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Authors *Deshwal VK<sup>1,2\*</sup>, Singh SB<sup>2</sup>, Chubey A<sup>2</sup> and Kumar P<sup>2</sup>*

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Full Length Research Paper

## Isolation and characterization of *Pseudomonas* strains from Potatoes Rhizosphere at Dehradun Valley, India

Deshwal VK<sup>1,2\*</sup>, Singh SB<sup>2</sup>, Chubey A<sup>2</sup> and Kumar P<sup>2</sup>

<sup>1</sup>Department of Microbiology, Doon (P.G.) Paramedical College, Dehradun, India,

<sup>2</sup>Department of Microbiology, Singhania University, Pachheri Bari, Jhunjhunu-Rajasthan, India

\*Corresponding author: *Deshwal VK*

### Abstract

*Pseudomonas* is well known Plant growth promoting rhizobacteria. 20 sites at dehradun were selected for present study. *Pseudomonas* strains were isolated from potato rhizosphere. 140 Strains were isolated and characterized on the basis of biochemical tests. Strains were isolated on King's medium B and cetrimide agar medium. And various biochemical tests were done. Out of 140 isolated *Pseudomonas* strains, 55 strains were *P. aeruginosa*, 26 were *P. cepacia*, 37 strains were *P. fluorescens* and 22 strains were *P. putida* were characterized.

**Key words:** Biochemical test, *Pseudomonas*

### Introduction

Use of chemical in agriculture affects the soil environment and soil fertility. So Scientists are searching alternative of chemical in agriculture. Usually microorganisms require simple form of chemical for their growth and metabolic activity. Environment like soil has complex material and microorganism has capability to recycle the elements by degradation. Other than this quality a group of microorganisms improve the plant growth activity and environmental friendly (Deshwal *et al.*, 2003a; 2003b; 2006).

Plant Growth promoting Rhizobacteria (PGPR) are classified according to their association with plant root i.e. extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Martinez-Viveros *et al.* 2010). PGPR strains increase plant growth by direct and indirect method. In direct method, PGPR secrete some plant growth promoting substance, siderophore, enzyme in rhizosphere but indirect method PGPR strains secrete antimicrobial substances like HCN, antibiotics, enzymes which inhibited the growth of plant pathogenic microorganisms (Deshwal *et al.*, 2013).

Genus *Pseudomonas* is Gram-negative motile aerobic rods that are widespread throughout nature and characterised by elevated metabolic versatility, presence of a complex enzymatic system (Franzetti and Scarpellini, 2007). Fluorescent pseudomonads are ePGPR and make up a dominant population in soil along with other PGPR like *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* belongs to ePGPR (Gray and Smith, 2005; Bhattacharyya and Jha, 2012). *Pseudomonas* strains produce fluorescent under UV light are known as fluorescent pseudomonads. These strains are characterized by biochemical test (Uğur *et al.*, 2012; Deshwal, 2012). Aim of present study is isolation and characterization *Pseudomonas* from Potato rhizosphere at dehradun.

### Material and Methods

**Isolation of *Pseudomonas*:** 20 different fields site at dehradun were selected for isolation of *Pseudomonas*. *Pseudomonas* strains were isolated from potato rhizosphere. Carefully uproot the plant and remove the excess soil. Collect 5g rhizosphere soil and make different dilutions upto 10<sup>-6</sup> of this rhizospheric soil by serial dilution technique. Transfer 0.2ml of sample from each dilution in King's B and cetrimide agar medium. Spread it by sterilized glass spreader. Invert the plates and incubate at 28°C for 24-48hrs.

**Biochemical characterization:** 140 strains were characterized according to Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

### Results and Discussions

Strains were isolated on King's medium B and cetrimide agar medium. Fluorescent and non-fluorescent strains were selected for further study. These strains were transferred on MacConkey agar medium. All the strains were gram staining rod shape bacteria. *P. aeruginosa*, *P. cepacia* failed to grow at 5°C and maximum *P. fluorescens*, *P. putida* strains showed poor growth as compared to growth at 28°C. Isolated strains showed growth at 42°C. Further, various biochemical tests were conducted. Out of 140 isolated strains, 55-*P. aeruginosa*, 26-*P. cepacia*, 37-*P. fluorescens* and 22- *P. putida* were identified (Table 1). Many strains produced acid from glucose, arabinose, glycerol, fructose, xylose but showed variation in other sugar for acid production (Table 2). Similar

observation has been mentioned in Bergey's manual of determinative bacteriology (Holt *et al.*, 1994). Uğur *et al.*, (2012) bio-chemically characterized 46 Gram negative bacteria from the seawater on the southwest coast of Turkey and found that 20 out of 46 isolates were *Pseudomonas*. Similar observation has been observed by (Deshwal *et al.*, 2012a, b). Rachid and Ahmed (2005) screened fluorescent *Pseudomonas* on King's B medium and 5 *Pseudomonas fluorescens* were confirmed on the basis of biochemical test. Noori and Saud (2012) bio-chemically characterized 20 *Pseudomonas* strains and found that 15 strains were identified as *Pseudomonas fluorescens*, 3 isolates belong to the species of *P. luteola*, one isolates to the *P. aeruginosa* and a single isolate showed a doubtful identification. All biochemical tests and available literature suggested that isolated strains were *Pseudomonas*.

**Table 1:** Characterization of *Pseudomonas* strains

Biochemical test	<i>Pseudomonas</i>			
	<i>aeruginosa</i>	<i>cepacia</i>	<i>fluorescens</i>	<i>putida</i>
Gram staining	-	-	-	-
Morphology	Rod	Rod	Rod	Rod
Growth at 5°C	-	-	E	E
Growth at 42°C	+	E	-	-
Growth on MacConkey agar	+	+	+	+
Fluorescence – King's medium B	E	-	E	E
Oxidase	+	+	+	+
Lysine decarboxylase	-	E	-	-
Oxidation in OF medium	+	+	+	+
Alkaline in OF medium	-	-	-	-
Nitrate reduced to nitrite	+	E	E	-
Simmon's citrate medium	+	+	+	+
Christensen's citrate medium	+	+	+	+
Urease	+	E	E	E
Gelatinase production	+	E	E	-
Growth in KCN medium	+	E	E	E
H <sub>2</sub> S (PbAc paper)	-	-	-	-
Gluconate	E	+	-	-
Malonate	+	E	E	E
ONPG	-	E	-	-
Starch hydrolysis	-	-	-	-
DNAase production	E	-	-	-
Phenylalanine	-	-	-	-
Tyrosine hydrolysis	+	+	+	+
Ornithine decarboxylase	-	E	-	-
Arginine dihydrolase	+	-	+	+
Casein hydrolysis	+	+	E	-
Selenite reduction	E	-	-	-
Thornley's arginine	+	-	+	+
Tween 20 hydrolysis	+	+	E	E
Tween 80 hydrolysis	+	+	E	-
3-ketolactose production	-	-	-	-
Lecithinase production	+	+	E	-
Nitrite reduced	+	-	-	-
PHBA growth	+	+	+	+
PHBA accumulation in cells	-	+	-	+
Aesculin hydrolysis	+	E	E	-
Growth in presence of cetrимide	+	E	+	+

E= more than 85% showed positive reaction

**Table 2:** Carbohydrate utilization test

Carbohydrates acid from	<i>Pseudomonas</i>			
	<i>aeruginosa</i>	<i>cepacia</i>	<i>fluorescens</i>	<i>putida</i>
10% glucose	+	+	E	+
10% Lactose (in peptone water medium)	E	+	E	E
Acid from glucose (in ammonium salt media ) acid	-	E	E	E
From Glucose	+	+	+	+
Arabinose	+	+	+	+
Cellobiose	-	+	E	-
Ethanol	+	-	E	E
Glycerol	+	+	E	E
Fructose	+	+	+	E
Mannitol	+	+	E	-
Rhamnose	-	-	E	-
Maltose	-	+	-	-
Lactose	-	+	-	-
Raffinose	-	E	-	-
Xylose	+	+	+	+
Sorbitol	-	+	E	-
Sucrose	-	E	E	-

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