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Morpho-cultural and biochemical identification of *Pseudomonas* sp. isolated from the rhizosphere of different vegetable crops and study its efficacy on *Solanum melongena* (Brinjal)

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Abstract
In the present study, seventeen bacterial isolates isolated from the rhizosphere of vegetable crops from different location of Jammu region. Out of seventeen isolates, seven isolates were identified to be *Pseudomonas* sp on the basis of their cultural, morphological and biochemical characters. To evaluate the efficacy of these isolates in respect to their plant growth promoting activity, the isolates were inoculated by seedling dip method and the result showed the significant increase in root and shoot length, root fresh and dry weight and shoot fresh and dry weight of brinjal plant in all the treatments as compared to control. Among the treatments, the plants inoculated with isolate 15 (I15) showed the maximum plant growth promoting activity followed by the isolate I-14. The screened isolates can thus be advantageously used in organic farming of brinjal.

Keywords: PGPR, Pseudomonas, Brinjal, rhizosphere

1. Introduction
Plant Growth Promoting Rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Kloepper*et al*., 1980; Glick, 1995) [12]. PGPR, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host (Gardner *et al*., 1984; Moeinzadeh *et al*., 2010) [25]. The direct mechanisms involve nitrogen fixation, phosphorus solubilization, Hydrogen cyanide, production of phytohormones such as auxins, cytokinins and gibberellins, and lowering of ethylene concentration (Ali *et al*., 2010; Hadad*et al*., 2010) [2]. Among the PGPR, *Pseudomonas* species stand out because of high level of genetic variability and competitiveness in soil (Appanna, 1997) [3]. The most effective strains of *Pseudomonas* have been *fluorescent Pseudomonas* spp which are characterized by their production of yellow green pigments, termed pyoverdines or pseudobactins, that fluorescence under UV irradiation and function as siderophore (Abdallah, 1991).
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Materials and Method
Collection of soil sample
Soil sample were taken from the rhizosphere (approximately 15cm deep) of different vegetable crops (brinjal, tomato, potato, pea and ladyfinger) from five locations *viz*., Chatha, R.S Pura, Vijaypur, Ramgarh and Supwal of Jammu region. The samples were mixed and air dried for further isolation of bacteria.

Isolation of bacteria
Isolation was done by serial dilution method. For isolation of *Pseudomonas* sp. King's B medium was used. 0.1ml of suspension from dilutions (10^{-5} to 10^{-8}) was added in sterilized petriplates containing 20ml of sterilized King's B medium. The plates were incubated at 28±2 ^{0}C for 48 hr and well separated individual colonies with yellow green and blue white pigments were marked after observing the plates under UV light.

Identification of rhizobacterial isolates

Morphological and colony characterization

The bacterial isolates obtained from the plant rhizosphere were examined for their Gram reaction, colony morphology, fluorescence and cell shape (Garrity*et al*., 2005). Microscopic observations in oil emersion were recorded on the basis of their shape, size, color, opacity and mucosity. Colony characterization was based on their shape, size, color, opacity and mucosity. Individual bacterial isolates were streaked on the media plates and incubated at 28±2 °C for 4 to 5days to record colony characteristics

Identification of selected isolates based on biochemical characterization

Different biochemical test viz, Catalase test, Starch hydrolysis, Triple sugar iron agar (TSIA), Indole production, Hydrogen sulphide production, Methyl red test (MR test) and Voges-Proskauer test (VP Test) were conducted to identify the selected isolates as described in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) [14].

Antibiotic sensitivity test

Antibiotic sensitivity was observed against three different antibiotic discs of Ciprofloxcin, Gentamicin and Ceftriaxone. The plates were incubated at 28±2 °C for 48 hours and were observed for the formation of zone of inhibition. Zone inhibition was indication of sensitivity of rhizobacterial isolates against the antibiotics tested.

Pot Experiment

A pot experiment was conducted to assess the growth promoting potential of selected *Pseudomonas fluorescens* isolates. The soil was mixed with FYM in 2:1 ratio and it was sterilized with formaline by thorough mixing followed by covering with polyethylene sheet for one week. After one week, soil was spread to release the residues of formaline and then filled in the pots. Selected *Pseudomonas* isolates grown in nutrient broth for 48hrs with a bacterial population maintained at 1×10^8colony forming unit (cfu) per ml, then the roots of seedling were dipped in that concentration for 15-20 minutes and then transplanted in pots. The experiment was conducted under Completely Randomized Design with three replications of each selected isolates and control. After thirty-days radial root length, shoot length, root and shoot fresh weight and root and shoot dry weight was recorded.

Results and Discussion

Morphological characterization

The seventeen isolates were isolated from the rhizosphere of different vegetable crops and all the seventeen isolates of *Pseudomonas* sp. grown on nutrient agar medium (NA) and incubated at 28±2 ºC to study various morphological characters. The growth in all the isolates initiated after 24 hours of incubation**.** Isolate I-2, I-3, I-6, I-7, I-10, I-14 and I-15 showed light green, circular, shining, slimy, irregular characteristics and isolate I-1 and I-12 were yellowish in colour, circular and regular whereas I-4 and I-5 were greenish, slimy and regular and isolate I-8, I-9, I-11, I-13, I-16 and I-17 were whitish creamy, slimy and regular (Table 1). Gram staining showed that these isolates were Gram negative bacteria and microscopic observation showed the out of seventeen isolates, seven were long rods whereas ten were short rods (Table 1). The colonies showed fluorescence on King's B Agar medium under ultraviolet light.

Table 1: Cultural characteristics of rhizobacterial isolates

Isolate	Colony Characteristics	Microscopic Observation
I-1	Yellowish green, circular, regular	Gram –ve, long rods
I-2	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-3	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-4	Greenish, creamy, slimy	Gram –ve, short rods
I-5	Greenish, regular	Gram –ve, long rods
I-6	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-7	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-8	Whitish, creamy, circular	Gram –ve, long rods
I-9	Creamy, slimy, regular	Gram –ve, short rods
I-10	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-11	Whitish, creamy, circular	Gram –ve, long rods
I-12	Yellowish green, circular, regular	Gram –ve, long rods
I-13	Creamy, slimy, circular, regular	Gram –ve, short rods
I-14	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-15	Light green, circular, shining, slimy, irregular	Gram –ve, short rods
I-16	Regular, slimy, spreading	Gram –ve, long rods
I-17	Circular, creamy slimy	Gram –ve, long rods

Biochemical characterization

The biochemical characterization of rhizobacterial isolates was done for identification of bacteria shown in Table-2. Catalase test, starch hydrolysis, MR-VP test, IAA Production, TSIA test, hydrogen sulphide and antibiotic sensitivity test was conducted. Out of seventeen isolates seven isolates (I-2, I-3, I-6, I-7, I-10, I-14 and I-15) showed positive for catalase, and starch hydrolysis test whereas negative for MR-VP and hydrogen sulphide. I-1, I-4, I-5, I-8, I-9, I-11, I-12, I-13, I-16 and I-17 showed positive MR-VP and hydrogen sulphide whereas negative for catalase and starch hydrolysis. All isolates showed negative result for IAA production. Out of seventeen only I-15 showed positive for triple sugar iron agar test (Plate 1). For Antibiotic Sensitivity test all isolates were sensitive against antibiotics used *viz*., Ciprofloxcin, Gentamicin and Ceftriaxone. Formation of zone of inhibition was observed around the antibiotic used (Plate-2). The largest zone of inhibition was observed in ciprofloxcin whereas smallest zone of inhibition was observed in ceftriaxone (Table-3).

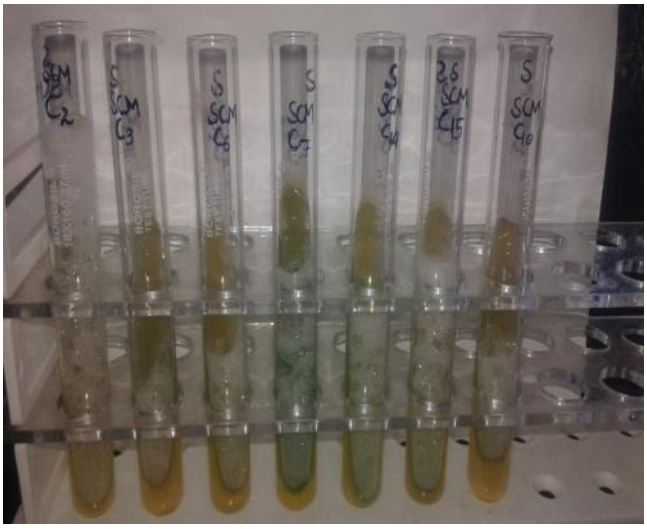
Table 2: Biochemical characteristic of rhizobacterial isolates

S. No.	Catalase Test	Starch hydrolysis	MR test	VP test	TSIA Test	IAA production	H_2S Production
I- 1	_	_	+	+	_	_	+
I- 2	+	+	_	_	_	_	_
I- 3	+	+	_	_	_	_	_
I- 4	_	_	+	+	_	_	+
I-5	_	_	+	+	_	_	_
I- 6	+	+	_	_	_	_	_
I- 7	+	+	_	_	_	_	_

I- 8	_	_	+	+	_	_	+
I-9	_	_	+	_	_	_	+
I-10	+	+	_	_	_	_	_
I- 11	_	_	_	+	_	_	+
I-12	_	_	+	_	_	_	+
I- 13	_	_	_	+	_	_	_
I- 14	+	+	_	_	_	_	_
I- 15	+	+	_	_	+	_	_
I-16	_	_	+	+	_	_	+
I-17	_	_	_	_	_	_	_

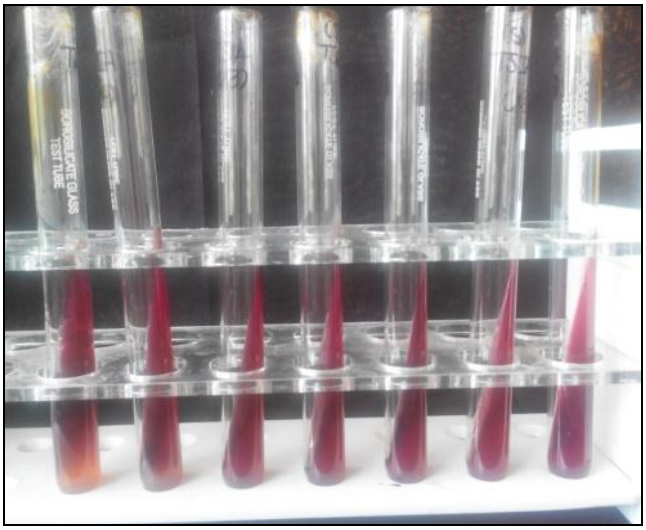
Table 3: Antibiotic sensitivity test for rhizobacterial isolates

S. No.	Antibiotic	Observation	Result	Zone of Inhibition in (mm)
1	Ciprofloxcin	Formation of zone of inhibition was observed	Sensitive	22
2	Gentamicin	Formation of zone of inhibition was observed	Sensitive	17
3	Ceftriaxone	Formation of zone of inhibition was observed	Sensitive	11



a) Catalase Test for Bacterial Isolates

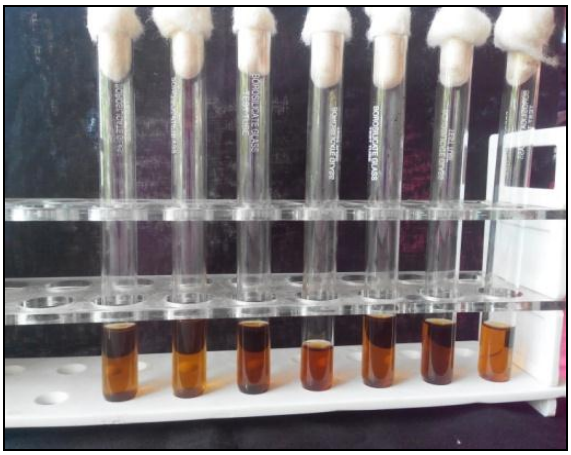
c) Indole Production Test



b) TSIA for Bacterial Isolates

d) Hydrogen sulphide test

e) Starch Hydrolysis Test



f) MR-VP test

Plate 4: Different Biochemical Test Conducted On Bacterial Isolates

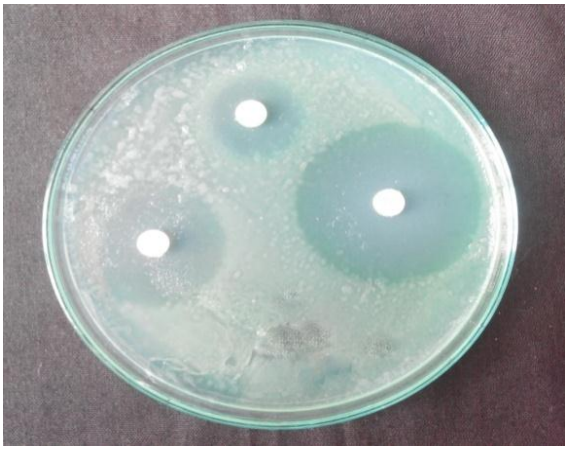
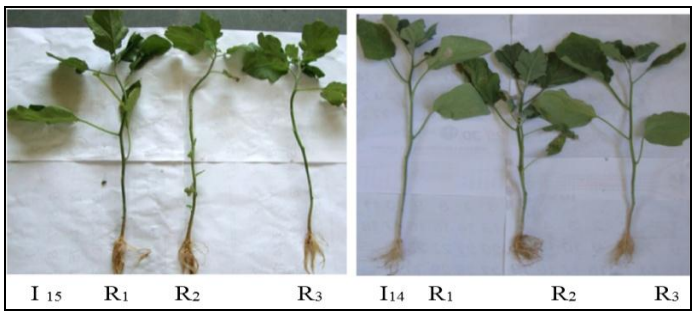


Plate-5: Antibiotic Sensitivity Test for Bacterial Isolates
Evaluation of *Pseudomonas fluorescens* isolates for gowth promoting traits in brinjal

On the basis of morphological and biochemical characterization seven isolates of *Pseudomonas* sp. (I-2, I-3, I-6, I-7, I-10, I-14 and I-15) were selected for the further evaluation of their growth promoting traits in brinjal. The isolates were inoculated in the brinjal plant by seedling dip method and the various parameters such as root length, shoot length, fresh root and shoot weight and dry root and shoot weight were recorded. All the seven isolates significantly showed plant growth promoting activity by increasing root and shoot length, root fresh and dry weight and shoot fresh and dry weight as compared to the control (Plate-2) but among the treatments I-15 showed the highest root length (18.52g±0.31), shoot length (45.03g±0.65), root fresh weight (3.50g±0.36), root dry weight (1.58g±0.11), shoot fresh weight (45.73±0.34) and shoot dry weight (8.64g±0.28)followed by I-14 whereas minimum root length(11.73g±0.34), shoot length (36.06±0.67), root fresh weight (1.71g±0.12), root dry weight (0.78g±0.03), shoot fresh weight (35.73g±0.34) and shoot dry weight (5.69g±0.04) was observed in I-6 (Table4 and Fig.1)

Statistical Analysis:
The results were analyzed at 5% level of significance using OPSTAT software.



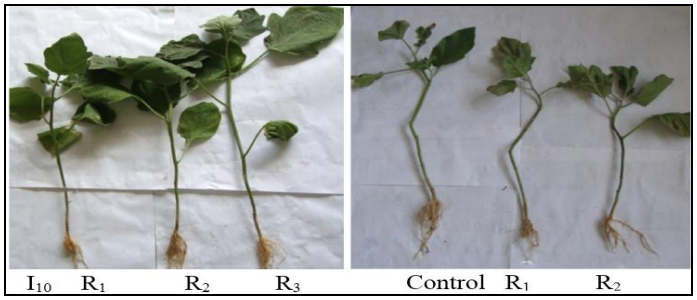


Plate 6: Efficacy of *Pseudomonas* in Brinjal plant

Table 4: Effect of *Pseudomonas* sp. on root and shoot length, root and shoot fresh weight & root and shoot dry weight

Treatments	Root length (cm)	Shootlength (cm)	Root fresh weight (g)	Root dry weigth (g)	Shoot fresh weight (g)	Shoot dry weight (g)
I-2	11.93±0.61	36.10±0.62	1.97±0.17	0.94±0.09	36.83±0.36	5.69±0.04
I-3	13.13±0.61	38.23±0.53	1.87±0.13	1.02±0.08	38.83±0.38	5.88±0.03
I-6	11.73±0.34	36.06±0.67	1.71±0.12	0.78±0.03	35.73±0.34	5.69±0.04
I-7	14.63±0.34	38.60±0.30	1.77±0.13	1.04±0.09	39.73±0.34	5.97±0.03
I-10	15.80±0.32	38.13±0.61	2.50±0.36	1.25±0.11	42.60±0.45	6.52±0.36
I-14	17.73±0.34	43.06±0.64	2.67±0.30	1.41±0.12	44.83±0.44	7.51±0.36
I-15	18.52±0.31	45.03±0.65	3.50±0.36	1.58±0.11	45.73±0.34	8.64±0.28
C.D(0.05)	1.28	1.81	0.71	0.17	1.10	0.63

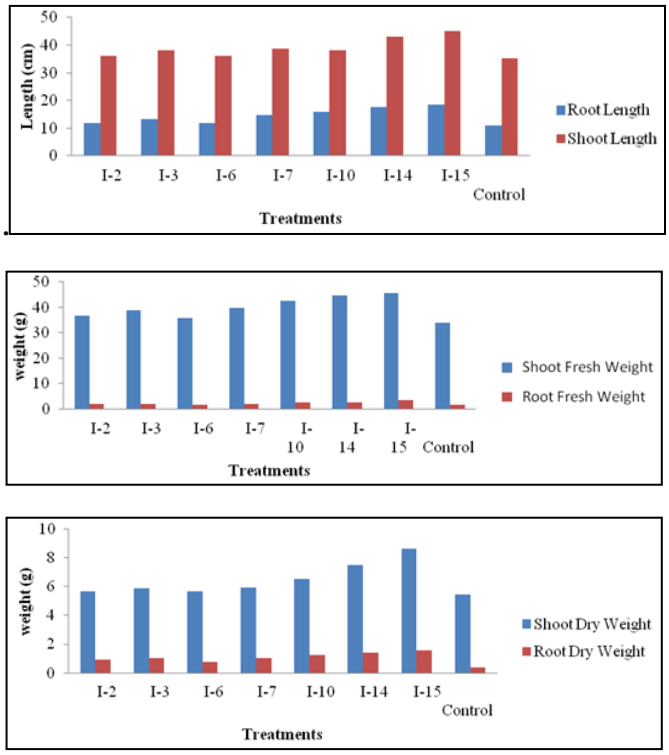


Fig 1: Effect of *Pseudomonas* sp. on growth parameters of *Solanum melongena*

Discussion

In the present study, plant growth promoting traits of *Pseudomonas* sp. isolates were studied on brinjal plants. Seventeen isolates were obtained from different vegetables crops (brinjal, tomato, potato, pea and ladyfinger), the isolates were purified by streak plate method and studied morphologically and microscopically. Morphological characteristics of seventeen isolates showed variations among the isolates collected from various locations of Jammu. All the isolates were Gram negative though vary in colony, colour and shape. A variation in the colony colour may be attribute to the production of different pigments metabolites. In the genus *Pseudomonas*, different species are known to produce various kinds of pigments, the allocation of which in the genus is uncertain (Palleroni *et al*., 1970). Majority of bacterial isolates showed fluorescence under UV light on King's B medium. These characteristics were regarded as taxonomically useful characteristics for *Pseudomonas* (Palleroni, 1983; Kremer *et al*., 1990 [20]; Cartwright and Benson, 1985). According to Stolp and Gadkari (1983) [37], colony color of *P. fluorescens* was depended on kind of media. Fluorescent color was initial color for *P. fluorescens* according to Raaijmakers and Weller (1998) [32] and Kremer *et al*., 1990 [20]. Microscopic study revealed that out of seventeen, seven were short rods and ten were long rods. Ayers and Papavizas (1963) [4] mentioned that pigmented rhizobacteria that were gram-negative rods belonged to *P. fluorescens.*

The rhizobacterial isolates were further tested for their biochemical characteristics *viz*., Catalase test, Starch hydrolysis, Hydrogen sulphide production, Indole Acetic Acid production, Triple sugar iron agar test and MR-VP test. These biochemical tests confirmed that out of seventeen isolates, seven isolates were *Pseudomonas fluorescens* as reported by earlier workers (Karkalas, 1985 [15], Nathan *et al*., 2011 [26], Meera and Balabaskar, 2012) [24].

The rhizobacterial isolates were also test against three different antibiotics *viz*., Ciprofloxcin, Gentamicin and Ceftriaxone. All isolates were sensitive against antibiotics used, formation of zone of inhibition was observed. Similar results were reported by Endimiani *et al*., 2002 [9]; Lambert, 2002; Shahcheraghi *et al*., 2003 [35]. The largest zone was observed in Ciprofloxacin which is 22 mm. Ciprofloxcin is a commonly used antibiotic in clinical practice (Chaudhry *et al*., 1999) [7]. It is a broad-spectrum inhibits enzyme deoxyribonucleic acid (DNA) gyrase needed for replication of DNA. It has fluoroquinolone with coverage against gram-positive and gram-negative organisms, including *P. fluorescens*, *P. aeruginosa* and many other microorganisms (Chaudhry *et al*., 1998 [7]; Synder and Kartz, 1992 [38]; Knauf *et al*., 1996 [19]; Kunimoto *et al*., 1999) [21]. Gentamicin has aminoglycoside which inhibits protein synthesis and Ceftriaxone contain cephalosporin that inhibits cell wall synthesis. Ciprofloxcin directly inhibits DNA synthesis that is why it is more efficient than Gentamicin and Ceftriaxone.

After morphological and biochemical study seven isolates of *Pseudomonas* sp viz., I-2, I-3, I-6, I-7, I-10, I-14 and I-15 were used for pot experiment and various plant growth parameters were recorded. All the treatments showed good plant growth promoting activity as compared to control. Among the treatments, Isolate (I-15) showed maximum root and shoot length (18.52cm and 45.03cm), followed by isolate I-14 with (17.73cm and 43.06cm). Meera and Balabasker (2012) reported similar result that *Pseudomonas fluorescens* showed maximum germination per cent, increase in the shoot length; root length and vigour index. Saravana *et al*. (2013) [34] also showed that inoculation with fluorescent *Pseudomonas* induced a significant increase in root and shoot length over the uninoculated control. *Pseudomonas fluorescens* has been shown to increase seed germination, root and shoot length, and seedling vigour in several instances (Ramamoorthy *et al*., 2001 [33]; Khalid *et al*., 2004 [17]; Egamberdieva, 2008) [8]. *Pseudomonas* spp. was reported to produce amino acids, salicylic acid and IAA (Sivamani and Gnanamanickam, 1988 [36]; O'Sullivan and O'Gara, 1992) which might have improved the plant growth and seedling vigour. Production of indole acetic acid by the strains of *Pseudomonas* spp. responsible for increasing root elongation was also reported (O' Dowling and O' Gara, 1994). Kaushal

et al. (2013) [13] reported that increased in root and shoot length in rice plant as compared to control might be due to *P. fluorescens* because of their plant growth promoting activity.

Conclusion

Studies concluded that seven isolates of *Pseudomonas* sp isolated from the rhizosphere of different vegetable crops showed good plant growth promoting activities. So, these isolates can be exploited for eco-friendly cultivation of brinjal crop

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