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In Vitro Study of *Pseudomonas* spp. Isolated from Soil

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Summary

Pseudomonas spp., a soil microorganism, associated in the rhizosphere zones of crop fields are powerful phosphate solubilizers. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases that play a major role in the mineralization of organic phosphorous in soil. (Rodríguez, H. and Fraga, R. 2000). Thus, *Pseudomonas* are eco-friendly and bring about solubilization of bound phosphates in soil with out any environmental hazard. This experiment was conducted to isolate soil *Pseudomonas* spp. from different agrobased areas of Korea district of C.G. and study its behavior taking different parameters. From 25 soil samples collected, bacterial cultures were isolated and four were identified as *Pseudomonas* spp. and further studied their cultural and microscopic characteristics *in vitro*.

Introduction

Advancement in agriculture technologies has brought about an increase in productivity but they have adverse effect on environment. Extensive use of chemical fertilizers to improve plant health, productivity and for control of pathogens has disturbed the ecological balance and has led to the depletion of soil nutrients. Hence there is a need to search for an alternative to improve soil health without causing ecological damage. Biofertilizers are therefore gaining importance as they are ecofriendly, non hazardous and nontoxic. Biofertilizer refers to products consisting of selected and beneficial living microbes, which are used as microbial inoculants in soil. Several organisms such as Cyanobacteria, *Azolla, Rhizobium*, endophytic diazotrophs and phosphate solubilizing microorganisms are presently being used as biofertilizers (Kannaiyan et al., 2004). These microbes are known to improve plant health by secretion of growth promoting substances and by increasing the availability of micronutrients. Phosphate Solubilizing Bacteria are slowly emerging as important organisms used to improve soil health by reducing phosphate deficiency in soil (Yosef et al., 1999)]. Bacteria belonging to family Pseudomonadales, genus *Pseudomonas* and species *P. striata* and *P. liquifaciens* is an important Phophorus Solubilising Biofertilizer (Motsara *et al.,* 1995). The genus *Pseudomonas* was described by Migula (1894) and is one of the most diverse and ubiquitous bacterial genera whose species have been isolated worldwide in all kinds of environments. Various species of *Pseudomonas* are thought to play an important role in plant growth promotion and disease suppression (Kloepper et al., 1980; Jayaswal et al., 1993). In particular, *P. fluorescens* (Howell & Stipanovic 1980, Weller & Cook, 1983) and *P. cepacia* (Homma et al., 1989; Hebbar et al., 1992; Jayaswal et al., 1993) have attracted considerable attention on account of their potential for biological control.

Materials and Method

Collection of soil samples

Soil samples were collected from selected agro-based areas of 5 blocks of Korea district of chhattisgarh by composite sampling method (Walworth, 2006).

Isolation of bacteria

Total of 28 bacterial cultures were isolated from soil samples. Isolation of rhizospheric bacteria was carried out by serial dilution of soil and plating 1 ml of selected dilution (10^{-9}) on Nutrient agar and Pseudomonas agar base (all from Hi Media, Titan and Merk).

Identifciation and Characterization of the isolated Bacteria

All total 28 bacterial isolates were characterized by various microscopic and cultural examinations. Four out of 28 bacterial cultures were tested for growing them on Pseudomonas agar base medium and Nutrient agar medium.

Result and Discussion

All 28 bacterial isolates were motile rods, out of which four isolates were identified as *Pseudomonas* (Gram negative) and others were *Bacillus subtilis* (Gram positive)(from IARI, Plant Pathology department, Delhi). Results of identifying tests performed are given in table below.

Table (a). Cultural characteristics, microscopic examinations of four bacterial isolates.

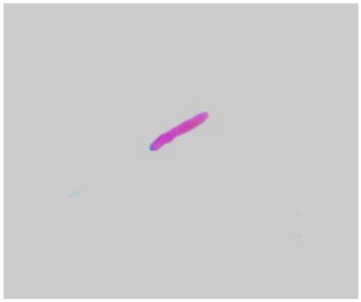
Characteristics	Bacterial isolates			
	P10	P11	P25	P27
Colony characteristics on nutrient agar media				
Colony size/ growth on solid medium, Nutrient agar medium	+	+	3+	3+
Surface	Mucoid	Mucoid	Slimy	Slimy
Margin	Undulate	Undulate	Undulate	Undulate
Elevation	Flat	Flat	Flat	Flat
Form	Irregular	Irregular	Regular	Regular
Colony colour	Cream	Cream	White	White
Density	Opaque	Opaque	Opaque	Translucent
Growth in liquid medium, Nutrient broth	Surface growth	Surface growth	Turbid	Surface growth

Note: P10, P11, P25, P27 : *Pseudomonas* isolates.
Growth : Abundant growth-(3^+), Moderate Growth-(2^+), Slight Growth-($^+$).

Four bacterial isolates showed specific cultural characteristics on solid media. Isolate P25 and P27 showed abundant growth and isolate P10and P11 showed slight growth, P10 and P11 has Mucoid colony surface where as P25 and P27 has slimy colony surface, all four isolate showed undulate colony margin, P10 and P11 showed irregular growth form while P25 and P27 showed regular growth form, isolate P10 and P11 colony colour is cream and isolate P25 and P27 colony colour is white, isolate- P10, P11,P25 showed opaque colony and P27 showed translucent colony, isolate- P10,P11 and P27 showed surface growth in liquid media while P25 showed turbid growth in liquid media.

Table (b)

Microscopic examination	Bacterial isolates			
	P10	P11	P25	P27
Gram staining	Negative	Negative	Negative	Negative
Capsule staining	Negative	Negative	Negative	Negative
Endospore staining	Negative	Negative	Negative	Negative
Motility	Motile	Motile	Motile	Motile
Morphology	Rod shaped	Rod shaped	Rod shaped	Rod shaped



Gram stained bacterial cell at 100x oil immersion

Bacterial culture on Nutrient agar media

All four *Pseudomonas* spp. are Gram negative, non-capsulating, non-spore forming, motile and rod shaped.

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