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Research Article

ISOLATION AND CHARACTERIZATION OF *PSEUDOMONAS FLUORESCENS* FOR PLANT GROWTH PROMOTION

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ABSTRACT
Soil dwelling microorganism near rhizosphere are good source of Plant growth promoting Rhizobacteria (PGPR). They are efficient in stimulating the nitrogen fixation and absorption of micronutrients from the soil. Siderophores produced by these bacteria are having antifungal activity and therefore can be used as best PGPR. Pseudomonas species are diverse and efficient phosphate solubilizer bacteria and can be isolated from many crop rhizospheres. They have promising role in promoting the crop plant growth and can be used as an effective biofertilizer. These microbes can thereby improve the soil physiochemical properties and can have direct impact on the nutrient availability and plant growth improvement. In the present investigation a preliminary attempt was done for the isolation of fluorescent *Pseudomonas fluorescens*.

Keywords: *Rhizospheres, Siderophores, Absorption*.

INTRODUCTION
Agriculture is the one of the main branch which decides the self-dependency of any county. In this scenario for the proper crop growth farmers depend on the fertilizers and pesticides. In supplying the nutrients now a days farmers are depending more on the synthetic chemical constitutents, which increases the pollution. We have to prevent pollution of the soil and water, but also increase production without causing damage to our ecosystem. Biofertilizers play a vital role in helping our farmers and decrease the pollution of soil and water. As the synthetic chemicals are the main cause of increased pollution and biomagnifications. Plant growth promoting rhizobacteria (PGPR) are the best soil microbes that enhance the luxuriant plant without any adverse effects. They help in improving the accessibility of the trace elements, phytohormones, and have antagonistic effect against the phytopathogens (Egamverdiyeva, 2005; Wani *et al.*, 2007; Mittal *et al.*, 2008). For many years many researchers have attempted to isolate and multiply these useful bacteria like *Bacillus, Enterobacter, Azospirllum, Azatobacter, Klebsiella, Pseudomonas* etc., (Glick, 2012). Mode of action of all these PGPR are diverse, with their application method also differs. So their usage in plant growth is also meager.
Conditions of the fields are different and influenced by soil, water, microbial communities that reside, pH, temperature etc., The successful performance of a bio-inoculant in field is governed by its ability to survive and interact with native microbiome. This is a challenging issue which the researchers face while preparing and supplying for the farmer community which is the final application after selection of the useful PGPR (Tabelsi and Mhamdi, 2013). Successful colonization of these plant microbes is necessary for a better performance and more beneficial effect. This colonization is very complex process and is influenced by various factors like, root exudates, biotic, and abiotic factors along with the rhizobacteria (Benizri *et al.*, 2001). PGPR can affect the plant growth by increased mineral nutrient solubilization, nitrogen fixation repression of soil borne pathogens, and making nutrients available for the plants (Gupta *et al.*, 2000).
Pseudomonas aeruginosa is one among the frequently isolated endophytic bacteria and it has antagonistic activity against *Phytophthora capsici* (Aravind *et al.*, 2009). Genus *Pseudomonas* is Gram negative, aerobic, flagellated rod shaped bacteria. These bacteria have antagonistic activity on plant pathogens, due to competition for nutrition, site along with antibiosis and siderophores production. So in the present

investigation we have isolated and identified a *Pseudomonas* sp from the rhizospere. We screened for antifungal activity against plant pathogenic fungi and attempted for the biochemical characterization of *Pseudomonas fluorescens*.

MATERIALS AND METHODS

Isolation and biochemical characterization of Pseudomonas sp from rhizosphere

Soil samples were collected from a depth of 5-10 cm from rhizosphere of different plantation crops like Banana (PS1) and Papaya (PS2) around the Anantapur, AP, India from three different location at a radius of 25 km. Roots were uprooted carefully and collected the soil sample in sterilized polybags. Later bacteria were isolated through serial dilution method. 1 gm of soil sample was dissolved in sterilized distilled water and separated the bacteria by serial dilution method and Morphological characterization done using Gram staining (Moyes *et al.,* 2009).

Biochemical characterization was done using Oxidase, catalase, urease test, methyl red test, Citrate utilization test, Voges Proskauer test, McConkey's lactose bile salt agar and Nitrate reduction test using standard procedure of Cappuccino and Sherman (1992).

Characterization of rhizobacteria for PGPR traits

Production of Indole acetic acid (NAA) was done using Gordon and Weber method (1951), Siderophore Production was detected according to Teintze *et al.,* (1981), Production of Hydrogen cyanide was done using Donate-Correa *et al.,* (2005), Production of Ammonia according to Cappuccino and Sherman (1992). Later Anti fungal activity was tested using Sindhu *et al.,* (1999).

RESULTS AND DISCUSSION

Beneficial microbes are present abundantly in the soil rhizosphere. We have to isolate these useful microbes from the soil surrounding the roots of different plants. Association of plant and microorganisms greatly influence the growth of the associated plants (Sahin *et al.,* 2004). Due to the safety of PGPR much attention has been attained to isolate and use in the bioformulations, so that farmers are benefited with these in a larger number. In the present study *Pseudomonas* isolated from soil samples showed fluorescent production. Gram staining was done to identify the positive and negative bacteria. When the morphology of this bacteria was observed it was round, raised with smooth shiny surface and smooth margin with light cream to white colour and fluorescent green (Figures 1-3). Siderophore production was orange in colour. Three different samples collected where cultured using different temperatures and pH to know the growing conditions of the isolated bacteria. Optimum temperature was 30^0C, among the tested range was

Figure 1: Morphology of *Pseudomonas* on agar medium

between 20–40^0C. At neutral pH there is luxuriant growth of the *Pseudomonas fluorescens.* Pseudomonas genus is most divergent and referred as Pseudomonads (Deshwal and Kumar, 2013). To

prevent the damage of the ecosystem by the synthetic chemicals and to improve the soil fertility, there is the need to encourage the isolation and formulating these as farmer usable resources in the future. Our present work makes it clear that there are useful PGPR in the banana and Papaya plantation and these *Pseudomonas* sp can be convert into usable formulation and make them available to the farmer community and also these strains can be improved using the biotechnological tools.

Figure 2: Formation of clear halo zone around fluorescent *Pseudomonas*

Figure 3. Antifungal activity of *Pseudomonas*

Table 1: Biochemical and characteristics of isolated Pseudomonas *sp*

Morphology and characteristics	***Pseudomonas fluorescens***
Gram staining	Positive
Shape	Rod
Flagella	Present
Colour	Cream to white
Oxidase	+
Catalase	+
Urease activity	-
IAA Production	+
Citrate Utilization	-
McConkey's Lactose Bile salt agar	+
Nitrate reduction test	+
Siderophore production	+

Table 2: The range of pH suitable for the isolated *Pseudomonas fluorescens* at 30^{o}C

	pH4	pH 5	pH7	pH8	pH9	pH10
PS1	-	+	+++	+	-	-
PS2	-	+	+++	+	-	-

Table 3: Effect of temperature on the growth of the *Pseudomonas fluorescens* collected samples

	20^{0}C	25^{0}C	30^{0}C	35^{0}C	40^{0}C	20^{0}C
PS1	-	+	++	+	-	-
PS2	-	+	++	+	-	-

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