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Isolation of phosphate solubilizing micoorganisms from rhizosphere of sugarcane

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Abstract

The rhizospheric soil of sugarcane was investigated for the study of phosphate solubilization by bacteria and fungi. In the present study the number of microorganism were isolated from the rhizosphere of sugarcane. Among this six microorganisms viz. Bacillus, Pseudomonas, Azotobacter, Arthrobacter, Penicillium and Mucor solubilize the inorganic phosphate. From the study it was observed that fungi viz.Penicillium and Mucor have **more solubilizing activity than bacteria both quantitatively and qualitatively. Hence the application of biofertilizer prepared by above mention bacteria and fungi should be helpful to increase the amount of phosphate into the soil because these microoeganisms release acid in very minut quantity in phosphate solubilization.**

Keywords: Phosphate solubilization, rhizospheric soil, sugarcane, phosphate solubilizing microorganisms, biofertilizer

Introduction

Phosphorus is one of the major nutrient second to nitrogen in requirement for plants. A greater part of soil phosphorus approximately 95-99% is present in the form of insoluble phosphate and cannot be utilized by plant (Vassileva *et al.*, 1996). Therefore, it is crucial to take advantage of the accumulated phosphate in soil improving the growth of plants. Hence there is enormous interest in isolating phosphate solubilizing microorganisms, including bacteria & saprophytic fungi due to their large biomass, metabolic activity and ability to maintain their solubilizing capacity for years. (Pandey, 2005) Phosphate solubilizing microorganisms particularly those belonging to the genera *Pseudomonas* Alcaligenes, *Arthrobacter, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia and Flavobacterium. (Rodriguez et al., 1999) ,Aspergillus* and *Penicillium*(Whitelaw *et al* ., 2000) and many others possess the ability to bring insoluble phosphate in the soil into soluble form by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. High proportion of phosphate solubilizing microorganism is concentrated in rhizosphere and they are metabolically more active than microorganism from other sources. Most of the researchers employed the Pikosvskaya's medium for the isolation and screening of phosphate solubilizing microorganisms. The present study was aimed for the isolation of phosphate solubilizing microorganisms from sugarcane on the basis of solubilization index and solubilization phosphate.

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Material and Methods

Isolation of phosphate solubilizing microorganisms

The rhizospheric soil samples of sugarcane were collected from sugarcane field at sarai Village of district Haridwar, Uttrakhand (India). The soil samples were collected in sterile polythene bags and immediately transported to the laboratory for processing. Phosphate solubilizing microorganisms were isolated on Pikovskaya's Agar medium by serial dilution method and incubate the plates at 28±30^{0}c for 4-5 days (bacteria & fungi). Phosphate solubilizing microorganisms showing phosphate solubilizing zone around them were considered as Phosphate solubilizing microorganism and pure culture of the isolates were made by repeated sub culturing for 2-3 times on fresh Pikovskaya's plate and were maintained on Pikovskaya's agar slants at 4°c temperature.

Identification of microorganisms: Identification of phosphate solubilizing bacterial strains was performed by morphological characteristics and biochemical analysis comparing with standard references (Holt *et al.*, 1994) including colony morphology, Gram staining, catalase test, Indole test, MR-VP test, citrate test, carbohydrate fermentation test as well as starch hydrolysis. The isolated fungi were identified by fungal staining.

Analysis of phosphate solubilizing activity

The quantitative as well as qualitative analysis of phosphate solubilizing activity of the selected isolates were conducted by plate screening method and broth culture method.

Qualitative measurement of phosphate solubilization

Bacterial & fungal isolates were screened for their tri- calcium phosphate (TCP) solubilizing activity on Pikovskaya's agar (PKV) plates. Isolates were spot inoculated on the centre of agar plate aseptically. Plates were then incubated at 28±2^{0}C for 3 days for bacteria and 4 days for fungi . A clear zone around a growing colony indicated phosphate solubilization and measured as phosphate solubilisation index (SI). Phosphate solubilization index was calculated as the ratio of the total diameter (colony+halo zone) to the colony diameter by using the following formula (Edi-Premono *et al.,* 1996) .

$$\text{Solubilizing index} = \frac{\text{Colony diameter} + \text{Clear zone}}{\text{Colony diameter}}$$

Quantitative measurement of phosphate solubilization

Bacterial & fungal isolates found to be positive for TCP solubilization were further analyzed for their ability to solubilize it in liquid medium. Microbial isolates were inoculated in Pikovskaysa's broth (100mL) in 250ml flask . and incubated at 28±2˚C for 6-17 days. After incubation the bacterial culture were filtered through Whatmann No.1 filter paper for bacteria and fungi through Whatmann No.2 filter paper. Filterate may be colored due to the fungal pigments and were clarified by centrifugation at 10,000 rpm for 10-15minutes. The soluble phosphorus was determined in clear filtrate using standard procedures. The intensity of blue color was measured in spectrophotometer at 430 nm wavelength (Dubey and Maheshwari *et al.*, 2012).

Results and Discussion

Isolation and Identification of PSB: In the present study, the collected soil samples were evaluated for Phosphate solubilizing bacteria & fungi in Pikovskaya's (PKV) plates . Initially 10 bacterial isolates were isolated on the basis of clearance around their colonies on Pikovskaya's agar plates. Out of 10 bacterial isolates , 3 bacterial isolates showed higher phosphate solubilization index (SI) ranged from 3.0-3.5 and designated as PS1,PS2 and PS3 were selected for further studies(Table 3). All isolates were rod shaped and 90% of them were Gram negative . All bacterial isolates were further characterized by a series of biochemical reactions and identified as genus *Bacillus sp.* , *Pseudomonas sp.* and *Azotobacter sp.* (Table 1)

Out of 8 Fungal isolates , 2 isolates showed phosphate solubilizing activity on Pikovskaya agar media and showed higher phosphate solubilizing index ranged (SI) from 3.5-5 and designated as PF1 ,PF2 (Table 3). All the fungal isolates were identified by observing its macroscopic(colour, texture and appearance) and microscopic(microstructures) characterized by fungal staining. (Aneja K.R.*et al.*, 2010) as *Penicillium sp.* , and *Mucor sp.*

Analysis of phosphate solubilizing activity

Qualitative measurement of phosphate solubilization: All the selected bacterial isolates were found to be potent phosphate solubilizer showing clear halo zone around their colonies. Among these 3 potent isolates, strains PS1 *(Bacilluss sp.)* and PS2 (*Pseudomonas sp)* showed the maximum phosphate solubilization activity than PS3 (*Azotobacter sp.*) as visualized by the size of clear zone developed around the colony which showed solubilization index of 3.5-3.0 respectively(table 3). The zone formation could be due to the activity of phosphatase enzyme in bacterial isolates. The experimental PKV slants with phosphate solubilizing microbes were stored at 4°C to arrest their growth and activity. After 4 days of incubation it was observed that strain PF1 (*Penicillium* sp.) , solubilized more phosphate than PF3 (*Mucor sp)* which showed the solubilization Index of 5.0- 4.1 as shown in table 3.

Table 1 : Seasonal variations of Physico-chemical parameters from different reservoirs of Nasik district and highest permitted value for drinking water (WHO standard, 1993 mg/l)

S.N.	Identification and characterization	Bacterial isolates		
		PS1	PS2	PS3
1	Gram Staining	Gram Positive	Gram Negative	Gram Negative
2	Colony & color	White Irregular Margin	White green Fluorescence	Milky Slimy circular raised
3	Cell Shape	Rods	Rods	Rods
4	Cell Arrangement	In chain	Single	Single
5	Catalase	+	+	+
6	Dextrose	A	-	-
7	Lactose	A	-	AG
8	Sucrose	A	-	AG
9	Citrate utilization	-	+	+
10	Starch Hydrolysis	+	-	+
11	Methyl Red	-	-	+
12	Vogue Proskouer	+	-	-

+=Positive, -= Negative, A=Acid, and AG = Gas, MR = Methyl Red, VP= Vogas- Proskauer

Table2 . Morphological Characterization of Fungal isolates from Rhizosphere of Sugarcane.

S.N.	Fungal isolates	Morphological characteristics				Remark
		Colour	Shape	Mycelium	Fruiting Body shape	
1	PF1	White Red	A Brush like	Septate	Conidia in chain	*Penicillium sp.*
2	PF2	White to Dark Gray	Ball like	Non-Septate Mycelium without Rhizoids	Single Columellate Sporangiophore	*Mucor sp.*

Table 3. Showing phosphate solubilizing ability of bacterial and fungal isolates .

S.No.	Isolates	Name of the isolates	Phosphate solubilizing ability	Solubilizing Index (SI)
1.	Bacteria	*Bacillus sp.*	+++	3.5
		Pseudomonas sp.	++	3.1
		Azotobacter sp.	+	3.0
2.	Fungi	*Penicillium sp.*	+++	05
		Mucor sp.	+++	4.1

Maximum=+++, Average=++, Minimum=+

Quantitative measurement of phosphate solubilization

The solubilzation levels of TCP varied with different isolates, all the 3 isolates were capable of solubilizing tri calcium (TCP) in broth medium. It was observed that all the bacterial strains showed concentration of phosphate solubilization from 0.340-0.520. *Bacillus sp.* shows maximum phosphate solubilizing activity.(0.520µl/g) than *Pseudomonas sp.(0.*420µl/g) and *Azotobacter sp.* (0.340µl/g) Where as Percent phosphate solubilized from TCP containing liquid medium by fungal isolates ranged from 0.78-0.93. *Penicillium sp.* (0.930µl/g)shows maximum concentration of phosphate solubilization than *Mucor sp.*

(0.780µl/g)**.**The acidification of the broth medium coincided with phosphorus solubilization. Furthermore, M.M.Ball *at al.,* 2007 also suggested that acidification of culture supernatants can be the main mechanism for P solubilization. Several species of bacteria (such as *Bacillus* sp, *Pseudomonas* sp.)degrade and solubilze the insoluble phosphate into soluble forms through the mechanism of secretion of organic acid like acetic acid , glycolic acid, formic acid etc. (Scheffer *et al.*,1998) this may indicate that our isolates might have used the same mechanism to solubilise TCP.

In the present study , phosphate solubilizing five isolates (*Bacillus sp. , Pseudomonas sp. , Azotobacter sp. ,Penicillium sp. and Mucor sp.*) were identified. Species of *Penicillium*, *Aspergillus* and Yeast have widely reported to solubilize various form of inorganic phosphate (Whitelaw *et al.*, 2000) . Various worker observed that fungi have more efficiency to solubilize phosphate than bacteria (Sanjotha *et al.*, 2011). Similar results were obtained in the present study. Hence there is need to develop the strain of fungi as phosphate fertilizer, also the application of the biofertilizer prepared by fungi should be helpful to reduce the salinity of soil by neutralization phenomenon, because these microorganism release the acid in very minute quantity in phosphate solublilzation .

Table 4 : Phosphate solubilization activity of isolates

S.No.	Isolates	Name of the isolates	Con. Of phosphateµl/g
1.	Bacteria	*Bacillus* spp.	0.520
		Pseudomonas spp.	0.420
		Azotobacter spp.	0.340
2.	Fungi	*Penicillium* spp.	0.930
		Mucor spp.	0.780

Conclusion

The microbial strains PS1and PS2 (*Bacillus sp.* and *Pseudomonas sp.* respectively) and fungal isolates PF1 and PF2 (*Penicillium sp.* and *Mucor sp.*) are significant phosphate solubilizer. *Penicillium and Mucor* have more solubilizing ability than bacteria both quantitatively and qualitatively . Hence the application of biofertilizer prepared by above mention bacteria and fungi should be helpful to increase the amount of phosphate into the soil because these microorganisms release acid in very minute quantity in phosphate solubilization and reduce environmental pollution and promotes sustainable agriculture.

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