

Evaluation of Rhizospheric Bacteria from *Ocimum* sp. as Potential Pgp

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Abstract

In the present investigation, 24 rhizospheric soil samples of *Ocimum* sp. were collected from different vicinities of Delhi, Kurukshetra and Haridwar (India). A total of 266 bacterial strains were isolated and screened for in vitro plant growth promoting trait. About 86.46% bacterial isolates showed ammonium production, 89.09% exhibited phosphate solubilization and 87.59% for catalase production whereas only 7.14% showed positive reaction for HCN production. Five isolates viz., CHII(II)K7, CHIII(I)Y6, DDI(I)1, UHI(II)7 and CHII(I)NA4 were found to exhibit maximum number of plant growth promoting traits.

Keywords: PGPR; PGP traits; *Ocimum*; IAA; Medicinal plant; Bio-inoculant

Introduction

Micro-organisms play a vital role in recycling of nutrients. They offer an attractive way for sustainable agricultural system by reducing the use of chemical fertilizers [1-3]. Most of the world population still relies mainly on herbal products and medicines for health care [4,5]. India has a rich heritage of medicinal plants. These are storehouse of antimicrobial agents. They offer advantage of being safer and pose lesser side effects [6]. The relationship between PGPR and medicinal plants is yet to be explored. PGPR are beneficial bacteria that help in plant growth. Mechanisms of PGPR are solubilization of phosphate, N₂ fixation, siderophore production, phytohormone synthesis, ACC deaminase activity, ISR, production of antibiotics and enzymes that lyse cell wall of fungal pathogens [7-11]. PGPR traits have been recorded in several bacteria and cyanobacteria species belonging to *Acinetobacter*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Rhizobium* and *Serratia* [12-15]. Understanding interactions between PGPR and plants will be helpful for developing strategies for plant growth enhancement. Chemical fertilizers being highly expensive and extremely hazardous to environment may pose a serious threat to human health. A system is therefore required to replace these chemical means so that ecologically sustainable biocontrol strategy can be developed and this can be achieved by use of efficient PGPR strains that can be used for the management of plant pathogens as well as for achieving good yields of crops.

One of the most valuable medicinal plants is *Ocimum* (tulsi or basil) that belongs to family Lamiaceae and bears a high medicinal value [16]. *Ocimum sanctum* is also known as "The Incomparable One", "The Mother Medicine of Nature", and "The Queen of Herbs"[17]. Tulsi improves digestive system and possess properties such as anti-ulcer activity, anti-stress activity, anti-carcinogenic, anti-oxidant, antimicrobial, anti-diabetic and anti-inflammatory. It provides protection against cardiac and neurological disorders. Tulsi provides strength to the immune system [16]. Traditionally, it is used to treat asthma [18] with growing interest in finding eco-friendly methods for sustainable agriculture, it is necessary to explore soil microbial diversity for PGPR having combination of plant growth promoting traits. Keeping these points in mind, the aim of our work was to evaluate various microbial (bacterial) isolates from *Ocimum* rhizosphere for their plant growth promoting traits and suitability for their application to improve the yield of this very important medicinal plant i.e. *Ocimum* sp.

Materials and Methods

Sampling sites and collection of soil sample

For isolation of potential rhizobacterial strains, sampling of rhizospheric soil with intact root system was done carefully with the help of sterile equipments. The rhizospheric soil samples (twenty-four) of *Ocimum* plants were collected from different localities in Delhi, Haridwar and Kurukshetra (India) during the month of June-July (Table 1). The top soil containing dry matter was removed from the sampling site and entire root system along with the rhizosphere soil was collected digging up to 15 cm in depth. Samples were taken from the upper as well as lower region of rhizosphere. The samples were then placed in sterile plastic bags and stored at 4°C (Figures 1-3).

Isolation and characterization of bacteria from *Ocimum* rhizosphere

A total of 266 bacterial isolates were obtained from 24 rhizospheric soil samples of *Ocimum* sp. Isolation was done by Serial Dilution technique on different media such as Nutrient agar medium, Kings B medium, YEMA medium, Ashby medium and Pikovaskaya medium by incubating plates at 28°C for 3 days (Table 2). About 10 gm of rhizosphere soil was mixed with 90 ml of sterile distilled water in a flask and shaken for 10 minutes on a rotary shaker. Following this, 1 ml suspension from the flask will be added to 10 ml vial and successive dilutions were made upto 10⁻⁷ dilution. About 0.1 ml of this suspension was spread on respective media plates. The plates were observed for typical bacterial colonies and well isolated single colonies were picked up for streaking on fresh respective agar plates to get the pure cultures [19]. All the isolates were studied for their morphological characteristics. Different morphological characteristics of colonies such as color, elevation, shape, size, etc. were recorded after 3 days of incubation.

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S. No.	Sample	Locality	No. of isolates
1	CHI(I)	Haridwar	24
2	CHI(II)	Haridwar	25
3	CHII(I)	Haridwar	21
4	CHII(II)	Haridwar	18
5	CHIII(I)	Haridwar	27
6	CHIV(I)	Haridwar	26
7	KUKI(I)	Kurukshetra	22
8	KUKI(II)	Kurukshetra	08
9	DDI(I)	Delhi	04
10	DDI(II)	Delhi	07
11	DDII(I)	Delhi	05
12	DDII(II)	Delhi	04
13	DDIII(I)	Delhi	13
14	DDIII(II)	Delhi	10
15	DDIV(I)	Delhi	02
16	DDIV(II)	Delhi	09
17	DDV(I)	Delhi	04
18	DDV(II)	Delhi	06
19	DDVII(I)	Delhi	03
20	DDVII(II)	Delhi	02
21	UHI(I)	Haridwar	09
22	UHI(II)	Haridwar	08
23	UHII(I)	Haridwar	06
24	UHII(II)	Haridwar	03

Table 1: List of soil samples and isolates obtained.

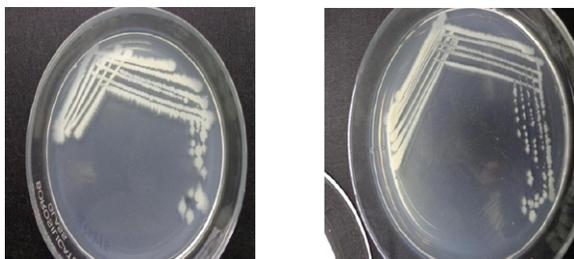


Figure 1: Isolates UHI(II)7 and CHIII(I)Y6 grown on nutrient media plates.

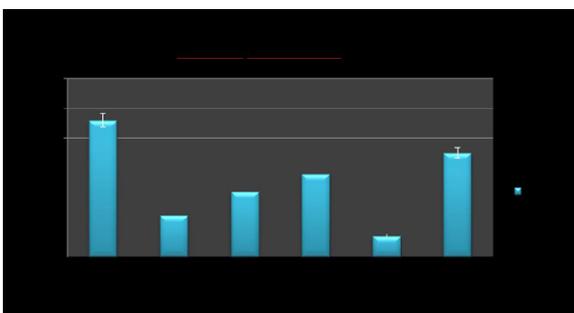


Figure 2: IAA production in the presence of tryptophan from *Ocimum* rhizosphere isolates.

In vitro screening of bacterial isolates for their plant growth promoting (PGP) activities

Production of Indole acetic acid: Indole acetic acid (IAA) production by the isolates was assayed colorimetrically [20]. Bacterial cultures were grown in LB medium amended with 100 mg/l tryptophan as the precursor of IAA by incubating in a shaker at 250 rpm at 28

± 2°C for seven days. After one week of growth, the cultures were centrifuged at 4000 rpm for 20 min and the supernatant collected and IAA in the supernatants were quantified by using colorimetric assay. Two milliliter of cell free extract was mixed with 4 ml Salkowski reagent (1 ml of 0.5M FeCl₃ in 50 ml of 35% HClO₄) and absorbance of the resultant pink color was read after 30 min at 535 nm in colorimeter. Appearance of pink color in test tubes indicated IAA production. The IAA production was calculated from standard curve and the result was expressed as µg/ml over control [21].

Production of ammonia: All isolates were tested for ammonia production. The cultures were inoculated in 10 ml peptone water and incubated for 72 h at 36°C. After incubation, 0.5 ml of nessler's reagent was added to each tube. Development of brown to yellow color was taken as a positive test for production of ammonia [22].

Production of HCN: The isolates were tested for HCN production [23]. Bacteria was inoculated on the nutrient media plates containing 4.4 g glycine per liter. To the top of the plate, whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed and sealed with parafilm. The plates were incubated at 36°C for 4 days. Plates were observed for the development of orange to red color of filter paper. This was recorded as positive test for HCN production.

Phosphate solubilization: All the bacterial isolates were tested *in vitro* for their phosphate solubilizing activity using Pikovskaya's medium. The culture was spot-inoculated on the Pikovskaya medium plates and incubated at 28°C for 7 days. The appearance of clear zone around bacterial growth was taken as a positive test for phosphate solubilization [24].

Catalase test: Catalase test was done by adding 3 drops of 3% hydrogen peroxide to the bacterial culture. Appearance of effervescence was taken as positive test for catalase activity [22].

Antifungal activity: A 9 mm PDA culture disc from the plates of *Fusarium oxysporum*, growing in petridishes was cut individually from 7-day-old culture. This was placed on one side of the previously plated sterilized modified PDA medium (g/500 ml PDA (Hi Media)-19.5, peptone-1, yeast extract-0.5, agar-2.5) approximately 1.5 cm away from the edge of the plate. Simultaneously, the bacterial isolate was streaked onto the opposite side of the petri plate. The plates were incubated at 28°C for 7 days and results were recorded [25]. The level of inhibition was calculated by subtracting the distance (mm) covered by the growth of fungus in the direction of the bacterial isolate from the fungal radius. The percent inhibition was calculated as:

% inhibition = $(R-r)/R \times 100$ where 'r' is radial growth of fungus opposite the bacterial growth and 'R' is the radial growth of fungus in control plate.

Siderophore production: Siderophores production by the isolates was assayed using plate assay. The tertiary complex (Chrome azural S (CAS)/Fe³⁺/hexadecyl trimethyl ammonium bromide) served as an indicator. The selected isolates were streaked on to the succinate medium mixed with indicator dye. Formation of bright zone with yellowish fluorescent color in the dark colored medium indicated siderophore production [26].

ACC-deaminase activity: Selected bacterial isolates were cultured in DF salt minimal medium [27] at 28°C for 2 days with shaking at 200 rpm. Centrifuged the culture at 5000 rpm for 5 min and washed with minimal medium. suspended the cell pellets minimal medium supplemented with 1 mM ACC and incubated at 28°C for 24 h with shaking at 200 rpm. ACC deaminase activity was measured according

to a modification of the method of Honma and Shimomura (1978). The standard concentration curve of α -ketobutyrate was generated. All series of known α -ketobutyrate concentrations was prepared in 200 μ l volume and mixed with 300 μ l of 2,4- dinitrophenylhydrazine reagent. Incubated the contents at 30°C for 30 min for the development of phenylhydrazone. The color of the phenylhydrazone was developed by the adding 2 ml 2M sodium hydroxide, following which, absorbance of the mixture was measured at 540 nm [28].

Heavy metal tolerance: The selected isolates were tested for their resistance to heavy metals namely Ni, Hg, Co, Cd, Cu, Pb, Zn and Cr by agar dilution method [29]. Nutrient agar plates amended with various soluble heavy metal salts at concentrations 25 μ g/ml, 100 μ g/ml and 400 μ g/ml were inoculated and incubated for 3 days at room temperature. Heavy metal tolerance was indicated by the appearance of bacterial growth and results recorded.

Effect of temperature on growth of isolates: Chosen isolates were streaked on the nutrient media plates. The plates were incubated at 10°C, 20°C, 28°C, 37°C and 45°C for 3 days. After incubation, the plates were observed for growth and results were noted.

Statistical analyses: Statistical analysis of the tests was carried out using SPSS 16.0 design. All the tests were conducted in triplicate. Data reported as mean \pm standard deviation (SD). Also, data was analyzed with standard error at 0.5% significance.

Biochemical characterization: The biochemical tests such as indole test, methyl-red test, vogues-proskauer test, citrate utilization, catalase test, oxidase test, H₂S production test, carbohydrate fermentation etc., were performed according to the standard procedures [30].

Results and Discussion

Isolation and morphological characterization of isolates

In the present study, 266 bacterial isolates were screened *in vitro* for PGP activities. Properties such as ammonia production and phosphorus solubilization are found among large no. of bacteria. About 230 (86.46%) bacterial isolates showed ammonium production, 179(89.09%) exhibited phosphate solubilization and 233 (87.59%) for catalase production. Only 19 (7.14%) showed positive test for HCN

Culture Medium	Bacteria isolated
Nutrient agar medium	<i>Bacillus</i> sp.
Kings B medium	<i>Pseudomonas</i> sp.
YEMA medium	<i>Rhizobium</i> sp.
Ashby medium	<i>Azotobacter</i> sp.
Pikovaskaya medium	Phosphate solubilizing bacteria

Table 2: Isolation of bacteria from respective culture medium.

Isolate(s)	Colour	Size	Shape	Elevation	Margin	Odor	Pigmentation	Surface	Opacity
CHII(II)K7	Yellowish green	Small	Round	Raised	Entire	None	Yellowish green	Smooth	Transparent
CHIII(I)Y6	Creamish	Medium	Irregular	Raised	Irregular	None	None	Shiny smooth	Opaque
DDI(I)1	Yellowish green	Small	Round	Raised	Irregular	None	Yellowish green	Smooth	Transparent
UHII(II)1	White	Small	Round	Raised	Entire	None	None	Smooth	Opaque
DDVII(II)1	White	Small	Round	Raised	Entire	None	None	Smooth	Opaque
KUKI(II)6	Creamish	Small	Irregular	Raised	Irregular	None	None	Smooth	Opaque
UHI(II)7	White	Medium	Irregular	Raised	Irregular	None	None	Shiny smooth	Opaque
DDII(II)1	Creamish	Medium	Round	Raised	Irregular	None	None	Rough	Opaque
CHII(I)NA4	Yellowish	Small	Round	Raised	Entire	None	Yellowish	Rough	Opaque
DDV(I)3	Yellowish	Small	Round	Raised	Entire	None	Yellowish	Smooth	Opaque

Table 3: Morphological characteristics of selected PGPR isolates.

production. All the isolates were morphologically characterized. Based on the properties, 10 best isolates were selected for further investigation. These isolates were characterized and their morphological characteristics have been studied. Different characteristics of colonies such as color, shape, size, elevation etc., were noted (Table 3).

PGP traits of the selected bacterial isolates

In the present study, some of the isolates could exhibit more than three or four PGP traits, which may promote plant growth directly or indirectly. As shown in Table 4, isolates CHIII(I)Y6, CHII(I)NA4, CHII(II)K7, DDI(I)1 were found to exhibit seven different PGP traits whereas isolate UHI(II)7, exhibit six PGP traits; DDII(II)1 exhibit five PGP traits whereas isolates UHII(II)1, DDVII(II)1, KUKI(II)6, and DDV(I)3 exhibit four PGP traits i.e., ammonia production, phosphate solubilization, catalase production and heavy metal tolerance.

IAA production, ammonia production and HCN production by selected isolates: Six isolates out of ten showed IAA production, viz., CHII(II)K7, CHIII(I)Y6, DDI(I)1, UHI(II)7, DDII(II)1 and CHII(I)NA4 (Figure 2). Ammonia production was observed in all selected isolates. Isolate CHII(II)K7 and CHIII(I)Y6 showed maximum ammonia production while other isolates showed moderate production of ammonia. Among the 10 isolates, three (CHIII(I)Y6, DDI(I)1 and CHII(I)NA4) were recorded positive for HCN production, CHIII(I)Y6 and DDI(I)1 isolates showing maximum HCN production by changing the color of filter paper orange to brown (Table 4).

Phosphate solubilization by selected isolates: All the selected isolates are phosphate solubilizing. Maximum zone was observed in isolate UHI(II)7 (28mm). Significant zones were also seen in isolate CHII(II)K7 (26mm), DDV(I)3 (26mm), KUKI(II)6 (24mm) and DDI(I)1(22mm) after 7 days of incubation (Figures 3 and 4; Table 5).

Antifungal activity: Several researchers have reported that increasing incidences of *Fusarium* wilt has causes major damage to production of basil [31-35]. More specifically, *Fusarium oxysporum* has been a major causal agent leading to disease throughout the world [31]. The symptoms include chlorosis, necrosis, wilt of stems and leaves, crown and root rot, formation of dark lesions and plant death. The antifungal activity of the isolates was tested against fungal pathogen i.e., *Fusarium oxysporum* in dual culture under *in vitro* conditions. Out of ten, only three isolates showed antagonistic potential (Figure 5; Table 5). The growth of the fungus was lesser as compared to the control plate. Isolate CHII(II)K7 exhibited greater inhibition followed by CHIII(I)Y6 and CHII(I)NA4 respectively.

Siderophore production and ACC-deaminase activity: Out of 10 selected isolates, isolate DDI(I)1 was able to produce siderophore and it is confirmed by the development of orange halos surrounding

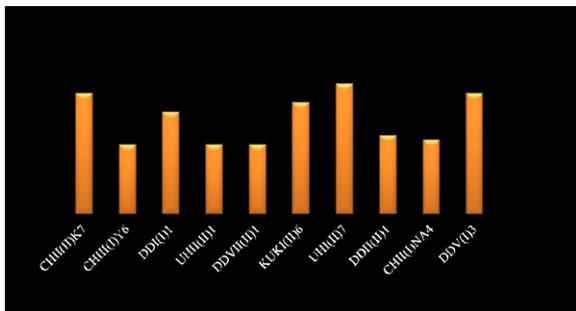


Figure 3: Phosphate Solubilization Index of *Ocimum* rhizosphere isolates.



Figure 4: Isolates showing solubilization of phosphate.



Figure 5: Antifungal activity exhibited by isolate CHII(I)NA4 against *Fusariumoxysporum*.

the colonies. ACC deaminase activity is helpful in maintaining plant growth and development, so its activity was measured. Isolate UHI(II)7 showed the highest ACC deaminase enzyme activity of 3.90 μmol α -ketobutyrate/mg protein/h followed by isolate CHII(II)K7 which showed enzyme activity of 2.92 μmol α -ketobutyrate/mg protein/h.

Heavy metal tolerance by selected isolates: Selected isolates are found to be more tolerant at lower concentration of 25 and 100 $\mu\text{g/ml}$ and less tolerant at concentration of 400 $\mu\text{g/ml}$. All selected isolates showed tolerance to Ni, Cu and Pb at concentration 25 $\mu\text{g/ml}$. But seven isolates were positive for Zn and Co; six for Cr; five for Cd and only four for Hg at concentration 25 $\mu\text{g/ml}$. All selected isolates showed tolerance to nickel, copper and lead except DDV(I)3 for Pb at conc. 100 $\mu\text{g/ml}$ (Figures 6 and 7; Table 6). 6(Zn, Co); 5(Cr); 4(Cd) and 3(Cr) no. of isolates were tolerant to 100 $\mu\text{g/ml}$ concentration. For higher concentrations, like 400 $\mu\text{g/ml}$; only CHII(II)K7 is tolerant to copper at concentration of 400 $\mu\text{g/ml}$. 6(Zn); 4(Pb); 3(Cd, Cr) and 2(Co, Hg) number of isolates were tolerant whereas all selected isolates were tolerant to nickel even at high concentration of 400 $\mu\text{g/ml}$.

Effect of temperature on growth of isolates: It was observed that the growth of isolates on nutrient agar plates varied with temperature. The growth of all selected isolates was good in the temperature range of 20°C to 37°C except DDVII(II)1 which was unable to grow at 20°C. In addition, all selected isolates were found to grow at 45°C (Figures 8-10). On the contrary, isolates CHII(II)K7, UHI(II)7 and DDII(II)1 were found to grow at 10°C but the growth was less. Out of 10 selected isolates, five isolates have shown maximum PGP traits (CHII(II)K7, CHIII(I)Y6, DDI(I)1, UHI(II)7, CHII(I)NA4) i.e., IAA production, ammonia production, phosphate solubilization, HCN production, catalase production, heavy metal tolerance etc. The biochemical characteristics of these five isolates are given in Table 7. All the isolates were found to be positive for catalase test. On the basis of morphological and biochemical characterization, it was found that two isolates CHII(II)K7 and DDI(I)1 belongs to *Pseudomonas* sp., whereas CHIII(I)Y6, UHI(II)7 and CHII(I)NA4 belongs to *Bacillus* sp.

Discussion

Plant rhizosphere can be considered as an ecological niche for many various soil microorganisms because of the high amounts of nutrients available in the rhizosphere region. Growth enhancement may take place due to plant hormone synthesis other PGP traits occurring in the rhizosphere [36-38]. PGPR with numerous PGP traits have been

Isolate(s)	IAA	HCN	AMM	PSB	AFA	HMT	SID	CAT	ACC
CHII(II)K7	+	-	+	+	+	+	-	+	+
CHIII(I)Y6	+	+	+	+	+	+	-	+	-
DDI(I)1	+	+	+	+	-	+	+	+	-
UHI(II)7	-	-	+	+	-	+	-	+	-
DDVII(II)1	-	-	+	+	-	+	-	+	-
KUKI(II)6	-	-	+	+	-	+	-	+	-
UHI(II)7	+	-	+	+	-	+	-	+	+
DDII(II)1	+	-	+	+	-	+	-	+	-
CHII(I)NA4	+	+	+	+	+	+	-	+	-
DDV(I)3	-	-	+	+	-	+	-	+	-

IAA: Indole Acetic Acid Production; HCN: Hydrogen Cyanide Production; AMM: Ammonia Production; PSB: Phosphate Solubilization; AFA: Antifungal Activity; HMT: Heavy Metal Tolerance; SID: Siderophore Production; CAT: Catalase Production; ACC: ACC Deaminase Activity; +: Positive Test; -: Negative Test.

Table 4: Characterization of selected bacterial strains for multiple plant growth promoting (PGP) traits.

Isolate(s)	Phosphate solubilization		Antifungal activity against <i>Fusarium oxysporum</i> (% inhibition)
	Diameter of Zone in mm	Solubilization index	
CHII(II)K7	26 ± 0.00	5.2	+++ (61.10 ± 1.34)
CHIII(I)Y6	15 ± 2.00	3.0	++ (58.88 ± 1.07)
DDI(I)1	22 ± 2.64	4.4	-
UHII(II)1	15 ± 0.00	3.0	-
DDVII(II)1	15 ± 2.00	3.0	-
KUKI(II)6	24 ± 2.64	4.8	-
UHI(II)7	28 ± 1.00	5.6	-
DDII(II)1	17 ± 2.64	3.4	-
CHII(I)NA4	15 ± 2.00	3.2	++ (42.21 ± 0.00)
DDV(I)3	26 ± 2.00	5.2	-

++: Moderate Activity; +++: Good Activity; -: No Activity

Table 5: Phosphate solubilization and antifungal activity by selected PGPR isolates.

IAA is reported to be involved in the epiphytic fitness of PGPR [41]. IAA produced by bacteria might modify the micro-habitates of epiphytic bacteria by enhancing the nutrient leakage of plant cells. Due to this availability of nutrients increases which may further help in the colonization of bacteria to the rhizosphere. In our study, the concentration of IAA produced ranges from 7.0 to 46.0 µg/ml. Similar observations for IAA production have been reported by Malleswari and Bagyanarayan, 2013. Production of hydrogen cyanide has been found in *P. fluorescens*, *P. aeruginosa* and *Chromobacterium violaceum* [6]. Out of 10, three isolates viz., CHIII(I)Y6, DDI(I)1 and CHII(I)NA4 showed HCN production [42] reported that IAA production is helpful in enhancement of plant growth and HCN production can be considered as defence regulator against phytopathogens. The most common way

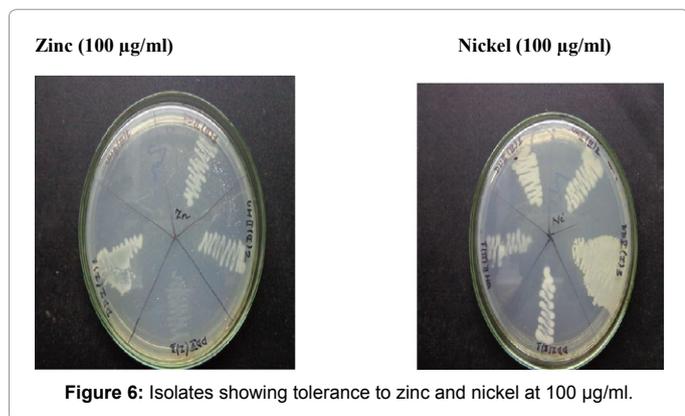


Figure 6: Isolates showing tolerance to zinc and nickel at 100 µg/ml.

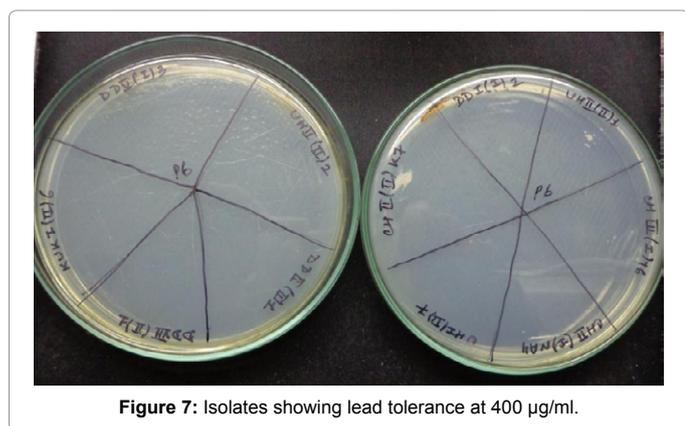


Figure 7: Isolates showing lead tolerance at 400 µg/ml.

reported by researchers but there have been few findings on indigenous isolates of India [39]. Isolated bacteria were screened for different plant growth promotion activities and characterized by biochemical tests. IAA, a phytohormone, is well known as the most important native auxin. It may act as important signal molecule in the regulation of plant development. Of ten isolates, six isolates are positive for IAA production (Table 4). The potential for IAA synthesis varies with different species and strains as well as cultural condition, growth stage and availability of substrate [40]. The rhizospheric bacteria are more efficient auxin producers than those from the bulk soil [37]. About 80% of soil bacteria are evaluated to possess IAA producing potential.

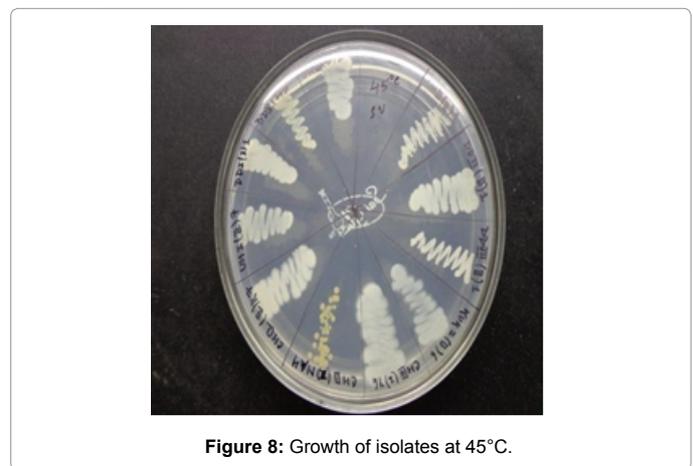


Figure 8: Growth of isolates at 45°C.



Figure 9: Isolates showing citrate utilization and indole production test.

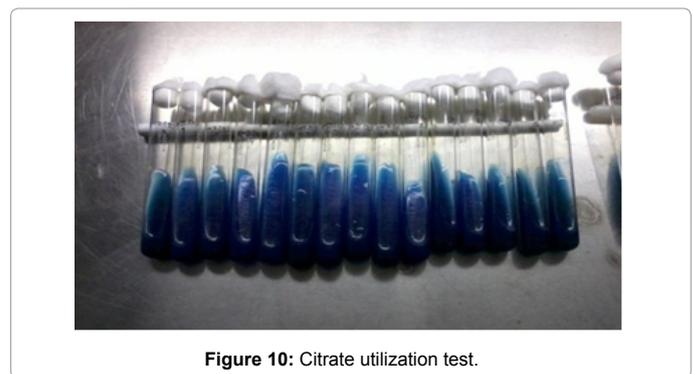


Figure 10: Citrate utilization test.

Heavy metal (µg/ml)	CHII(I) I) K7	CHIII(I)Y 6	DDI(I) 1	UHI(II)1	DDVII(I) I)1	KUKI(II) 6	UHI(II) 7	DDII(II) 1	CHII(I)NA 4	DDV(I) 3
Nickel (Ni)										
25	+	+	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+	+
400	+	+	+	+	+	+	+	+	+	+
Zinc (Zn)										
25	+	+	+	-	+	+	-	+	-	+
100	+	+	+	-	-	+	-	+	-	+
400	+	+	+	-	-	+	-	+	-	+
Cobalt (Co)										
25	+	+	-	+	-	+	+	+	-	+
100	+	+	-	-	-	+	+	+	-	+
400	-	-	-	-	-	-	+	+	-	-
Cadmium (Cd)										
25	+	+	+	-	-	-	+	+	-	-
100	-	+	+	-	-	-	+	+	-	-
400	-	-	+	-	-	-	+	+	-	-
Lead (Pb)										
25	+	+	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+	+	-
400		+	+	+	-	-	-	-	-	-
Copper (Cu)										
25		+	+	+	+	+	+	+	+	+
100		+	+	+	+	+	+	+	+	+
400		-	-	-	-	-	-	-	-	-
Chromium (Cr)										
25		+	-	+	+	-	+	+	-	+
100		+	-	+	+	-	+	-	-	+
400		-	-	+	+	-	+	-	-	-
Mercury (Hg)										
25		-	-	+	-	+	-	+	+	-
100		-	-	-	-	+	-	-	-	-
400		-	-	-	-	+	-	-	-	-

Table 6: Heavy metal tolerance of selected PGPR isolates.

Test(s)	CHII(II)K7	CHIII(I)Y6	DDI(I)1	UHI(II)7	CHII(I)NA4
Gram	-ve	+ve	-ve	+ve	+ve
Reaction					
Shape	Rods	rods	rods	rods	rods
Indole	-	-	-	-	-
Methyl-red	-	-	-	+	-
VP	-	+	-	+	+
Citrate	+	+	+	-	+
Catalase	+	+	+	+	+
Oxidase	-	-	+	+	-
H2S	-	-	-	-	-
Nitrate	+	+	+	+	+
reduction					

Table 7: Biochemical identification of selected PGPR isolates.

for increasing the availability of nutrients to the plants is solubilization of phosphorus in the rhizosphere by PGPR. The phosphate-solubilising *Pseudomonas* spp. increased growth as well as phosphorus content of maize. In our study, maximum zone of solubilization (28 mm) was observed for isolate UHI(II)7. O'Sullivan DJ et al. [43] reported *Bacillus* sp., *Providencia* sp., *Brevundimonas* and *Alcaligenes* as

phosphate solubilizers. Solubilization of phosphorus have been well documented in *Alcaligenes*, *Bacillus* sp. [44]. Siderophore production is another important trait of PGPR [45,46] and observed the siderophore production in *Pseudomonas* that exhibited antagonism to plant pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani*. In the present study, only isolate DDI(I)1 was able to produce siderophore. Among the isolates screened for ACC deaminase activity, only two isolates exhibited this trait viz., CHII(II)K7 and UHI(II)7. *Providencia* sp. (AW5) and *Alcaligenes* sp. (AW10) possess ACC deaminase activity (3.13 and 9.5 µmol α-ketobutyrate/mg/h, respectively) [2]. In our study, the two isolates showed activity of 3.90×10⁻⁶ µmol α-keto butyrate/mg protein/ h and 2.92×10⁻⁶ µmol α-keto butyrate/mg protein/h by isolate UHI(II)7 and CHII(II)K7. Similar findings have been reported by other workers [47,48]. Plant growth by ACC deaminase producing *Micrococcus* sp. NII-0909 was reported [49]. Three bacterial isolates showed positive test for growth inhibition of fungus *Fusarium oxysporum*. Production of lytic enzymes by *Pseudomonas* have been reported in several studies for control of plant pathogenic fungi [42,38]. All the test bacterial isolates in the present study showed positive test for catalase and ammonia production. It has been found that plant growth can be enhanced by decreasing the levels of toxic heavy metals and that the microorganisms have developed these mechanisms for increasing

their chances of survival in the heavy metal containing environment [50]. Microorganisms that possess plant growth promoting traits and are metal tolerant may be helpful in the recolonization of the plant rhizosphere in polluted soils.

Summary and Conclusion

These studies concluded that the five chosen isolates viz., CHII(II)K7, DDI(I)1, CHIII(I)Y6, UHI(II)7 and CHII(I)NA4 which were positive for maximum PGP traits have proven to be most promising and can be selected as effective PGPR strains. The isolates namely CHII(II)K7 and UHI(II)7 have been reported as potential PGPR with best activities and they were further characterized by 16S rDNA sequencing as *Pseudomonas* sp. CHII(II)K7 and *Bacillus licheniformis* UHI(II)7, respectively.

With the success story of this primary screening protocol, we can further move on to their assessment under field conditions which might be useful for the development of potential inoculants/biofertilizer for increasing the growth and productivity of medicinal plants.

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