IJPSR (2010), Vol. 1, Issue 9 (Suppl.) **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 May, 2010; received in revised form 24 July, 2010; accepted 25 August, 2010

BIOCHEMICAL CHARACTERISATION OF PHOSPHO BACTERIUM ISOLATED FROM RHIZOSPHERE OF *COSTUS SP*. WITH SPECIAL REFERENCE TO BACILLUS SPECIES

Sabiha Sultana M.*[1], C. Maya [2], Victoria Peter [1] and Omar Farooq [1]

Department of Botany, Microbiology & Biotechnology, Al-Ameen Arts, Science and Commerce College, Bangalore University [1], Karnataka, India
Department of Botany, Bangalore University[2], Karnataka, India

Keywords:

Phosphobacterium,

Phosphate fertilizers,

Biochemical analysis,

Costus sp

Correspondence to Author:

Sabiha Sultana M.

Department of Botany, Microbiology & Biotechnology, Al-Ameen Arts, Science and Commerce College, Bangalore University, Karnataka, India

E-mail: sultana_sabiha@yahoo.com

ABSTRACT

Soils generally contain adequate amount of inorganic and organic phosphorous but most of these remain unavailable to plants. The efficiency of water soluble phosphorus alone is usually low and soluble phosphorus when added to soil along with phosphate solubilizing microorganisms indicates utilization. The presence of soil microorganisms capable of transforming insoluble phosphorus to soluble form of phosphorus was studied Different phosphate dissolving bacterial strains are isolated from the rhizosphere soil of *Costus* plant and biochemical characterization and P_2O_5 uptake in liquid culture was studied.

INTRODUCTION: Phosphorus is applied to soils in inorganic fertilizers or in organic manures in the form of plant animal residues. Of all forms of phosphorus occurring in soils, the monovalent anion phosphate is the only one available to the growing plants when soluble forms of inorganic phosphorus fertilizers are applied to soils. It is found that high densities of phosphate dissolving bacteria harbor in soil. Fortunately, it is found that high densities of phosphate dissolving bacteria harbor in soils. Bacteria may play an important role in supplying the growing plants with their needs of phosphorus[1]. The USSR investigator had isolated an active phosphate dissolving bacteria which enhanced phosphorus uptake [2].

Costus pictus D. Don is an antidiabetic herb and its rhizosphere microflora was assessed. The role of plant growth promoting rhizo-microorganisms in improving plant growth is well documented [3]. The rate of metabolic activity of bacterium widely ranges and is based on the breakdown of organic material by the microbial enzyme which includes lyases, oxidoreductase, hydrolyase, amylase, catalase and transferase besides, cellulase. This is assessed by carrying out biochemical analysis of the *Bacillus* species isolated from rhizosphere soil of *Costus* plant.

MATERIALS & METHODS:

- Bacteria were isolated from rhizosphere soil by dilution plate technique, and *Bacillus* species are selected [4].

- Three strains of *Bacillus* species were selected [5], and labeled as B1, B2 and B3 strains.

- All the three strain are screened in liquid medium of Pikovskaya broth [6] using different phosphate like Tricalcim phosphate, Aluminium phosphate, Rock phosphate and commercial phosphate. Incubated for seven days at 28C in 250ml conical flasks and shaken for five minute on a rotary shaker. The isolates liberate different soluble phosphates (**Table 1**).

TABLE 1: RELEASE OF PHOSPHATE FROM 'P' FERTILIZER BY THE PSB IS LIQUID CULTURE

PSB	P_2O_5 mg/ 100 ml at 690 mm			
	TCP	R.P.	AP	CP
Control	0.000	0.000	0.000	0.000
Bacillus I	0.400	0.360	0.460	0.930
Bacillus II	0.310	0.180	0.400	0.560
Bacillus III	0.200	0.380	0.380	1.000

- Isolated strain B1, B2 and B3 are subjected to various biochemical analysis such as IMViC tests, starch hydrolysis, gelatin liquefaction tests [7], Acid and gas production was observed in sugar fermentation tests [8].

Observation & Chemical Analysis:

Chemical Analysis:

1. Phosphate solubilization by; Ammonium molybdate phosphoric blue color method [9].

2. Biochemical Analysis [10&11];

    - Indole test (I)
    - Methyl Red test (MR)
    - Voges Proskauer test (VP)
    - Citrate utilization test (CU)
    - Sugar Fermentation (SF)
        - {Dextrose (D), Mannitol (M) and Sucrose (S)}
    - Gelatin liquefaction (GL)
    - Starch hydrolysis (SH)

RESULTS & DISCUSSION: The results obtained by this study shows significantly high solubilization of commercial fertilizer than the other inorganic phosphate. All the *Bacillus* species show significant high solubilization of commercial fertilizer (Table 1). When compared with other phosphate fertilizer Aluminium phosphate shows enhanced results in all the three *Bacillus species*. The pH of liquid culture of phosphate solubilizing *Bacillus* strains has revealed that them they have released acid and hence the liquid broth culture shows p^H more towards the acidic range, *Bacillus species*-3 has not shown very good acid formation when compared to control and other strain in Aluminium phosphate fertilizer (**Table 2**).

TABLE 2: CHANGE OF PH VALUE BY THE PSB IN LIQUID CULTURE

PSB	pH			
	TCP	R.P.	AP	CP
Control	3	2	3	3
Bacillus I	3	3	4	3
Bacillus II	4	3	5	4
Bacillus III	5.6	5.6	5.6	5.6

The Biochemical characteristic shows that all the three *Bacillus species* utilize the monosaccharides and produce acid / gas, where as sucrose is negative i.e. disaccharides are not utilized by the *Bacillus species* (**Table 3**).

TABLE 3: BIOCHEMICAL CHARACTERISTICS OF THE ISOLATED *BACILLUS SPP*

Organism	Gram characteristics	Cultural plate characteristic	Gelatin liquefaction	Starch hydrolysis	Sugar Fermentation			Indole production	MR reaction	VP reaction	Citrate	Catalase activity
					Mannitol	Dextrose	Sucrose					
B1	Gram +ve rods with endo spores	Abundant opaque white growth	+ve	+ve	-	A/G	-	-	+ve	-ve	+ve	+ve
B2	Gram +ve rods with endo spores	Abundant opaque filamentous growth	+ve	+ve	-	A/G	-	-ve	+ve	-ve	+ve	+ve
B3	Gram +ve rods with endo spores	Abundant opaque creamish white growth	+ve	+ve	Acid	Acid	-	+ve	+ve	-ve	-ve	-ve

Acid soils shows deficiency of phosphorus is plants, under such condition inoculate soil with phosphate dissolving microorganisms along with phosphate fertilizer. Also phosphate solubilizing bacteria are proved beneficial in biological control in root diseases[12]. Phosphate solubilizing microbes has proved to enhance the growth of the antidiabetic plant *Costus sp.* The Biochemical Analysis reveals that all the *Bacillus species* were capable of hydrolysing gelatin and starch. The Methyl red shows positive results and Voges Proskauer test show negative results *Bacillus species* 1 and *Bacillus* 2 utilized citrate as the sole carbon source whereas *Bacillus* 3 did not. Indole test showed negative for B1 and B2 species where as it gave a positive result for B3. Catalase test also showed positive for B1 and B2 and negative for B3. The results indicate a decrease in the pH due to the formation of organic acids and support the view that the acidic end products produced as the result of the microbial activity affect the solubilization of phosphate Table 1 & 2. The results of Table 3 correlated with the results of Table 1 & 2, a significantly enhanced production of acid and gases in biochemical analysis was revealed. Soils with pure cultures of indigenous phosphate dissolving bacteria may assist in rendering more phosphorus to the plant, especially in soils which may not have an adequate number of efficient phosphorus solubilizing bacteria. Study of the morphology, cultural characteristics and biochemical

properties helped to identify these organisms as *Bacillus species*. These biochemical tests also revealed the diverse enzymatic activity of the soil bacteria and therefore capable of solubilization of different phosphates.

REFERENCES:

1. Taha SM, Mahmood SAZ, Halin, EL, Damaty A. and Hafez EL: Total microbial flora-acid producing and phosphate dissolving bacterium and rhizosphere under different manuring treatment. Plant and soil 1967.
2. Samtsevish SA: Preparation, use and effectiveness of bacterial fertilizers in Ukrainian SSR Translated Mikrobilogiya, USSR National Sci Foundation, New York. 1963; 31:747-755.
3. Murthy NA: Effect of Azospirilum and phosphobacterium in improving seeds germination and vigour of Alma. J .Non. Timber, forest products 1998; 5: 34-36.
4. Bergey: Manual of Systematic Bacteriology. Williams & Wilkins, London. 1989.
5. Woese CR: Bacterial Evolution. Microbial, rev 1987; 51, 221 – 271.
6. Pikovakaya RI: Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologia 1948; 17:362-70.
7. Vishwanath W and Li-Labati H: Biochemical and microbiological quantity of iced stored cat fish wallagoatty of Imphal market. Fish. Technol, 1995; 32: 113-117.
8. Morean R: The biological liberation of soluble phosphate from non soluble phosphorus compounds in soil. Compt. Rend 1960; 249:1802-1806.
9. Jackson HL: Soil chemical analysis, Prentice Hall of India (P) Ltd. New Delhi. 1971.
10. James G: Cappuccino and Natalie Sherman Microbiology. A Laboratory Manual 6th Edn. pp: 133-195:2004.
11. Aneja KR: Experiments in Microbiology, Plant Pathology and Biotechnology 4th Edition, pp: 245 – 275:2003.
12. Subba Rao NS: Soil microorganisms and plant growth. Soil Microbiology 1995; 103-104.

****