute, New Delhi. PP. 45-46.

[DOR: 20.1001.1.16807073.2010.12.1.12.8]

5. Gaur, A. C. 1972. Role of Phosphate Solubilizing Microorganisms and Organic Matter in Soil Productivity. *Proc. of Symposium on Soil Fertility*, National Academy of Science, India. PP. 259- 268.
6. Haile, W., Fassil, A. and Asfaw, H. 1999. Studies on Phosphate Solubilizing Ability of Bacteria Isolated from Some Ethiopia Soils. *Proc. of the 9th Annual Conference of the Biological Society of Ethiopia*, Awassa, Ethiopia, PP. 31.
7. Hameeda, B., Harini, G., Rupela, O. P., Wani, S. P. and Reddy, G. 2006 a. Growth Promotion of Maize by Phosphate Solubilizing Bacteria Isolated from Composts and Macrofauna. *Microbiol. Res*., **163**(2)**: 234-242**
8. Hameeda, B., Rupela, O. P., Reddy, G. and Satyavani, K. 2006 b. Application of Plant Growth Promoting Bacteria Associated with Compost and Macrofauna for Growth Promotion of Pearl Millet (*Pennisetum glaucum L.*). *Biol. Fertil. Soils*, **43**:221-227.
9. Han, H. S. and Lee, K. D. 2005. Phosphate and Potassium Solubilizing Bacteria Effect on Mineral Uptake, Soil Availability and Growth of Eggplant. *Res .J. Agric. Biol. Sci.,* **1(2)**: 176-180.
10. Mamo, T., Haque, I. and Kamara, C. S. 1988. Phosphorus Status of Some Ethiopian High Land Vertisols, In: "*Management of Vertisols in Subsaharan Africa*", Jutz, S. C., Haque, I., Macinter, J. and Stares, J. E. S. (Eds). *Proc. of ILCA Conference Held at Addis Ababa*, 31st Aug-4th Sept, 1987, Addis Ababa, Ethiopia, PP. 232-252.
11. Pal, S. S. 1998. Interactions of an Acid Tolerant Strain of Phosphate Solubilizing Bacteria with a Few Acid Tolerant Crops. *Plant Soil*, **198**:169-177.
12. Pandey, A., Trivedi, P., Kumar, B. and Palani, L. M. S. 2006. Characterization of a Phosphate Solubilizing and Antagonistic Strain of *Pseudomonas putida* (B0) Isolated from a Sub-alpine Location in the Indian Central Himalaya. *Curr. Microbiol.,* **53**:102-107.
13. Paul, N. B. and Sundura Rao, W. B.1971. Phosphate Dissolving Bacteria in Rhizosphere of Some Cultivated Legumes. *Plant Soil*, **35**: 127-132.
14. Pikovskaya, R. I.1948. Mobilization of Phosphate in Soil in Connection With Their Vital Activities of Some Microbial Species, *Microbiologiya,* **17**:362-370.
15. Ponmurugan, P. and Gopi, C. 2006. Distribution Pattern and Screening of Phosphate Solubilizing Bacteria Isolated from Different Food and Forage Crops. *J. Agron.,* **5**:600-604.
16. Rdresh, D. L., Shivprakash, M. D. and Prasad, R. D. 2004. Effect of Combined Application of *Rhizobium*, Phosphate Solubilizing Bacterium and *Trichoderma* spp. on Growth, Nutrient Uptake and Yield of Chickpea (*Cicer aritenium* L.). *Appl. Soil Ecol.,* **28:**139-146.
17. Sertsu, S and Ali, A.1983. Phosphorus Sorption Characteristics of Some Ethiopian Soils. *Eth. J. Agric. Sci.,* **34**: 28-407.
18. SubbaRao, N. S. 1993. *Biofertilizeres in Agriculture and Forestry*. 3rd Edition. Oxford and IBH Publishing Co. Pvt. LTD., New Delhi. PP: 129-135.
19. Sundram, K. P. 1994. Fertilizers News, Fertilizer Association of India. New Delhi.
20. Vikram, A., Alagawadi, A. R., HAmzehzarghani, H. and Krishnaraj, P. U. 2007. Factors Related to the Occurrence of Phosphate Solubilizing Bacteria and Their Isolation in Vertisols. *Int. J. Agri. Res.,* **2(7)**: 571-580.
21. Vikram, A. and Hamzehzarghani, H. 2008. Effect of Phosphate Solubilizing Bacteria on Nodulation and Growth Parameters of Greengram (*Vigna radiata* L. Wilczek). *Res. J. Miocrobiol.*, **3(2)**: 62-72.

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جداسازی باکتری‌های حل کننده فسفات از ریزوسفر لوبیا در اتیوپی و بررسی توانمندی آنها در قابل استفاده نمودن فسفر غیر محلول

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چکیده

باکتری‌های حل کننده فسفات بومی (PSB) از چهار منطقه انکوبر، کیت، مهالدا و مولال در اتیوپی جداسازی و توانمندی آنها در حل نمودن فسفر غیر قابل استفاده، بررسی گردید. بیشترین تعداد جمعیت باکتری ($۲/۶ \times ۱۰^{۳}$ در هر گرم خاک) در منطقه کیت و حداقل جمعیت باکتری (۱۵ در هر گرم خاک) از منطقه مولال بدست آمد. از میان این باکتری‌ها پنج سوش مؤثر بومی PSB برای تحقیقات بعدی انتخاب شد. این سوشها شناسایی تماماً از جنس *Psudomunas* sp بوده و با اسامی Anb-105، Meh-008، Meh-101، Meh-103 و Meh-305 نامگذاری شدند. اثربخشی این سوشها همراه با سوش Jim-41 از مرکز ملی تحقیقات خاک در قابل استفاده نمودن فسفر غیر قابل استفاده موجود در چهار منبع تری‌کلسیم فسفات (TCP) اتیوپی، خاک فسفات مصر (ZRP)، خاک فسفات بی‌کیلال (BRP)، و استخوان‌های کهنه (OB) تلقیح شدند. نتایج نشان داد که تمام سوشهای PSB به طور معنی‌داری ($P \leq 0.01$) توانستند فسفرغیر قابل استفاده موجود از سه منبع TCP، ZRP و OB در مقایسه با شاهد، بصورت قابل استفاده گیاه آزاد نمایند. بیشترین اثربخشی (۳۹ میلی‌گرم در ۵۰ میلی‌لیتر) متعلق به سوش Meh-305 و در ردیف بعدی (۳۱ میلی‌گرم در ۵۰ میلی‌لیتر) متعلق به سوش Meh-101 بر روی منبع TCP در pH های ۳/۸۲ و ۳/۰۰ بدست آمد. گرچه سوشهای Meh-008، Jim-41 توانستند در طول مدت ۲۰ روز تلقیح از منبع BRP، مقداری فسفر قابل استفاده گیاه را آزاد نمایند لیکن این مقدار در مقایسه با آزادسازی سوش‌های Meh-101, Meh-305 بسیار ناچیز بود.

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