Research in Biotechnology, 5(2): 01-05, 2014 ISSN: 2229-791X
www.researchinbiotechnology.com

Regular Article

Isolation of Phosphate solubilizing bacteria from Sorghum bicolor rhizosphere soil inoculated with arbuscular mycorrhizae fungi (*Glomus* sp)

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The presence of phosphorus in soil material is abundant but the availability is limited for plant. Some soil micro-organisms enhance solubility of phosphate in soils. This study investigated the coexistence of phosphate solubilizing microorganism in the rhizosphere soil inoculated with mycorrhizal fungi (AMF) *Glomus* sp in *Sorghum bicolor*. The rhizosphere soil samples were collected and processed for the presence of phosphate solubilizing microorganisms. The microbes isolated were screened for their efficiency to solubilize the phosphate present in pikovskaya's medium. Based on the solubilization index five bacterial isolates were chosen for the study. The maximum solubilization index was recorded as 1.75 cm by the bacterial isolates 2 and 5 on 6th day of incubation further total inorganic phosphorus produced by the bacterial isolates were determined and it was found to be 1.578 µg/mL by isolate 5 and 186 µg/mL by isolate 2. The five bacterial isolates having the efficiency to solubilize phosphorus and produce inorganic phosphorus was identified based on the morphological and biochemical tests and found to be *Acinetobacter* sp, *Bacillus* sp, *Pseudomonas fluorescence, Pseudomonas aeruginosa* and *Micrococcus* sp respectively. The maximum solubilization index and inorganic phosphorus production was with *Bacillus* sp and *Micrococcus* sp.

Key words: Phosphate solubilization index, pikovskaya's medium, *Bacillus sp, Micrococcus sp.*

The transition of the insoluble forms of phosphorous to an accessible form by plants is an important attribute of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi. Soil microbiota develops important fractions in the ecosystem since it influences growth, mineral nutrition and plant health. Phosphorous is normally the most limiting nutrient in soil and of the important groups of the soil microbial community are the phosphate solubilizing microorganisms and the arbuscular mycorrhizal fungi (AMF).

These microorganisms have the ability to solubilize and mineralize P from inorganic and organic pools of total soil P, making the element available for plants (Gyaneshwar *et al.,* 2002). The P-solubilizing microorganisms are ubiquitous in soils and could play an important role in supplying P to plants in a more environmentally sustainable manner.

AMF improve the absorption of P and other nutrients by plants increasing the contact surface and the explored soil volume (Clark and Zeto 2000) and possibly facilitating nutrient transport among plants (Chen *et al.* 2005). Moreover, plants colonized by AMF have alternative mechanisms to meet their nutritional demands and to maintain their physiological functions under abiotic stress conditions, such as drought stress (Ruiz-Lozano et al. 2001) and salinity (Azcón and El-Atrash 1997). Although potentials of AMF and PSB clearly exists their widespread application remains limited mainly by a poor understanding of microbial ecology and population dynamics in soil. Therefore, it is interesting to quantify these organisms in rhizosphere soil for their possible use as combined inocula, in order to obtain the maximum benefit to the development of plants.

The purpose of the study is to investigate the presence of phosphate solubilizing bacteria in the AMF (*Glomus sp*) inoculated rhizosphere soil of sorghum bicolor.

Materials and methods

Collection and screening of phosphate solubilizing bacteria from rhizosphere soil sample:

The rhizosphere soil sample was collected from the *Sorghum bicolor* inoculated with *Glomus sp*. The samples were processed for the screening of phosphate solubilizers with pikovskaya's medium. The screening was carried out several times to obtain efficient phosphate solubilizers with pikovskaya's medium. Further the solubilization index of the isolates were determined by the zone of clearance in the pikovskaya's agar plates and the colony diameter was calculated with the formula given below:

Solubilization index (SI) = colony diameter + Diameter of the halozone/ colony diameter.

Based on the solubilization index five bacterial isolates were selected for further study (Pikovskaya, 1948).

Table 1 Solubilization index of five PSB isolates

Isolates	Incubation period	Solubilization index (cm)
Isolate 1	1st day	Nil
	2nd day	Nil
	3rd day	Nil
	4th day	1.33
	5th day	1.33
	6th day	1.42
	7th day	1.37
Isolate 2	1st day	Nil
	2nd day	Nil
	3rd day	1.33
	4th day	1.50
	5th day	1.50
	6th day	1.75
	7th day	1.66
Isolate 3	1st day	Nil
	2nd day	Nil
	3rd day	Nil
	4th day	1.25
	5th day	1.25
	6th day	1.60
	7th day	1.50
Isolate 4	1st day	Nil
	2nd day	1.33
	3rd day	1.33
	4th day	1.23
	5th day	1.40
	6th day	1.42
	7th day	1.37
Isolate 5	1st day	Nil
	2nd day	1.33
	3rd day	1.66
	4th day	1.66
	5th day	1.66
	6th day	1.75
	7th day	1.57

Estimation of soluble inorganic phosphorus:

The 5 selected bacterial isolates were cultured in pikovskaya's broth and kept in rotary shaker for 7 days. Bacterial culture broth was collected at a regular interval of 24 hrs and centrifuged at 10,000 rpm for 10

minutes. 5ml of supernatant was collected and added with 5 ml of Borrs reagent. The final volume was made upto 50 ml with distilled water and hold it for 10 minutes. The development of yellow color was read in calorimeter at 430nm (Koening and Johnson, 1942).

Identification of phosphate solubilizing bacteria:

The bacterial isolates screened for phosphate solubilization was identified based on the standard morphological and biochemical characteristics (Cappucino and Sherman, 1999).

Results

The rhizosphere soil sample collected from *Sorghum bicolor* is processed for the presence of phosphate solubilizing bacteria using pikovskaya's medium incubated for a period of 7 days at 37^{0}C. Five predominant colonies are selected for the study based on the solubilization index. The solubilization index of the five isolates is recorded at a regular interval of 24 hrs till 7 day of incubation and the results are recorded in Table 1.

Determination of soluble inorganic phosphorus released by the bacterial isolates at different incubation period

Inorganic phosphorus production by the five bacterial isolates is determined by for a period of 7 days and the results are recorded in Fig 1.

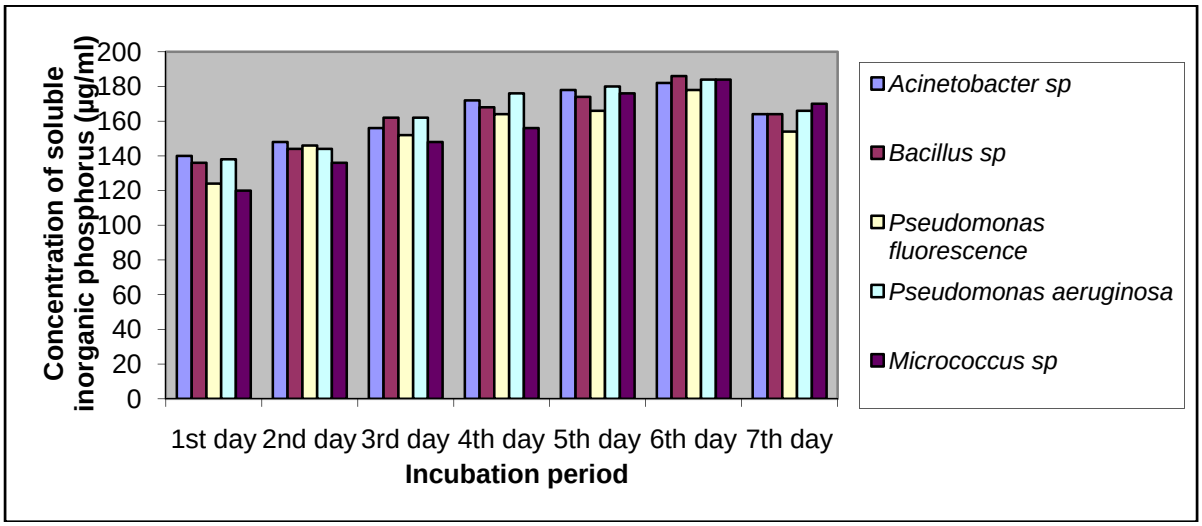

Fig 1. Soluble inorganic phosphorus released by the five PSB isolates at different incubation period

Table 2. Morphological characteristics of the five PSB isolates

Isolates	Colony morphology	Gram's reaction	Cell shape	Motility
Isolate 1	Opaque white	+	Cocco bacilli	Motile
Isolate 2	Waxy white	+	Rod	Non motile
Isolate 3	Yellowish white	-	Rod	Motile
Isolate 4	Thin white	-	Rod	Motile
Isolate 5	Soft, smooth, yellow	+	Cocci	Non motile

Table 3. Biochemical characteristics of five PSB isolates

Isolates	Indole	Methyl Red	Voges Proskauer	Citrate	H_2S	Urease	Growth in selective media	Results
Isolate 1	-	-	-	+	-	-	Macconkey agar (without Nacl)	*Acinetobacter sp*
Isolate 2	-	-	-	-	-	-	Skim milk agar	*Bacillus sp*
Isolate 3	-	-	-	+	-	-	King's B	*Pseudomonas fluorescens*
Isolate 4	-	-	-	+	-	-	King's B	*Pseudomonas aeruginosa*
Isolate 5	-	-	-	+	-	+	Mannitol salt agar	*Micrococcus sp*

*Note: PSB : Phosphate Solubilizing Bacteria

Identification of bacterial isolates:

The five bacterial isolates having the potentials to solubilize phosphorous present in the medium as well as the production of inorganic phosphorous is identified based on their morphological and biochemical characteristics (Cappucino and Sherman, 1999). The results for the morpho-logical and biochemical characteristics of the five bacterial isolates are recorded and tabulated in Table 2 and 3.

Discussion

Many soil microorganisms are able to solubilize phosphate ions from sparingly soluble inorganic or organic P compounds in vitro (Barea *et al*., 1983). Nevertheless, the contribution of this process to plant nutrition is unclear because of the possible refixation of solubilized phosphate ions on their way to the root surface. The microbiologically solubilized phosphate could, however, be taken up by a mycorrhizal mycelium, thereby developing a synergistic microbial interaction (Barea *et al*., 1997).

The rhizosphere soil dominates more number of microbial populations which is capable of solubilizing phosphate and the rhizosphere soil represents the number of bacteria (Sperber, 1958).

Solubilization index of the five bacterial isolates were calculated and it was found to be maximum on 6th day of incubation at 37˚C by isolate 2 (1.75cm), isolate 5 (1.75cm), isolate 3 (1.60cm), isolate 1 (1.42cm), isolate 4 (1.42cm).

The bacterial isolates *Bacillus sp*. (186µg/ml) and *Micrococcus sp* (184µg/ml) released maximum level of inorganic phosphorus on 6th day of incubation at 37^{0}C (Schinner, 1992).

Five potential phosphorous solubilizing bacterial isolates were identified from the rhizosphere soil based on the morphological and biochemical character-istics. viz *Acinetobacter sp, Bacillus sp, Pseudomonas fluorescens, Pseudomonas aeruginosa, Micrococcus sp*.

References

Azcón R and El-Atrash F. 1997. Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and N2 fixation (15N) in *Medicago sativa* at four salinity levels. Biology and fertility of soils 24:81-86..

Barea, J. M., C. Azco´n-Aguilar, and R. Azco´n**.** 1997. Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant systems. *In* A. C. Gange and V. K. Brown (ed.), Multitrophic interactions in terrestrial systems. Blackwell Science, Cambridge, England 65–77.

Barea, J. M., R. Azco´n, and C. Azco´n-Aguilar. 1983. Interactions between phosphate solubilizing bacteria and VA mycorrhiza to improve plant utilization of rock phosphate in non acidic soils, p. 127–144. *In* 3rd International Congress on Phosphorus Compounds, Brussels, Belgium, 4 to 6 October 1983.

Cappuccino, J.G. and N. Sherman, 1999. Microbiology: A Laboratory Manual, 4th Ed. Addison Wesley Longman, Inc. Harlow, England, 199–204.

Chen X, Tang Jj, Zhi Gy and Hu SJ. 2005. Arbuscular mycorrhizal colonization and phosphorus acquisition of plants: effects of coexisting planta species. Appl Soil Ecol 28: 259–269.

Clark RB and Zeto SK. 2000. Mineral acquisition by arbuscular mycorrhizal plants. J Pl Nutr 23:867–902.

Gyaneshwar P, Kumar GN, Parekh LJ and Poole PS. 2002. Role of soil microorganisms in improving P nutrition of plants. Pl Soil 245: 83–93.

Koenig, R. A., and C. R. Johnson. 1942. Colorimetric determination of phosphorus in biological materials. Anal. Chem. 14:155.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Microbiologia. 17: 362-370.

Ruiz-Lozano Jm, Collados C, Barea Jm and Azcón R. 2001. Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. N Phytol 151: 493–502.

Schinner, 1992: Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biol. Bioehem. 24, 389-395.

Sperger, J.I., 1958. The incidence of apatite – solubilizing organisms in the rhizosphere soil. Australian Journal of Agricultural Research. 9: 778-781.