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Pseudomonas fluorescens: A Proficient Plant Growth Promoting Rhizobacteria Isolated from Organic Farm

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A B S T R A C T

In the present study, strains of *Pseudomonas fluorescens* designated as OrgS63 and OrgS49 isolated from soybean rhizosphere of an organic farm. Siderophore production was detected by using modified Fiss Minimal medium and estimation was done by CAS assay. Both the strains were found to produce good amount of siderophores. These *Pseudomonas* strains were screened for their other plant growth promoting traits like IAA production, production of Ammonia, HCN production, Phosphate solubilization and antifungal activity. OrgS63 was the most efficient phosphate solubilizer on NBRIP plates with a solubilization index of 6.10. In liquid NBRIP medium, significantly higher Phosphate solubilization was recorded for both the strains OrgS63 and OrgS49 (440 and 450 µg/ml) after 5 days of incubation. Both the strains OrgS63 (90.00%) and OrgS49 (90.44%) has shown high % inhibition against *Fusarium oxysporum.* These isolates also showed positive results for Indole 3-acetic acid (IAA), ammonia production, hydrogen cyanide (HCN) production and ammonia production. Thus, the present study concludes that these Siderophore producing *Pseudomonas fluorescens* strains could serve as a proficient *Plant growth promoting Rhizobacterial* inoculants for improving the cropping systems and enhancing metal remediation of polluted environments.

Introduction

Due to excessive use of chemical fertilizers in agricultural field, the soil loses the microbial activity. It is reported that Organic applications in agricultural farms increases the microbial activity drastically as compare to soil with chemical fertilizers [3]. In rhizosphere, siderophore producing *Pseudomonas* plays a central role in improvement of the plant development. These bacteria act as biocontrol agent by limiting iron nutrition to the pathogen. Many bacteria synthesize siderophore that has an high affinity for ferric iron, resulting in ferric-siderophore complex which is transported into the cell and thus facilitates iron uptake under low iron condition making unavailable to the other organisms and restrict the growth of deleterious bacteria and fungi at the root [1].

Siderophore producing bacteria showing metal resistant property is useful in bioremediation of metal polluted iron depleted soils [2].

Pseudomonas sp. is abundant in agricultural soils which make them perfect suitable as Plant growth promoting Rhizobacteria (PGPR) [4].

The present study was conducted to isolate and check plant growth promoting along with metal resistant abilities of *Rhizobacteria* from an Organic Farm.

Materials and Methods

Bacterial strains

Strains of *Pseudomonas fluorescens* designated as OrgS63 and OrgS49 was isolated from soybean (*Glycine max L*) rhizosphere of an organic farm.

The pure culture isolates were maintained on modified King's B agar(for 1 litre, proteose peptone, 20 gm; KH_2PO_4, 1.0 gm; $MgSO_4$, 0.4gm; Glycerol, 8ml; agar 20 gm) slants at 4ºC and used for further studies.

Detection of siderophore production

Both the strains were grown overnight at 28ºC in modified King's B Broth. The bacterial isolates were spot inoculated on Chrome Azurol S Agar plates and visualized for the appearance of pink halo zones against blue background after 72 hrs of incubation at 28 ºC[4].

Plant growth promoting traits

Phosphate solubilization

Phosphate solubilization by the strains was detected qualitatively on NBRIP agar plates and also measured quantitatively by using NBRIP medium and solubilization index was calculated by using the following formula (Edi-Premono *et al.*, 1996) [5].

$$SI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

HCN production

HCN production by the isolates was detected by the method of Baker and Schipper, 1987 [6].

IAA production

Indole acetic acid production by the isolates was quantitatively measured by the method given by Gordon and Weber (1951) [6].

Ammonia production

Ammonia production was detected by adding 1 ml Nessler's reagent to a 72 h old culture grown in peptone broth) and recorded the presence of the deep yellow to orange colour [6].

Antifungal activity

For determining the Antifungal activity, *Pseudomonas* cultures was streaked on Potato Dextrose Agar 1.5 cm from the edge of the each plate along with fungus (*Fusarium oxysporum*) and were incubated at 28 ± 2°C. Each test was replicated three times. Radial growth was measured and percent of radial growth inhibition was calculated [6].

Percent of radial growth inhibition
$= (R1 - R2 / R1) \times 100$

R1 = Radial growth of *Fusarium oxysporum* in control plate

R2 = Radial growth of *Fusarium oxysporum* interacting with antagonistic bacteria

Metal resistant ability

The isolates were tested by agar dilution method for their resistance to heavy metals (Co and Zn). Metal concentration ranging from 25 to 200 µg/ml were inoculated with overnight grown culture on Nutrient agar plates. Metal Resistant ability was observed after incubating the plates for 24 to 48 hours[7].

Effect of *Pseudomonas fluorescens* on seedling growth of *Glycine max* L (Soybean seeds)

The strains were subjected for their efficacy on seed germination and seedling growth of Soybean seeds (variety JS 335). The bacterial suspension were prepared and tested for their plant growth promoting efficiency, which was carried out by the standard paper Roll towel method (ISTA, 1993).

The soybean seeds were mixed with bacterial suspension. Plant growth promotion of soybean seedling was assessed using Vigour Index (Baki and Anderson, 1973) [8].

Vigour index = Percent Germination X seedling length (shoot length + root length)

Result and Discussion

The strains isolated in this study were Gram negative short rods and colonies produced fluorescence under uv light. On CAS Agar plates they produced pink color halo zones and tested positive for Siderophore production. These *Pseudomonas* strains were screened for their other plant growth promoting traits like IAA production, production of Ammonia, HCN production, Phosphate solubilization and antifungal activity. OrGS63 was the most efficient phosphate solubilizer on NBRIP plates with a solubilization index (SI) of 6.10 whereas OrGS49 has SI 5.16 (showing clear halo zone of solubilization 31 mm and 24 mm respectively)In liquid NBRIP medium, significantly higher Phosphate solubilization was recorded for isolates OrGS63 and OrGS49 (440 and 450 µg/ml) after 5 days of incubation

Both the strains OrgS63 (90.00%) and OrgS49 (90.44%) has shown high % inhibition against *Fusarium oxysporum.* Positive results indicate that HCN production by rhizospheric microbes has been proposed as a possible defense mechanism against soil borne plant pathogens (Walia *et al.*, 2013) (Fig. 1–9; Table 1 and 2).

Isolate OrGS63 and OrGS49

Isolate OrGS63 and OrGS49 also shows positive results for ammonia production and revealed significant level of Indole acetic acid (IAA) 19.00 µg/ml and 16.4µg/ml.

Both the strains shows resistance to Heavy metals such as Co and Zn. OrGS63 and OrGS49 shows resistance in all concentration (25 to 200 µg/ml) of both the metal.

OrgS63 and OrGS49 showed higher SVI 2349 and 2002 after 8^{th} day of germination period. The results revealed that seed treatment with *Pseudomonas* strains improved seed germination and seedling vigour index over the control (SVI 787).

Thus, the present study concludes that these Siderophore producing *Pseudomonas fluorescens* strains may serve as a proficient *Plant growth promoting Rhizobacterial* inoculants for improving the cropping systems and enhancing metal remediation of polluted environments.

Table.1 Plant growth promoting characteristics of both the strains

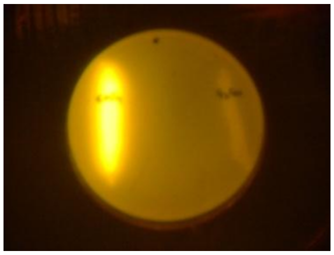
***Pseudomonas* Strains**	**Plant growth promoting characteristics**			
	Phosphate solubilization	HCN Production	IAA Production	Ammonia production
OrGS63	+	+	+	+
OrGS49	+	+	+	+

Table.2 Efficacy of *Pseudomonas* strains on seedling growth and SVI

S.N	**PSB Isolates**	**On 2nd day**		**On 4th day**		**On 8th day**	
		Seedling length	Seedling Vigour Index(SVI)	Seedling length	Seedling Vigour Index(SVI)	Seedling length	Seedling Vigour Index(SVI)
1	OrGS63	9.1 cm	805	13.7 cm	1233	26.1 cm	2349
2	OrGS49	9.8 cm	852	13.4 cm	1192	22.5 cm	2002
4	Control (uninoculated)	4.5 cm	337	7.3 cm	547.5	10.5 cm	787

Fig.1 *Pseudomonas* strains under UV light

Fig.2 *Pseudomonas* strains on King B Agar Plates



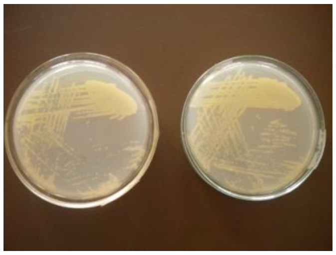


Fig.3 Siderophore production of *Pseudomonas* strains on CAS Agar plates showing pink color halo zone along with the control plate

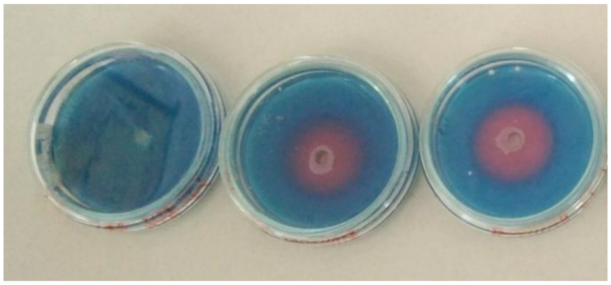


Fig.4 Phosphate solubilization

Fig.5 HCN Production

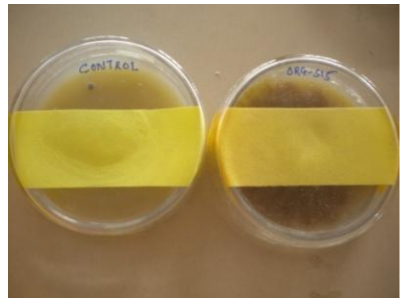


Fig.6 IAA production

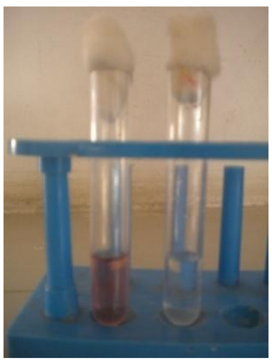


Fig.7 Ammonia Production

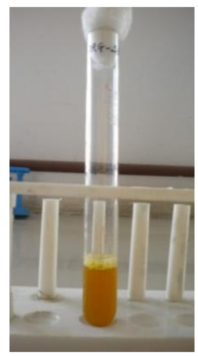


Fig.8 Antagonistic activity of *Pseudomonas* strains against *Fusarium oxysporum*

Control plate

Experimental Plate

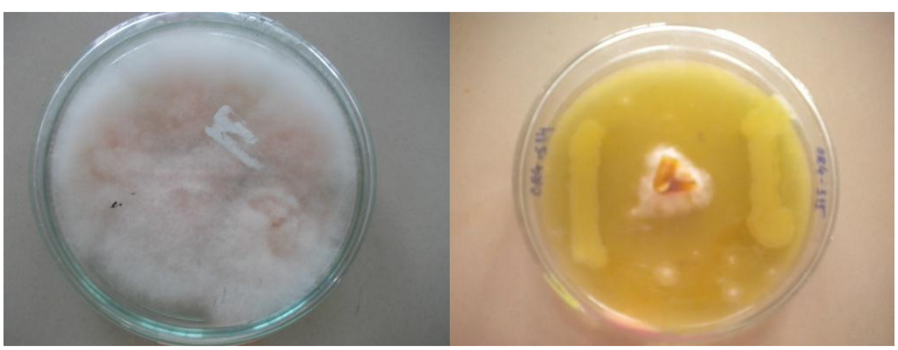


Fig.9 seedling growth along with Control

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