Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2013, 4(1):269-272

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Isolation and characterization of heavy metal Resistant *Pseudomonas spp.* and their plant growth promoting activities

Yogendra Singh, P.W. Ramteke[#] and Pradeep K. Shukla[#]

Department of Microbiology and Fermentation Technology, SHIATS, Allahabad
[#] Department of Biological Sciences, SHIATS, Allahabad

ABSTRACT

Interactions between plants and microorganisms in the rhizosphere (rhizobacteria) can clearly affect crop yields. Rhizobacteria that benefit plant growth and development are called PGPR. the most studied PGPR belong to gram-negative genera, and the greatest number of strains are members of the fluorescent pseudomonads in our present investigation was to study the plant growth promoting (PGP) activities and heavy metal resistant Pseudomonas spp. from wheat & pigeon pea fields of Allahabad district. From nine different rhizospheric soil of wheat & pigeon pea different Pseudomonas spp. were isolated. Among the 21, four Pseudomonas spp. In four different Pseudomonas spp isolates (YSY-13, YSY-15,YSY-17 and YSY-19) exhibited maximum plant growth promoting and heavy metal tolerant activities The isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Further, rhizobacteria tolerant to multiple heavy metals exhibited a couple of PGP activities.

Keywords: *Pseudomonas*, Heavy metal, Plant Growth Promoting Activities, Rhizospheric

---

INTRODUCTION

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria [1].known as plant growth promoting rhizobacteria (PGPR) [2].PGPR can stimulate plant growth indirectly by inhibiting other deleterious microbes or root pathogens [3,4]. For example fluorescent pseudomonads can influence biological control of root crop diseases, by modulating competitiveness, production of antibiotics, siderophores or HCN [5].The interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals,making the microorganisms an important component of phytoremediation technology [6,7,8]. [9].demonstrated that the exposure of *P. fluorescencs* ATCC 948 to three different heavy metals separately resulted, in differential expression of cellular proteins, but also in disappearance of the fluorescence, the basic characteristic shown by the fluorescent *Pseudomonas*, indicating the action of the metals on global regulatory components, controlling the siderophore molecules imparting this bacterial quality. Although many soil bacteria are tolerant to heavy metals and play important roles in mobilization or immobilization of heavy metals [10].Thegood results obtained *in vitro* cannot always be dependably reproduced under field conditions [11,12]. The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plant. The environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil.

MATERIALS AND METHODS

Collection of Sample

Nine different rhizospheric soil samples were collected from wheat & pigeon pea field grown in Allahabad district of Uttar Pradesh. The sample was collected in 1cm depth and it was packed in a sterile polythene bag and labeled properly.

Isolation of *Pseudomonas sp.* Isolates [13].

The isolation of *Pseudomonas spp*. from soil samples, 1g of soil sample was serially diluted in sterile distilled water, 0.1 ml of soil suspension from 10^{-1} to 10^{-5} was spreaded on the King's B medium agar plate. plates were incubated at 35^0C for 2-4 days in inverted position.

Identification of *Pseudomonas spp.*

The bacterial isolates were identified by using cultural, morphological and biochemical characteristics features described in Bergey's manual of determinative bacteriology [**14].** and stored at 4°C on slants and maintained through sub-culturing. The isolates were characterized by Gram staining, motility test, Methyl Red, Voges Proskauer, Citrate, oxidase test, catalase test, H_2S production and starch hydrolysis as per the standard methods [15].

In vitro* Screening of Multiple Plant Growth Promoting Activities of *Pseudomonas sp.

Production of Indole acetic acid

Indole acetic acid (IAA) production was detected as described by [**16].** Bacterial cultures were grown for four *Pseudomonas spp*. on their respective media at 36±2 °C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production.

Production of HCN and catalase

All the isolates were screened for the production of hydrogen cyanide by adapting the method of [17]. Briefly, nutrient broth was amended with 4.4 g glycine/l and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 36±2 °C for 4 days. Development of orange to red colour indicated HCN production. Bacterial cultures were grown in a nutrient agar medium for 18-24 h at 36±2 °C. The cultures were mixed with appropriate amount of H_2O_2 on a glass slide to observe the evolution of oxygen.

Phosphate Solubilization

Phosphate Solubilization Bacterial isolates were evaluated from the ability to solubilize inorganic phosphate. Pikovskaya's agar medium (HiMedia, Mumbai) containing calcium phosphate as the inorganic form of phosphate was used in this assay. A loopful of bacterial culture were placed on the plates and kept for incubation at 28°C for 7 days. The presence of clear zone around the bacterial colonies indicates the solubilization of phosphate.

Heavy Metal Tolerance

The selected bacterial strains were tested for their resistance to heavy metals by agar dilution method [18].Freshly prepared agar plates were amended with various soluble heavy metal salts namely Cr, Pb, Hg, Cd, Zn, Co and Cu, at various concentrations ranging from 25 to 200 µg ml^{-1} were inoculated with overnight grown cultures. Heavy metal tolerance was determined by the appearance of bacterial growth after incubating the plates at room temperature for 24-48h.

RESULTS AND DISCUSSION

Rhizosphere soil samples from Wheat & Pigeon pea plants grown in different sites situated at in Allahabad district of Uttar Pradesh, were collected and used for the isolation of *Pseudomonas spp*. using specific media. The attempts yielded 21 *Pseudomonas* isolates. Among the 21 *Pseudomonas spp*. 4 species of *Pseudomonas spp*. were exhibited efficient plant growth promoting activities by screening methods.

The isolates were identified based on morphological and biochemical characteristics and were tested for their beneficial traits like ability to solubilization of insoluble inorganic phosphate and production of plant growth promoting substances. Efficient isolates selected based on the above characters were examined for their *in vitro* screening methods. The results obtained on these aspects are presented as follows.

Isolation and Identification of *Pseudomonas spp.*

On the basis of cultural, morphological and biochemical characteristics a total of 21 *Pseudomonas* isolates were identified from nine rhizospheric soil samples as described in Bergey's Manual of Determinative Bacteriology [**19].**Among the 21 isolates, 4 (YSY-13, YSY-15, YSY-17 and YSY-19) were selected for further studies based on the efficiency of multiple plant growth promoting activities.

General Characterstics of the test isolates are illustrated in Table 1.

Table 1. Morphological and cultural characteristics of *pseudomonas* isolates from the rhizospheric soil of wheat & pigeon pea.

Biochemical and cultural characterization	*Pseudomonas* species
Number of isolates	Four
Gram staining	Negative
Motility	Motile
Methyl Red	Negative
Voges Proskauer	Negative
Citrate	Positive
Oxidase	Positive
H_2S production	Negative
Catalase test	Positive
Starch hydrolysis	Negative

Table 2. Multiple plant growth promoting activities of *Pseudomonas* isolates from the rhizospheric soil of wheat & pigeon pea..

Pseudomonas isolates	IAA	NH_3	HCN	PO^-_4 Solubilization
YSY-13	+	-	+	-
YSY-15	+	+	-	-
YSY-17	+	+	+	-
YSY-19	+	-	+	-

Multiple Plant Growth Promoting Characteristics of Test Isolates.

Screening results of PGP traits are depicted in Table 2. IAA production was shown in all the isolates of *Pseudomonas* (100%) were showing Positive PGP activities in relation to indole acetic acid (IAA) Ammonia production was detected in (50%) of isolates of *Pseudomonas.* and hydrogen cyanide was detected in (75%) of test isolates. None of the test isolates of *Pseudomonas spp*. produced phosphate.

Table 3. Heavy metal tolerance among *Pseudomonas spp* isolates from Rhizospheric soil of wheat & pigeon pea fields.

HEAVY METAL TOLERANCE (µg/ml $^{-1}$)							
***Pseudomonas spp* isolates**	**Cr**	**Pb**	**Hg**	**Cd**	**Zn**	**Co**	**Cu**
YSY-13	100	200	100	100	100	100	200
YSY-15	200	100	50	100	50	50	100
YSY-17	200	100	100	100	50	100	100
YSY-19	100	200	50	200	200	100	25

Screening results of multiple Heavy metal showed tolerance among *Pseudomonas spp* isolates from Rhizospheric soil of wheat & pigeon pea fields. Among the 21 isolates of four isolates, *Pseudomonas spp* isolate (YSY-19) showed tolerance in all concentrations (25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml) . whileas (Table 3). (YSY-13, YSY-15,YSY-17) isolates showed tolerance in (50µg/ml, 100µg/ml, 200µg/ml).

Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals.[20]. found that by decreasing the heavy metal toxicity, PGPR increases plant growth. The selection of microorganisms both metal tolerant and efficient in producing PGPR compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils. In addition to these traits, plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil. Unfortunately, the interaction between associative PGPR and plants can be unstable. The good results obtained *in vitro* cannot always be dependably reproduced under field conditions. The ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities [21, 22].. Production of IAA by *Pseudomonas* is a general characteristic of our test isolates Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia. 50 % of the isolates were able to produce ammonia. Many different bacteria could produce HCN which is toxic for fungi [23].HCN production by *P.fluoresens*, *P.aeruginosa* and *Chromobacterium uiolaceum* was reported by many researchers [24].Most of the *Pseudomonas spp*. isolated (YSY-13, YSY-17 and YSY-19) from soils of

Rhizospheric soil of wheat & pigeon pea are produced HCN as potent antifungal agent. All the test bacterial isolates in the present study were able to produce catalase. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stress.

Acknowledgements
We are thankful to the Department of Biological Sciences of the SHIATS, Allahabad, for providing all facilities for conducting the research work.

REFRENCES

[1]Villacieros M, Power B, Sanchez-Contreras M, Loret J, Oruzebal RT, Martin M, Franandez-Pinas F, Bouile I, Whelan C, Dowling DN, Rivilla R, *Plant Soil*.,**2003** 251:47-54.
[2] Kloepper JW, Leong J, Teintze M, Schroth MN, *Nature*., **1980** 268:885-886.
[3] Lemanceau P, *Agronomie*.,**1992**, 12: 413–437.
[4] Kloepper JW, *Dekker*, New York, pp **1993**, 255–274.
[5] O'Sullivan DJ, O'Gara F, *Microbiol*. Rev.**1992**, 56,662,676.
[6] Wenzel WW, Lombi E, Adriano DC, Springer, Heidelberg, Berlin, New York,**1999,** Pp. 273-303.
[7] Glick BR, *Biotech. Adv*.,**2003,** 21, 5, 383,393 (11 pages).
[8]Nouri J, Lorestani B, Yousefi N, Khorasani N, Hasani AH, Seif S, Cheraghi M, *Environ. Earth Sci*.,**2011,** 62 (3), 639-644 (6 pages).
[9]Sharma S, sundaram CS, luthra PM, Singh Y, R. Sirdeshmukh YR, gade WN, *J Biotechnol.*, **2006**, 126, p 374–382.
[10]Gadd GM, *Experientia*,**1990,** 46 (8), 834–840 (7 pages).
[11]Chanway CP, Holl,FB, Can. *J. Microbiol*.**1993,** 39, 1084–1088.
[12]Zhender GW, Yao C, Murphy JF, Sikora ER, Kloepper JW, Schuster DJ, Polston JE, **1999**, *Biochemistry, Ecology and Agriculture*. APS Press, St Paul, MN, p. 33.
[13] King EO, Ward MK, Randey DE, *J. Lab. and Clin. Med***.1954,** 44: 301-307
[14]Holt JG, Krieg NR, Sneath PHA, J.T. Staley and S.T. Williams, In: **1994,** Bergy's Manual of Determinative Bacteriology, 9th ed., Williams and Wilkins Pub., MD: USA.
[15] Cappuccino JC, Sherman N, In: Microbiology: A Laboratory Manual, New York, **1992,** 125,179.
[16]Brick JM, Bostock RM, Silverstone SE, *Appl. Environ. Microbiol*.,**1991**, 57 **:** 535–538.
[17] Lorck H, *Plant Physiol*.,**1948,** 1, 142 -146.
[18] Cervantes C, Chavez J, Cardova NA, De Na Mora P, Velasco JA, *Microbiol*, **1986**, 48,159- 163.
[20]Burd JM, Bostock RM, Silverstone SE, *Appl. Environ. Micribiol.* **2004**, 64, 3663-3668.
[21]Arshad M Frankenberger WT, *Jr. Microbial production of plant growth regulators*. Marcel and Dekker, New York, pp.**1993**, 307–347.
[22]Glick BR, *Can. J. Microbiol*.,**1995**, 41, 109–114.
[23] Blumer C, & Hass D, *Microbiol*,**2000,** 173 3: 170-177.
[24] Siddiqui IA, Shaukat SS, Khan GH, & Ali NI, *Soil.Biol.Biochem*,**2003,** 35:1625-1634.