Research & Reviews

ISSN: 2320-3528

Research and Reviews: Journal of Microbiology and Biotechnology

# Biochemical Characterization of Phosphate Degrading *Pseudomonas Cichorii* Isolated From Forest Soils In Seshachalam Hills.

G Prasada Babu[1], D Chakravarthy[2], K Jaya Kumar[2] and Chinthala Paramageetham[2]*

[2]Department of Botany, Sri Venkateswara University, Tirupati – 517 502. Andhra Pradesh, India.
[1]Department of Microbiology, Sri Venkateswara University, Tirupati – 517 502. Andhra Pradesh, India.

Short Communication

Received: 20/02/2013
Revised: 28/02/2013
Accepted: 06/03/2013

***For Correspondence**

Department of Botany, Sri Venkateswara University, Tirupati – 517 502. Andhra Pradesh, India

**Keywords:** Phosphatase, Levan production, pikovskaya,s medium, carbohydrate utilization profiles

**ABSTRACT**

*Pseudomonas cichoriiisolates were* obtained from forest soils in Seshachalm hills using selective medium. The isolates were screened for phosphate activity and all the isolates were gram negative and showed bright fluorescence under UV light. The cultural and biochemical characteristics confirmed that the isolates were *P.cichorii.* Carbohydrates utilization profiles confirmed that the isolates were able to utilize Lactose, Xylose, Fructose, Glycerol, Trehalose, Mannitol and Ribose. However, the isolates were unable to utilize glucose and sucrose. The isolates showed positive results for levan production, oxidase and HCN. However they were negative for citrate utilization, ONPG and Gelatin hydrolysis.

## INTRODUCTION

In soils phosphorous is present in insoluble form complexed with cations like iron, aluminium and calcium. In soil it is abundant and exists in inorganic and organic forms. It is a major plant – growth – limiting nutrient after nitrogen (N). Plant mineral nutrition depends mainly in the phosphorous content of the soil, which can be assimilated only as soluble phosphate. Phosphorous plays many important photosynthesis and development of good root system, phosphorous deficiency leads to browning of leaves accompanied by small leaves, weak stem and slow development [1]. In natural ecosystem phosphorous enters in to the soil during the decay of natural vegetation and animals' excretion [2]. The assimilation of organic phosphorous by plants and microorganisms takes place through the enzyme phosphatese which is present in a wide variety of soil microorganisms .Fertile soil have high proportion of Phosphate solubilising microorganisms(PSM). The rhizobacteria plays a prominent role and they are termed as plant growth promoting rhizobacteria and among them are strains from genera such as *Alcaligens, Pseudomonas, Rhizobium, Azospirillum, Bacillus, Erwinia, Flavobacterium, Paenibacillus* and *Serratia* [3,4,5].

In the present study *P.cichorii* isolates were isolated from forest soil a fertile soil and screened for phosphatase activity. The isolates were characterized by their cultural, biochemical tests and carbohydrate utilization profiles.

## MATERIALS AND METHODS

### Sample Collection and Isolation of Microorganisms

Forest samples were collected from Seshachalam Hills, a part of Eastern Ghats from different sites using randomized block design. All the samples were pooled and subjected to serial dilution plating technique to isolate *P. cichorii* using King's B agar medium [6]. The isolates which give fluorescent were isolated and purified. These isolates were preserved in 20% glycerol stock until further use at -20°C.

ISSN: 2320-3528

**Phosphatase Assay**

All the purified isolates were grown in nutrient broth were subjected to phosphatase assay. This test was done to detect the *P.cichorii* isolates that has the ability to secrete phosphatase. Pure cultures of the bacterial isolates were spread on to the Pikovskaya's agar medium (PVK) [7] containing insoluble tricalcium phosphate and incubated at 27° C – 30° C for 7 days. Colonies showing halo zones were considered as phosphatase producer. These phosphatase producing isolates were subjected to cultural and biochemical tests for confirmation.

**Cultural Studies**

Cultural studies were performed to confirm the isolates as *P.cichorii.* Pure cultures of all the three isolates were streaked on to King's B, Pseudomonas agar [8], for colony development and growth pattern. The individual colonies were examined for size,shape ,colour, elevation, margin, pigmentation and reaction to UV light. Gram's reaction and levan production of all the isolates was recorded.

**Biochemical Studies**

Biochemical properties of all the *P.cichorii* isolates were carried for Citrate utilization, Gelatine hydrolysis, production of Oxidase, β- galactosidase, Catalase, HCN and Siderophore.

**Carbohydrate Utilization Profiles**

Carbohydrate utilization profiles were tested with lactose, xylose, Fructose, Galactose, Glycerol, Trehalose, Mannitol, Ribose, Glucose and Sucrose.

## RESULTS AND DISCUSSION

A total of 8 *Pseudomonas* isolates were obtained from forest soils. Among them three isolates were able to secrete phosphatase enzyme on Pikovskaya's medium. *P.cichorii* was studies in detailed for cell form, colony type, elevation, margin, colony colour, pigmentation levan production and reaction to UV light. It was evident from the observation that all the three isolates were rod shaped and gram negative in reaction. Isolates $PCTPT_2$ showed dull reaction to UV light, whereas isolate $PCTPT_1$ and $PCTPT_3$ showed bright reaction to UV light. It was evident from the observation that all the isolates were rod shaped and gram negative in reaction. All isolates produced greenish yellow colour colonies on King's B medium. The cultural characteristics were presented in (Table – 1)

**Table 1: Cultural and morphological characteristics of *P.cichorii* isolated from forest soil.**

| S.No | Forest soil | PCTPT 1 | PCTPT 2 | PCTPT 3 |
|---|---|---|---|---|
| 1 | Colony size | Moderate | Moderate | Moderate |
| 2 | Colony form | Circular | Circular | Circular |
| 3 | Colony margin | Undulate | Undulate | Undulate |
| 4 | Colony elevation | Convex | Raised | Convex |
| 5 | Cell shape | Rod | Rod | Rod |
| 6 | Gram's reaction | Negative | Negative | Negative |
| 7 | Fluorescence | Positive | Positive | Positive |

All the three isolates produced levan, Oxidase and HCN. All the three isolates showed optimum growth at 25°C. All the isolates were negative for gelatine hydrolysis, Citrate utilization and ONPG. However all the isolates were positive for Oxidase, HCN, Catalase, Casein hydrolysis. Siderophore production was observed for two isolates only namely $PCTPT_2$ and $PCTPT_3$. While isolates $PCTPT_1$ was unable to produce siderophore (Table-2).

Isolate $PCTPT_1$ produced acid from Lactose, Fructose, Glycerol. Isolate $PCTPT_2$ produced acid from Lactose, Xylose, Glycerol and the $PCTPT_3$ produced from acid from Mannitol and Ribose only (Table-3).

## DISCUSSION

Microorganisms substantially influence the soil productivity by solubilising this insoluble P through their metabolic processes in soil [9]. In soil phosphatases are responsible for release of soluble P from soluble organic P esters. Thus released P

ISSN: 2320-3528

entertains a possibility of remobilization in insoluble inorganic forms (mineralization). Production of phosphatase from *Aspergilli* is well known [10].

Thus the findings suggest that the *Pseudomonas cichorii* has the ability to produce phosphatase. Therefore these strains can be developed as Bio-fertilizers to augment plant growth promotion.

**Table 2: Biochemical characterstics of *P. cichorii* isolated from forest samples**

| S.No | Biochemical test | PCTPT 1 | PCTPT 2 | PCTPT 3 |
|---|---|---|---|---|
| 1 | Growth at - | 25°C | 25°C | 25°C |
| 2 | Gelatin Hydrolysis | - | - | - |
| 3 | Citrate Utilization | - | - | - |
| 4 | Oxidase | + | + | + |
| 5 | ONGP | - | - | - |
| 6 | HCN | + | + | + |
| 7 | Catalse | + | + | + |
| 8 | Casein Hydrolysis | + | + | + |
| 9 | Siderophore production | + | + | + |

**Table 3: Carbohydrates utilization by *Pseudomonas cichorii***

| S. No | Carbohydrate | Isolate-4 | | Isolate-5 | | Isolate-6 | |
|---|---|---|---|---|---|---|---|
| | | Acid | Gas | Acid | Gas | Acid | Gas |
| 1 | Lactose | + | - | + | - | - | + |
| 2 | Xylose | - | + | + | - | - | + |
| 3 | Fructose | + | - | - | + | - | + |
| 4 | Galactose | - | + | - | + | - | - |
| 5 | Glycerol | + | - | + | - | - | - |
| 6 | Trehalose | - | + | - | + | - | + |
| 7 | Manitol | - | + | - | - | + | - |
| 8 | Ribose | - | - | - | + | + | - |
| 9 | Glucose | - | - | - | - | - | - |
| 10 | Sucrose | - | - | - | - | - | - |

## CONCLUSION

A total of three isolates showed phosphatase production. This was evident by observing a clear halozones on the pikovskaya's agar medium. On the basis of cultural, morphological characteristics like rod shaped, gram negative characteristics the isolates were confirmed as bacteria. On comparision of our results with biochemical tests and literature the isolate was tentatively identified as *P.cichorii.*

**References:**


1. Sonam Sharma, Ram Babu Tripathi. Isolation of phosphate solubilising microorganisms (PSMs) from soil. J. Microbiol. Biotech. Res. 2011; 1 (2): 90-95.
2. Kannaiyan S, Kumar K, Govindarajan K. Biofertilizers Technology. Scientific Pub (India), Jodhpur, 2004.
3. Das, Katiya KV, Goel R. Phospahte solubilisation potential of plant growth promoting *Pseudomonas* mutants at low temperature. Microbiol Res. 2003; 158:359- 362.
4. Illmer P, Barbato A and Schinner F. Solubilisation of inorganic phosphatases by microorganisms isolates from forest soil. Soil. Biol. Biochem. 1992; 24, 389 – 395.
5. Sahu, Maloy kumar, Sivakumar K Kannan L. Phosphate solubilising actinoycetes in the estuarine environment: an inventory J. Environ. Biol. 2007; 28, 795 – 798.

ISSN: 2320-3528

6. King EO, Ward MK, Raney DE. Media for the demonstration of pyocyanin and fluorescein. J. Lab Clin. Med. 1954; 44:301-307.
7. Pikovskaya, RI. Mobilization of phosphorous in soil in connection with vital activity of some microbial species. Mikrobiologiya. 1948; 17:362 – 370.
8. Ravikumar SP, Williams P, Shanthy S, Anitha N, Gracelin A, Babu S, et al. Effect of heavy metals (Hg and Zn) on the growth and phosphate solubilising activity in halophilic phosphobacteria isolated from Manakudi mangrove. J. Environ. Biol. 2007; 28:109 -114.
9. Tarafdar JC, Rao AV, and Bala K. Production of phosphatase by fungi isolated from desert soils. Folia. Microbiol. 1988; 33:453 – 457.
10. Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. Bergey's manual of determinative bacteriology. 9thEd. William R. Hansyl, Editor. Baltimore, Maryland ( USA): Williams and Wilkins;1994.