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Isolation and Molecular Characterization of Abiotic Stress Tolerant Plant Growth Promoting *Pseudomonas* spp. from Different Rhizospheric Soils of Telangana State, India

K. Damodarachari[1*], R. Subhash Reddy[1], S. Triveni[1], CH. V. Durga Rani[2] and M. Sreedhar[3]

[1]Dept. of Agricultural Microbiology & Bioenergy, College of Agriculture, [2]Dept. of Molecular Biology & Biotechnology, Institute of Biotechnology, [3]Quality Control Laboratory, Professor JayashankarTelangana State Agricultural University, Rajendra nagar, Hyderabad, Telengana State (500 030), India

Corresponding Author

K. Damodarachari
e-mail: damuagmicro2012@gmail.com

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Abstract

The present investigation was carried out to isolate rhizobacteria from different rhizospheric soils of Telangana state, India and screened for plant growth promoting properties such as mineral nutrient solubilization (P, K, Zn), IAA production, ACC deaminase, EPS production, biocontrol activity and tolerance to different abiotic stresses such as pH, temperature, salt, drought and heavy metals *in vitro* conditions. Based on cultural, morphological and biochemical characterization, it was found that forty four were of *Pseudomonas* spp. Among the forty four (44) *Pseudomonas* isolates, twenty eight (28) isolates were showing plant growth promoting properties. The results of different *in vitro* abiotic stress tolerance of *Pseudomonas* isolates, four isolates were showed growth at pH range from 4-10 (PS 1, PS 4, PS 26, PS 40). Five isolates were showed tolerance to 1.5 to 20% of NaCl concentration (PS 4, PS 15, eight isolates were showed tolerance from 1.5 to 15% of NaCl concentration (PS 1, PS 11, PS 18, PS 26, PS 30, PS 31, PS 32, PS 40), three isolates showed tolerance to temperature from 20 °C -50 °C (PS 11, BS 15, BS 40), nine isolates were showed tolerance to temperature from 20 °C -45 °C (PS 1, PS 4, PS 21, PS 23, PS 28, PS 30, PS 32, PS 34, PS 37), three isolates showed tolerance to water potential from -0.05 Mpa to -0.73 Mpa (PS 4, PS 15, BS 34).PS 15 was found efficient in Zn- solubilization with considerable other PGP properties. It was tolerant to pH stress (4, 6), temperature stress, salt stress, drought stress and heavy metals As and Cd only. PS 1 showed good P-solubilization, K-solubilization, Zn solubilization with biocontrol activity. It tolerated pH stress, temperature stress, salt stress, drought stress and tolerant to heavy metal toxicity. Performing environmental parameters for bacterial growth is also showing that bacteria can easy to survive in different environmental condition.

Keywords: *Pseudomonas,* PGPR, pH, temperature, drought, heavy metal tolerance

1. Introduction

Increasing crop productivity and enhancing resistance or tolerance against various stress factors has become major aim for modern agriculture (Farooq et al., 2009). In sustainable agriculture, integrated pest management is considered the most efficient strategy to manage stress causing agents, such strategy rely on combining several approaches including using resistant varieties, crop rotation, monitoring pests, biocontrol and in severe situations employing pesticides in an attempt to keep stress agents under control (Wegulo, 2012). Biological control forms an integral part of the IPM strategy (Landaet al., 2004).

Root zone bacteria that have been found to have beneficial effects on various plants include species of the genera *Arthrobacter, Azotobacter, Azospirillum, Bacillus, Enterobacter, Pseudomonas* and *Serratia* (Gray and Smith, 2005), as well as *Streptomyces* spp. (Tokala et al., 2002; Dimkpa et al., 2008). Although the exact mechanisms of plant growth stimulation remain largely speculative, it is known that they differ between bacterial strains and most certainly depend on the various compounds released by the different microorganisms. The literatureis replete with reports describing the production of the main phytohormone classes–auxins, cytokinins, gibberellins, abscisic acid and ethylene by plant growth promoting rhizobacteria (PGPR). The subject of PGPR elicited tolerance to abiotic stresses has been reviewed (Venkateswarlu et al., 2008). It has been shown that certain PGPR enhance plant stress tolerance through 1-aminocyclopropane-1-carboxylate deaminase and provide significant protection to a wide range of plant species from the damage caused by various abiotic stress conditions. ACC breakdown and ethylene synthesis inhibition by ACCdeaminase decreases the damage of various stress situations by enhancing homeostasis in and around the plant root, especially at early stages of stress exposure (Ali et al., 2009).

Increased incidence of abiotic and biotic stresses has become major cause for stagnation of productivity in principal crops. Besides high temperature, droughts, elevated CO_2, extreme rainfall events, more floods, cold waves, heat waves, and cyclones are the other important natural disasters that cause serious economic losses, are likely to be witnessed as a result of global warming. These factor sare likely to cause serious negative impact on crop growth and yields and impose severe pressure on our land and water resources (Grover et al., 2011). Exopolysaccharides producing plant growth- promoting rhizobacteria can also bind cations including Na^+. Therefore, an increase in the population density of EPS producing bacteria in the root zone is expected to decrease the content of Na^+ available for plant uptake, and thereby alleviate salt stress in plants growing in saline environments (Alamiet al., 2000). The present investigation is on the isolation of abiotic stress tolerant plant growth promoting rhizobacterial isolates followed by *in vitro* screening for tolerance to high pH, temperature, salt conditions, osmotic stress, metal toxicity etc. and molecular identification of few efficient isolates. Such PGPR will be helpful for efficient of management of abiotic stress in crop production.

High saline soils deprive plants of water and sodic soils affect nutrient availability. No reports are available on the diversity of Pseudomonas spp. occurring in such stressed soils of India. Presence of fluorescent pseudomonads is ubiquitous and some reports are available on their occurrence in stressed environment like saline, sodic and semi arid soils (Djedidi et al., 2011; Egamberdieva, 2011). *Pseudomonas fluorescens* can produce a wide range of enzymes and metabolites that help plants withstand varied biotic and abiotic stresses (Saravanakumar et al., 2011). In India, commercial preparations of *P. fluorescens* is widely used for disease management in pulses, rice and vegetables. Presently *P. fluorescens, P. putida*. *P. chloraphis, P. aureofacians* and other species are widely used in agriculture as they play a crucial role in soil health and plant development (Weller et al., 2007).

2. Materials and Methods

2.1. Soil samples, Bacterial isolation, and culture media

The present experiment was carried out during 2016-17 at department of Agricultural microbiology and bioenergy, college of Agriculture, PJTSAU, Rhizospheric soils were collected from different places of Telangana such as normal soils, salt affected, drought soils. The details of soil samples were presented in Table 1. Forty four strains of *pseudomonas* were isolated from these soils. The strains were coded as PS1 to PS-44.For isolation of rhizobacteria, the method proposed by Vlassak et al. (1992) was followed. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. 0.1 ml of respective dilutions was spread on sterilized Petri plates containing specific media i.e.nutrient agar. The bacterial isolates were identified on the basis of morphological, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (Holt and Kreig, 1984).

Table 1: Physico-chemical properties of soil samples

Sampling site		pH	Electrical conductivity (dS m^{-1})	Organic carbon (%)	Heavy metal concentrations (µg ml^{-1})			
					Arsenic	Cadmium	Mercury	Manganese
Rangareddy	Koheda	8.1	0.30	0.40	3.08	3.80	17.00	11.20
	Ibrahimpatnm	8.0	0.41	0.42	3.11	2.80	12.00	9.20
	Choutuppal	7.9	0.50	0.43	3.00	2.00	17.00	12.20
	College farm, PJTSAU	7.9	0.35	0.39	-	-	-	3.21
Mahabubnagar	Kalvakurthy	7.8	0.40	0.41	-	-	-	-
	Bijenpaally	7.2	0.24	0.36	3.08	3.80	-	-
Wanaparthi	Wanaparthi	7.5	0.28	0.42	-	-	-	
	Pebbair	8.0	0.45	0.37	-	-	-	-
Nagarkurnool	Kollapur	7.4	0.24	0.50	-	2.00	-	-
Shamshabad	Maheswaram	7.6	0.30	0.39	3.08	3.80	12.00	12.20
	Kandhukur	7.5	0.26	0.38	3.08	3.80	8.00	-
Yadagirigutta	Bhongir	7.8	0.30	0.43	-	8.00	-	-
	Yadagirigutta	8.0	0.34	0.40	-	-	3.80	-

2.2. Determination of mineral solubilization, IAA production, ACC Deaminase activity, EPS production, Siderophore production and antagonistic activity

Phosphate solubilization activity was determined using Pikovskaya's agar medium containing 0.5% (W/V) $Ca_3(PO_4)_2$

(Pikovskaya, 1948), Potassium solubilization determined using Aleksandrov medium containing 0.2% potassium aluminum silicate (Prajapati and Modi, 2012), Zinc solubilization determined using Tris mineral salt medium containing 0.1% ZnO (Saravanan et al., 2003). IAA production (Duby and Maheswari, 2012), EPS production at stress induced conditions were checked (Ali et al., 2013), bacterial utilization of ACC as sole nitrogen source was screened using qualitative assay(Jacobson et al., 1994). Siderophore production was determined by the Chrome Azurol S plate assay (Schwyn and Neilands, 1987), antagonistic activity was verified by following dual culture technique (Skidmore and Dickinson, 1976)

2.3. Screening for abiotic stress tolerance

2.3.1. Influence of pH

The pH of the culture medium was adjusted to 4, 6, 7, 8, 10 and 12 using sterile using buffers. 5 ml TSB (Trypticase soya broth) culture medium having different pH, and 0.1 ml bacterial suspension (10^8-10^9 cells ml^{-1}) was poured in sterile culture tubes in three replicates for each pH and incubated in shaker incubator at 120 rm^{-1} was measured at 600 nm.

2.3.2. Influence of salt concentration

Screening for salinity tolerance of isolated bacterial isolates by the inducing of different % of NaCl concentrations from 1.5%, 5%, 10%, 15% and 20% were checked.

2.3.3. Influence of temperatures

0.1 ml of bacterial suspension (10^8-10^9 cells ml^{-1}) was poured into the vials containing 5 ml TSB culture medium and culture in incubators at 20 °C, 30 °C, 40 °C, 45 °C and 50 °C in three replicates for each. After 24 hrs of culture, their absorbance was measured at 600 nm.

2.3.4. Influence of drought

Trypticase soya broth (TSB) with different water potentials (−0.05, −0.15, −0.30, −0.49, and −0.73 MPa)was prepared by adding appropriate concentrations of polyethylene glycol (PEG 6000) (Michel and Kaufmann 1973; Sandhya et al., 2009) and was inoculated with 1% of overnight raised bacterial cultures in TSB. Osmotic potential of broth media was measured by osmometer Three replicates of each isolate with each concentration were prepared. After incubation at 28 °C under shaking conditions (120 rpm) for 24 h, growth was estimated by measuring the optical density at 600 nm using a spectrophotometer.

2.3.5. Influence of heavy metals

Freshly prepared agar plates were amended with various soluble heavy metal salts namely As, Cd, Hg and Mn at concentration of 50 and 100 µg ml^{-1} were inoculated with overnight grown cultures. Heavy metal tolerance was determined by appearance of bacterial growth after incubating the plates at room temperature for 24-48 hours.

2.4. Molecular identification of efficient stress tolerance plant growth promoting bacterial isolates

The bacterial strains were identified using standard method of 16S rRNA gene sequencing. DNA template was prepared by picking individual colony of each strain and amplification of 16S rRNA gene was carried out by PCR. PCR amplification of DNA was done using universal primers: 27 F AGAGTTTGATCCTGGCTCAG and 1492 R :TACGGTTACCTTGTTACGACTT. Reaction mixture (25 µL), prepared for full-length 16S rRNA geneamplification was initially denatured at 94 °C for 2 min, followed by 30 cycles consisting of denaturation at 94 °C for 1 min; primer annealing at 52 °C for 1:30 min. and primerextension at 72 °C for 2 min and finally extension at 72 °C for 10 min in a thermocycler. Amplified PCR products of 16 Sribosomal gene were separated on 1% agarose gel in 0.5×TE (Tris-EDTA) buffer containing 2 µL ethidium bromide (20 mg ml^{-1}) and sequencing of their 16S rRNA gene.

3. Results and Discussion

3.1. Growth and colony morphology of isolates

Total forty bacterial isolates took about 48h to establish their growth on King's B agar. All the isolates developed small, medium, smooth and shiny colonies. About twenty isolates showed yellowish green, irregular, spreading, glistening, convex, opaque, viscid colonies. The remaining twenty isolates showed dull white irregular, spreading, glistening, convex, opaque, viscid colonies. Under microscopic studies, these isolates exhibited $gram^{-ve}$ nature with single, isolated, rod shaped cells with no endospores.

Among forty four *Pseudomonas* bacterial isolates, all the isolates showed positive results for gelatin hydrolysis, citrate utilization, oxidase test, catalase test. Nineteen isolates were positive for starch hydrolysis, thirty four isolates were positive for indole production, thirty isolates showed positive for methyl red test, thirty isolates were positive for Vogespraskauer test. Almost all isolates showed positive for H_2S production capability.

3.2. Plant growth promoting properties

Table 2 shows the Plant growth promoting properties of *Pseudomonas* isolates. Among forty four isolates, twenty eight isolates showed maximum PGP properties Among the twenty eight isolates, PS 21 showed highest P-solublization zone (24.00±1.15 mm), followed by PS-3 (23.66±0.33 mm), PS-1 (18.66±0.88 mm). Data on potassium solubilization activity show that out of twenty four isolates, PS 3 showed highest solubilization zone (22.67 mm), followed by, PS 14 (19.66±0.33), PS 35 (18.66±0.33 mm), PS 1 (17.66±0.33 mm). Out of twenty eight isolates IRP-7 showed highest Zinc solubilization zone (27.00±1.73 mm), followed by PS 15 (25.66±0.33 mm), PS 17 (25.00±1.73 mm), PS 32(24.00±1.15 mm), PS 28 (22.66± 0.33 mm).The indole acetic acid production results revealed that, out of twenty eight isolates, PS 20 showed highest IAA production (19.25 ±1.15 µg ml^{-1}), followed by PS 7 (19.23±0.57 µg ml^{-1}), PS 1 (19.20±0.5 µg ml^{-1}), PS 14 (18.70±0.14 µg ml^{-1}). Among twenty eight isolates, fourteen isolates (50%) were

Table 2: Screening of Pseudomonas isolates for plant growth promoting properties

Isolates	P solubilization (mm)	K solubilization (mm)	Zn solublization (mm)	IAA production (µg ml^{-1})	HCN production	ACC deaminase activity	Exo polysaccharides production	Siderophore production	Antagonistic activity (mm)
PS 1	18.66±0.88	17.66±0.33	19.66±0.33	19.20±0.5	++	+++	+++	++	19.66± 2.02
PS 3	23.66±0.33	22.66± 0.88	13.667±0.33	14.00 ±1.15	++	-		++	23.66±0.33
PS 4	14.00±1.52	15.00±0.57	19.00±0.57	18.33±1.15	++	+	+	++	25.00±0.57
PS 5	10.33±0.88	12.66±0.88	14.00±1.15	11.48±0.26	++	-	-	++	24.33±0.33
PS 7	17.33±0.33	8.33±0.88	27.00±1.73	19.23±0.57	++	++	-	++	19.33 ±0.33
PS 8	12.00±1.15	3.33±0.33	3.33±0.33	8.40± 0.30	++	-	++	++	12.66±0.88
PS 10	7.00 ±1.00	15.00±1.00	15.00 ±1.15	16.5±0.57	++	-		++	22.00 ±1.15
PS 11	13.66±0.33	16.00±1.155	8.00±1.52	6.50±0.28	++	+++	++	++	17.66 ±1.45
PS 12	4.00±1.00	1.00±0.00	11.33±0.33	15.97±2.50	++	+	-	++	11.33 ±0.33
PS 14	14.00±0.57	19.66±0.33	8.00± 1.15	18.70±0.14	++			++	18.00 ±1.15
PS 15	4.33±0.33	7.33±0.33	25.66±0.33	4.23±0.00	++	+++	+++	++	6.33 ±1.20
PS 17	14.00±1.73	6.00±0.57	25.00±1.73	5.21±1.15	++	-	-	++	26.00 ±1.15
PS 18	22.00±1.15	15.00±1.732	14.66±0.33	18.20 ±1.15	++	+	-	++	13.00 ±2.30
PS 20	18.33±0.33	4.00±1.00	13.00±1.15	19.25 ±1.15	++	-	-	++	3.33± 0.33
PS 21	24.00±1.15	12.33±0.33	19.66±0.33	15.57±0.21	++	+	+	++	16.00±1.15
PS-23	15.33±1.66	13.00±1.15	3.00±0.57	12.23± 0.57	++	++	+	++	23.33 ±1.45
PS 26	6.33±0.88	3.00±0.57	13.33±0.33	5.36±0.57	++	-	-	++	23.00 ±1.73
PS 28	17.33±0.33	12.66±0.33	22.66±0.33	1.50±0.25	++	-	-	++	12.66±0.33
PS 29	18.00±1.15	3.00±0.57	3.00± 1.00	9.25 ±1.155	++	+	-	++	13.00 ±1.15
PS 30	11.33±0.33	9.33±0.33	18.33±0.33	14.52 ±1.62	++	+++	+++	++	7.66± 0.333
PS 31	8.00±1.00	13.00±1.15	11.00±1.73	13.00±2.51	++	-	-	++	20.00±0.57
PS 32	3.00±0.57	13.66±0.33	24.00±1.15	8.32 ±1.73	++	-	-	++	13.66 ±1.45
PS 33	13.33±0.33	12.00±1.15	12.00±0.00	15.21±0.48	++	-	-	++	10.00±1.52
PS 34	5.00±1.00	7.00±1.00	4.33±0.33	18.30±1.00	++	++	++	++	24.66± 1.33
PS 35	14.33±0.33	18.66±0.33	13.00±2.08	12.26 ±0.57	++	-	-	++	12.66±2.02
PS-37	15.00±0.57	17.33±0.33	16.00±1.15	15.547±0.22	++	+	++	++	26.00±1.15
PS 39	12.66±0.88	6.00±1.732	10.33±0.33	10.89±4.78	++	-	-	++	10.33±2.33
PS 40	12.00±1.15	4.33±0.33	5.00±1.52	13.63±0.18	++	++	+	++	22.33±1.45
SEm±	0.924	0.829	0.928	1.373					1.285
CD (p=0.05)	2.624	2.354	2.636	3.901					3.650

positive for ACC deaminase production by utilization of ACC as the sole nitrogen source. Among fourteen isolates, four isolates showed strong (+++) ACCd production (PS 1, PS 11, PS 15, PS 30). Among the twenty eight isolates, eleven isolates (39 %) positive for EPS production. Among eleven, three isolates showed strong (+++) for EPS production (PS 1, PS 15, PS-30).

Similar results were reported by Fakedu (2013), that *Pseudomonas fluorescens* possessed promising properties which made it better biofertilizer bacteria. Twelve *Pseudomonas fluorescens*were isolated from rhizospheric soil of faba bean and tested for phosphate solubilization. The results were in agreement with the findings of Norkina and Pumpynaskaya (1956) whoisolated two strains of *Bacillus* spp. and *Pseudomonas* from rhizosphere soils different crop plants as mineral potassium soluiblizers. Bapiri et al. (2012) evaluated zinc solubilizing ability of *Pseudomonas fluorescens*

using zinc oxide, zinc carbonate and zinc sulphide in both plate and broth media assays. Zn solubilizing ability of 40 mentioned strains was studied with ZnO and $ZnCO_3$ solutions in broth assay. The soluble zinc and pH were measured after five days. The results showed, that only 8 of 40 strains could form clearing zone in plate assay. Upadhyay and Srivastava (2010) reported that *Pseudomonas fluorescens* strain solubilized phosphorus and synthesized IAA. Renuga (2005) reported that plant growth promoting *Pseudomonas pittida* can utilize 1-aniinocyclopropanc- l-carboxylase as a sole nitrogen source because it possessed the unusual enzyme ACC dcaminase, which hydrolysis ACC to ammonia and a-ketobutyrate. Ali et al. (2013) isolated drought tolerant *Pseudomonas* isolates and these were screened for under both no stressed conditions as well as under minimum water potential (−0.30 MPa). The strain Rdgp10 produced maximum amount of EPS (3.22±0.04 mg mg protein^{-1}) under non-stressed condition, closely followed by isolate SorgP3 (2.85±0.07 mg mg protein^{-1}), sorgP4 (2.75±0.06 mg mg protein^{-1}), SunfP12 (2.71±0.10 mg mg protein^{-1}) and BriP15 (2.18±0.25 mg mg protein^{-1}).

All the isolates showed moderate amount (++) of HCN production. Among twenty eight isolates, eight isolates showed strong (+++) siderophore production (PS 1, PS 3, PS 4, PS 11, PS 20, PS 35, PS 37, PS 40) and remaining twenty isolates showed moderate (++) siderophore production. Antifungal activity of 28 isolates was checked against *Rhizoctonia solani* and under *in vitro* conditions using PDA media. Based on inhibition zone out of 28 isolates, PS 17, PS 37 showed highest inhibition zone with 26.00±1.15 mm, followed by PS 4 (25.00±0.57 mm), PS 34 (24.66±1.33 mm), PS 5(24.33±0.33 mm), PS 3 (23.66±0.33 mm). Siderophores such as pseudobacin and pyoverdin (yellow green fluorescent pigment of *Pseudomonas* bacteria) present high antimicrobial activity and affinity to ions of trivalent iron (Maksimov et al., 2011).

3.3. Stress tolerance of Pseudomonas isolates

Table 2 shows the abiotic stress tolerant selected *Pseudomonas* isolates. The results of different *in vitro* abiotic stress tolerance of *Pseudomonas* isolates, four isolates were showed growth at pH range from 4-10 (PS 1, PS 4, PS 26, PS 40), four isolates showed tolerance to pH range from 4-8 (PS 5,PS 7, PS 17, PS 20), three isolates showed tolerance to pH range from 4-7 (PS 15, PS 34, PS 39), three isolates showed tolerance to pH range from 6-10 (PS 11, PS 18, PS 32), two isolates showed tolerance to pH range from 6-8 (PS 14, PS 29), two isolates showed tolerance to pH range from 7-8 (PS 30, PS 31).

The results of salt tolerance ability reveals that, two isolates were showed tolerance to 1.5 to 20% of NaCl concentration (PS 4, PS 15), eight isolates were showed tolerance from 1.5 to 15% of NaCl concentration (PS 1, PS 11, PS 18, PS 26, PS 30, PS 31, PS 32, PS 40), six isolates were showed tolerance from 1.5 to 10% of NaCl concentration (PS 5, PS 8, PS 14, PS 21, PS 23, PS 29), three isolates were showed tolerance from 1.5 to 5% of NaCl concentration (PS 7, PS 17, PS 34, PS 37, PS 39).

The results of temperature tolerance ability of *Pseudomonas* isolates revealed that, three isolates showed tolerance to temperature from 20 °C -50 °C (PS 11, PS 15, PS 40), nine isolates were showed tolerance to temperature from 20 ºC-45 °C (PS 1, PS 4, PS 21, PS 23, PS 28,PS 30, PS 32, PS 34, PS 37), one isolate was showed tolerance to temperature from 30 °C-50 °C (PS 7), two isolates were showed tolerance to temperature from 30 °C-45 °C (PS 12, PS 18).

The results of drought tolerance ability of *Pseudomonas* isolates revealed that, three isolates showed tolerance to water potential from -0.05 Mpa to- 0.73 Mpa (PS 4, PS 15, PS 34), eight isolates were showed tolerance to water potential from - 0.05 Mpa to -0.30 Mpa (PS 1,PS 7, PS 12, PS 18, PS 21, PS 23, PS 29, PS 37), seven isolates were showed tolerance to water potential at -0.05 Mpa (PS 10, PS 17, PS 20, PS 28, PS 31, PS 35,PS 39).

Similar results reported by Bhakthavatchal et al., 2013, Strain *Pseudomonas aeruginosa* FP6 was able to grow on up to 4.5 M NaCl, between 20 and 60 °C and at pH 5–10. Multifarious plant growth promoting activities of *P. aeruginosa* suggested its potential use in developing a cost-effective eco-friendly multifunctional biofertilizer.

Bacillus and *Pseudomonas* spp. with stress tolerance had proven ability to inhibit the growth of potential phytopathogenic fungi. Screening of bacterial strains for high temperature (50 °C), salinity (7% NaCl), and drought (−1.2 MPa) showed that stress tolerance was pronounced less in *Pseudomonas* isolates than in *Bacillus* strains. The reason behind this could be the formation of endospores by *Bacillus* isolates. Tolerance to drought was high in *Pseudomonas* strains than the other two stresses. Three strains, P8, P20 and P21 showed both salinity and temperature tolerance. P59 strain possessed promising antagonistic activity and drought tolerance (Praveen et al., 2014).

3.4. Effect of heavy metals on Pseudomonas isolates

The results of heavy metals tolerance of *Pseudomonas* revealed that, out of 28 isolates, fourteen isolates (50%) showed growth on 50 µg ml^{-1} (PS 28 (122×10^7 cfu ml^{-1}) >PS 7 (120× 10^7 cfu ml^{-1}), PS 23 (120×10^7 cfu ml^{-1}), PS 4 (115×10^7 cfu ml^{-1}) > PS 1 (112×10^7 cfu ml^{-1}), IGP 8 (112×10^7 cfu ml^{-1}) >PS 33 (102×10^7 cfu ml^{-1}) and twelve isolates (43%) showed growth on 100 µg ml^{-1} (PS 29 (123×10^7 cfu ml^{-1}) >PS 28 (50×10^7 cfu ml^{-1}), PS 4 (37×10^7 cfu ml^{-1}) on arsenic (As) enriched trypticase soy agar.

Fourteen isolates (50%) showed cfu on 50 µg ml^{-1} (PS 32 (125×10^{7}cfu ml^{-1}), PS 5 (112×10^7 cfu ml^{-1}), PS 20 (112×10^7 cfu ml^{-1}), PS 23 (112×10^7 cfu ml^{-1}), PS 1 (102×10^7 cfu ml^{-1}) and eleven isolates (43%) were showed cfu on 100 µg ml^{-1} PS 28 (86×10^7 cfu ml^{-1}), PS 20 (63×10^7 cfu ml^{-1}) on cadmium (Cd) enriched trypticase soy agar respectively (Table 3).

Six isolates (21%) and two isolates (7%) showed cfu on 50

Table 3: *In vitro* stress tolerance ability of the *Pseudomonas* isolates

Isolates	pH range	NaCl concentration (%)	Temperature (°C)	Drought (Mpa)
PS 1	4.0-10.0	1.5-15	20-45	-0.05 to -0.30
PS 3	7.0	-	20-30	-
PS 4	4.0-10.0	1.5-20	20-45	- 0.05 to -0.73
PS 5	4.0-8.0	1.5-10	30	-
PS 7	4.0-8.0	1.5-5.0	30-50	-0.05 to -0.30
PS 8	7.0	1.5-10	20-30	-
PS 10	7.0	-	20-30	- 0.05
PS 11	6.0-10.0	1.5-15	20-50	
PS 12	7.0	-	30-45	-0.05 to -0.30
PS 14	6.0-8.0	1.5-10	20-30	-
PS 15	4.0-7.0	1.5-20	20-50	-0.05 to -0.73
PS 17	4.0-8.0	1.5-5.0	30	-0.05
PS 18	6.0-10.0	1.5-15	30-45	- 0.05 to -0.30
PS 20	4.0-8.0	-	20-30	-0.05
PS 21	7.0	1.5-10	20-45	- 0.15 to -0.30
PS-23	6.0-7.0	1.5-10	20-45	- 0.05 to -0.30
PS 26	4.0-10.0	1.5-15	30	-
PS 28	7.0	-	20-45	-0.05
PS 29	6.0-8.0	1.5-10	30	- 0.05-0.30
PS 30	7.0-8.0	1.5-15	20-45	-
PS 31	7.0-8.0	1.5-15	30	-0.05
PS 32	6.0-10.0	1.5-15	20-45	-
PS 33	7.0	-	20-30	-
PS 34	4.0-7.0	1.5 -5.0	20-45	-0.05 to -0.73
PS 35	7.0-8.0	-	30	- 0.05
PS-37	7.0	1.5-5.0	20-45	-0.15-0.30
PS 39	4.0-7.0	1.5-5.0	30	- 0.05
PS 40	4.0-10.0	1.5-15	20-50	-

µg ml^{-1} (PS 5 58×10^7 cfu ml^{-1}), PS-14 (46×10^7 cfu ml^{-1}), PS 20 (42×10^7 cfu ml^{-1}), PS 37 (42×10^7 cfu ml^{-1}) and 100 µg ml^{-1} PS-37 (32×10^7 cfu ml^{-1}) PS 32 (30×10^7 cfu ml^{-1}) on mercury (Hg) enriched trypticase soy agar respectively.

Twelve isolates (43%) and seven isolates (25%) showed cfu on 50 µg ml^{-1} (PS 32 (120×10^7 cfu ml^{-1}), PS 20 (113×10^7 cfu 10^7 ml^{-1}), PS 18 (103×10^7 cfu ml^{-1}), PS 3 (80×10^7 cfu ml^{-1}), PS 5 (72×10^7 cfu ml^{-1}) and 100 µg ml^{-1} (PS 18 (43×10^7 cfu ml^{-1}), PS 32 (43×10^7 cfu ml^{-1}),PS 3 (33×10^7 cfu ml^{-1}), PS 5 (33×10^7 cfu ml^{-1})) on manganese (Mn) enriched trypticase soy agar respectively. Similar results reported by Kowalczyk et al. (2016) tested ability to remove Hg from the liquid medium and the effect of the various pH and mercury concentrations in the environment on bacterial strains growth kinetics. The selected strains were identified by analysis of the 16S ribosome subunit coding sequences as *Pseudomonas syringae*. It was demonstrated that *P. syringae* was able to remove almost entire metal from medium after 120 hours of incubation. Huixu et al. (2012) isolated a mercury resistant strain 2 from waste treatment plant. It was identified as *Pseudomonas putida* and designated as *Pseudomonas putida*on the basis of morphological and biochemical analysis in combination with phylogenetic analysis. Strain could tolerate and aerobically grow in the medium containing up to 50 mg dm^3 Hg (II). Raja et al. (2006) stated that growth rate of the sewage isolates in the presence of heavy metal (Cd, Ni, As and Pb) were consistently slower than that of the control, In this study, *Pseudomonas* sps were resistance to cadmium 7 mM in TY agar plate (Table 4).

Table 4: Tolerance of *Pseudomonas* isolates at different concentrations of heavy metals

Isolates	Heavy metal tolerance (×10^{7}cfu ml^{-1})							
	As		Cd		Hg		Mn	
	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
PS 1	112	32	102	50	-	-	70	30
PS 3	-	-	-	-	-	-	80	33
PS 4	115	37	53	-	-	-	-	-
PS 5	79	31	112	50	58	-	72	33
PS 7	120	30	-	-	-	-	-	-
PS 8	112	30	-	-	-	-	-	-
PS 10	-	-	-	-	-	-	-	-
PS 11	-	-	100	35	-	-	65	30
PS 12	-	-	-	-	-	-	-	-
PS 14	86	31	70	30	46	-	30	-
PS 15	112	32	56	-	-	-	-	-
PS 17	-	-	-	-	-	-	-	-
PS 18	58	32	-	-	-	-	103	43
PS 20	42	-	112	63	42	-	113	30
PS 21	-	-	-	-	-	-	-	-
PS-23	120	32	112	50	-	-	52	-
PS 26	-	-	100	35	-	-	65	-
PS 28	122	50	85	86	-	-	58	-
PS 29	98	123	43	50	-	-	56	-
PS 30	-	-	-	-	-	-	-	-
PS 31	-	-	-	-	30	-	-	-
PS 32	36	-	125	50	20	30	120	43
PS 33	102	32	78	32	-	-	-	-
PS 34	-	-			58	-	-	-
PS 35	-	-	-	-	-	-	-	-
PS-37	-	-	-	-	42	32	-	-
PS 39	-	-	-	-	-	-	-	-
PS 40	-	-	-	-	-	-	-	-

For PGP properties, among *Pseudomonas* isolates, PS 1 showed efficient P-solubilization, K- solubilization, Zn solubilization, and biocontrol activity. PS 15 showed efficient Zn-solubilization, and other PGP properties. IRP-7 showed good P-solubilization, Zn-solubilization, IAA production and other PGP properties. For plant growth promoting properties and abiotic stress, PS 15 was found efficient in Zn- solubilization with considerable other PGP properties. It was tolerant to pH stress (4, 6), temperature stress (40 °C, 45 °C, 50 °C), salt stress (5%, 10%), drought stress (-0.15 MPa, -0.30 MPa, -0.73 Mpa), and heavy metals As and Cd only. PS 1 showed good P-solubilization, K- solubilization, Zn solubilization withbiocontrol activity. It tolerated pH stress (4, 6, 8, 10, 12), temperature stress (40 °C, 45 °C), salt stress (5%, 10%, 15%), drought stress (-0.15 MPa, -0.30 MPa) and tolerant to heavy metal toxicity As, Cd and Mn

Identification of bacterial strains based on 16S rRNA gene sequence is given in Table In the present study the sequenced PCR products of effective bacterial isolates were matched with the available sequences in the GenBank database. BLAST Search results through NCBI showed 99% similarity of PS 15 with *Pseudomonas fluorescens*, 99% similarity of PS 1 with *Pseudomonas fluorescens*.

4. Conclusion

In regard to isolates having PGP properties and biocontrol

activity, PS-3 can be considered efficient isolate. However, none of the isolates showed commonly PGP properties, biocontrol activity and tolerance to abiotic stress conditions. A combination of isolates PS-3 and PS-1 could be a good combination for the purpose. Effective *Pseudomona*s isolates were identified as *Pseudomonas fluorescens*. The plant growth promoting *Pseudomonas* isolates identified from the research work presented could be screened further under *in vivo* conditions of abiotic stress conditions from different soils with different crops for confirming their use as bioinoculants.

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