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## Isolation of Multiple Metal and Antibiotic Resistant *Mesorhizobium* species and Their Plant Growth Promoting Activity

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### ABSTRACT

Heavy metal contamination has accelerated due to the rapid industrialization world wide. Accumulation of metals in excess can modify the structure of essential protein or can replace an essential element. Aim of this study is to check the *Mesorhizobium* species for their tolerance towards multiple metals as well as antibiotics and further check whether these metal resistant microbes are producing any plant growth promoting substances. *Mesorhizobium* strains were isolated from the nodules of chickpea, were tested for their tolerance to both heavy metals and antibiotics by agar plate dilution method and by the disc diffusion method respectively. Plant growth promoting activity was checked by the standard methods. All of the isolates showed tolerance to cadmium, chromium, nickel, lead, zinc and copper. All the isolates showed maximum tolerance towards lead and zinc which was followed by nickel and chromium. *Mesorhizobium* species also showed tolerance towards antibiotics, 70% of the isolates were tolerant to nalidixic acid, 60% were tolerant to each tetracycline and nitrofurantoin whereas no isolate was found to be resistant towards cloxacillin, ciprofloxacin and chloramphenicol. *Mesorhizobium* strains were also tested for their Plant Growth Promoting (PGP) substances, all the isolates produced good amount of indole acetic acid and were positive for ammonia and Hydrogen Cyanide (HCN) but only three isolates (RC1, RC3 and RC4) produced siderophore on the plates as well as in the liquid culture. In conclusion *Mesorhizobium* strains can be used for the growth promotion, as well for the detoxification of the heavy metals in metal polluted soils.

**Key words:** *Mesorhizobium*, heavy metal tolerance, antibiotic resistances, plant growth promoting activity

### INTRODUCTION

Contamination of the environment by heavy metals is the wide spread problem due to their use in industries and agricultural purposes (Fernandes and Henriques, 1991). It adversely affect about 12% of the world's agricultural land (Moffat, 1999). Heavy metal pollution has increased from the start of the industrial revolution. The primary source of this pollution includes the industrial operations such as, smelting, mining, metal forging, manufacturing of alkaline storage batteries, combustion of fossil fuel and sewage sludge of industrial/domestic origin (Ibekwe *et al.*, 1995). The application of sewage sludge in agronomic practices is often the most economical means of disposal.

It is beneficial because it increases the organic matter content and water holding capacity of soil (Pagliai *et al.*, 1981) and also provides plant with sufficient nutrients. However, sewage sludges from industrial sources, often contain variable amounts of potentially toxic heavy metals, such as, lead, cadmium, nickel, chromium, copper and zinc (McGrath, 1987). When these sludges are repeatedly applied to agronomic lands, heavy metals accumulate and persist in the top cultivated layer (0-20 cm) (McGrath, 1987). The persistence of these metals in soil adversely affect the agro-ecosystem (McIlveen and Negusanti, 1994; Broos *et al.*, 2004, 2005). Despite the reports of availability of larger quantities of heavy metals in sewage water, it is widely applied in agronomic practices for irrigation purposes. Though, a large number of reports on the effects of sewage sludge having multiple metals on microbial communities (McGrath *et al.*, 1988; Giller *et al.*, 1998) and plants (Ibekwe *et al.*, 1995) are available, yet there is discrepancy in the reported results (Ramirez *et al.*, 2008). And hence, a firm conclusion on the toxicity of heavy metals on plants including legumes and their symbiotic partners or plant growth promoting rhizobacteria can not be drawn. Moreover, the majority of the adverse effects observed in sludge treated soils are possibly due to the factors other than metals (e.g., contaminants, excess N supply) which increases the toxicity.

The elevated concentration of such metals adversely affects the quantitative and qualitative composition of microbial communities in soil including those bacterial populations that aggressively colonize plant roots and termed as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper and Schroth, 1978), leading to an altered microbial equilibrium in rhizosphere (Gray and Smith, 2005). Also, the enhanced concentrations of metals affect growth, metabolisms and consequently the total biomass of naturally occurring beneficial microbes.

To survive under metal stress conditions, plant growth promoting bacteria have evolved several mechanisms to tolerate the uptake of heavy metal ions (Nies, 1999). These mechanisms include precipitation of metal as insoluble salts by chemical transformation, accumulation and sequestration of the metal ions inside the cell, biotransformation-transformation of toxic metal to less toxic forms (Thacker and Madamwar, 2005). Almost all known bacterial resistance mechanisms are encoded on plasmids and transposons (Silver and Walderhaug, 1992) and the bacteria acquire resistance to heavy metals either by the gene transfer or spontaneous mutation. Metal ions are known to cause oxidative stress, stress response genes are induced as metal ion concentrations increase from starvation to toxic level. It is studied that genes are expressed under specific metal stress (Singh *et al.*, 2001). For example, in Gram-negative bacteria (e.g., *Ralstonia eutropha*), the *czc* system is responsible for the resistance to Cd, Zn and Co. The *czc*-genes encode for a cation-proton antiporter (CzcABC) which exports Cd, Zn and Co. Similarly in *Alcaligenes xylosoxidans*, *ncc* system was found which is resistant to Ni, Cd and Co. On the contrary, Cd-efflux ATPase was responsible for Cd resistance in Gram-positive bacteria (e.g., *Staphylococcus*, *Bacillus* or *Listeria*). Cu resistance systems observed in *Pseudomonas syringae* pv. tomato is *cop* whereas, in *Escherichia coli* it is *pco* which encode for different Cu-binding proteins which allow Cu to bind in the periplasm or in the outer membrane. In contrast, the *pco* system is an ion-dependent Cu antiporter (Kunito *et al.*, 1997). Additionally, a few naturally occurring microorganisms with high metal resistance in plant rhizosphere significantly reduced metal toxicity (Kamaludeen and Ramasamy, 2008). Generally, plant growth promoting rhizobacteria release plant growth promoting substances (Sheng and Xia, 2006; Ahmad *et al.*, 2008), vitamins, enzymes, siderophores and antibiotics (Noordman *et al.*, 2006; Burd *et al.*, 2000) which result in the over all improvement in the growth of the plants. They also promote the growth of plants by alleviating the stress induced

by ethylene-mediated impact on plants by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Madhaiyan *et al.*, 2007; Glick *et al.*, 2007). The present study was therefore, undertaken to check the resistance pattern of plant growth promoting rhizobacteria to heavy metals and antibiotics and their phytohormone production.

## **MATERIALS AND METHODS**

**Collection of samples:** Plant samples for the isolation of bacteria were collected from the soils of the experimental sites of Faculty of Agricultural Sciences, Aligarh Muslim University Aligarh, U.P., India.

**Evaluation of bacterial strains for metal tolerance:** *Mesorhizobium* strains were isolated from the nodules of chickpea plants grown in the soils of the experimental sites of Faculty of Agricultural Sciences, Aligarh Muslim University Aligarh, U.P, India. *Mesorhizobium* strains were tested for their resistance to six metals like cadmium, chromium, nickel, lead, zinc and copper not by agar plate dilution method (Holt *et al.*, 1994) using YEM agar medium. The freshly prepared agar plates amended with increasing concentration of cadmium (0-400  $\mu\text{g mL}^{-1}$ ), chromium (0-500  $\mu\text{g mL}^{-1}$ ), nickel (0-500  $\mu\text{g mL}^{-1}$ ), lead (0-1500  $\mu\text{g mL}^{-1}$ ), zinc (0-1500  $\mu\text{g mL}^{-1}$ ) and copper (0-400  $\mu\text{g mL}^{-1}$ ) were spot inoculated (100  $\mu\text{L}$ ) with  $10^8$  cells  $\text{mL}^{-1}$ . Plates were incubated at  $28\pm 2^\circ$  for 3-5 days. Lowest concentration of metals inhibiting bacterial growth on nutrient agar plate was defined as a minimum inhibitory concentration. Each experiment was replicated three times.

**Determination of antibiotic resistance:** To determine resistance to antibiotics, the plant growth promoting *Mesorhizobium* were tested for their sensitivity to ten antibiotics. The reactions to antibiotics were determined by the disc diffusion method (Bauer *et al.*, 1966). *Mesorhizobium* species were grown in Yeast Extract Mannitol (YEM) broth respectively, at  $28\pm 2^\circ\text{C}$  for 24 h. A 0.1 mL of the over night grown culture was spread on the surface of yeast extract mannitol agar. The antibiotic discs of known potency were then placed on the agar surface and the plates were incubated at  $28\pm 2^\circ\text{C}$  for 24 h and the zones of inhibition around the antibiotic discs were measured to the following antibiotics (all from Hi-media Mumbai): ampicillin (25  $\mu\text{g}$ ), chloramphenicol (25  $\mu\text{g}$ ), ciprofloxacin (30  $\mu\text{g}$ ), cloxacillin (30  $\mu\text{g}$ ), gentamycin (30  $\mu\text{g}$ ), methicillin (30  $\mu\text{g}$ ), nalidixic acid (30  $\mu\text{g}$ ), nitrofurantoin (100  $\mu\text{g}$ ), streptomycin (25  $\mu\text{g}$ ) and tetracycline (30  $\mu\text{g}$ ).

**Plant growth promoting activities:** Indole-3-acetic acid was quantitatively assayed by the method of Gordon and Weber (1951) later modified by Bric *et al.* (1991). For the activity of indole acetic acid bacteria were grown in Luria Bertani (LB) broth ( $\text{g L}^{-1}$ : tryptone 10; yeast extract 5; NaCl 10 and pH 7.5). Luria Bertani broth (100 mL) supplemented with 0, 20, 40, 60, 80 and 100  $\mu\text{g mL}^{-1}$  of tryptophan was inoculated with  $10^8$  cells  $\text{mL}^{-1}$  of one mL culture and was incubated for 24 h at  $28\pm 2^\circ\text{C}$  with shaking at 125 rpm. After 24 h, 5 mL of each culture was centrifuged (10,000 rpm) for 15 min and an aliquot of 2 mL supernatant was mixed with 100  $\mu\text{L}$  of orthophosphoric acid and 4 mL of Salkowsky reagent (2% 0.5 M  $\text{FeCl}_3$  in 35% per-chloric acid) and incubated at  $28\pm 2^\circ\text{C}$  in darkness for 1 h. The absorbance of pink color developed was read at 530 nm. The Indole Acetic Acid (IAA) concentration in the supernatant was determined using a calibration curve of pure IAA as a standard (Bric *et al.*, 1991). The experiments were repeated three times on different time intervals.

The bacterial strains were further assayed for siderophore production on the Chrome Azurol S (CAS) agar medium by the method of Alexander and Zuberer (1991). Chrome Azurol S agar plates were prepared separately and divided into equal sectors and spot inoculated with 100  $\mu\text{L}$  of

$10^8$  cells  $\text{mL}^{-1}$  and incubated at  $28\pm 2^\circ\text{C}$  for 72-96 h. Development of yellow orange halo around the growth was considered as positive for siderophore production. Each individual experiment was replicated three times. The siderophore produced by the test strains were further quantitatively assayed using Modi medium ( $\text{K}_2\text{HPO}_4$  0.05%;  $\text{MgSO}_4$  0.04%; NaCl 0.01%; mannitol 1%; glutamine 0.1%;  $\text{NH}_4\text{NO}_3$  0.1%). Modi medium was inoculated with  $10^8$  cells  $\text{mL}^{-1}$  of bacterial cultures and incubated at  $28\pm 2^\circ\text{C}$  for 5 days. Catechol type phenolates were measured on ethyl acetate extracts of the culture supernatant using a modification of the ferric chloride-ferricyanide reagent of Hathway. Ethyl acetate extracts were prepared by extracting 20 mL of supernatant twice with an equal volume of solvent at pH 2. Hathway's reagent was prepared by adding 1 mL of 0.1 M ferric chloride in 0.1 N HCl to 100 mL of distilled water and to this was then added 1 mL 0.1 M potassium ferricyanide (Reeves *et al.*, 1983). For the assay, one volume of the reagent was added to one volume of sample and absorbance was determined at 560 nm for salicylates with sodium salicylate as standard and at 700 nm for dihydroxy phenols with 2, 3-dihydroxy benzoic acid as standard. Hydrogen cyanide production by bacterial isolates was detected by the method of Bakker and Schippers (1987). For HCN production, the bacterial strains were grown on an HCN induction medium (30 g tryptic soy broth, 4.4 g glycine, 15 g agar  $\text{L}^{-1}$ ) at  $28\pm 2^\circ\text{C}$  for four days. For each bacterial isolate, 100  $\mu\text{L}$  of  $10^8$  cells  $\text{mL}^{-1}$  was placed in the centre of the petri plates. A disk of Whatman filter paper No. 1 dipped in 0.5% picric acid and 2%  $\text{Na}_2\text{CO}_3$  was placed at the lid of the petri plates. Plates were sealed with parafilm. After four days incubation at  $28\pm 2^\circ\text{C}$ , an orange brown colour of the paper indicating HCN production was observed. For ammonia production, the rhizobial strains were grown in peptone water (g  $\text{L}^{-1}$ : peptone 10 g, NaCl 5 g, pH 7) and incubated at  $30\pm 2^\circ\text{C}$  for four days. One milliliter of Nessler reagent was added to each tube and the development of yellow color indicating ammonia production was recorded (Dye, 1962).

**Statistical analysis:** Data of three replicates were subjected to statistical analysis using pair samples T test with significance level of  $p < 0.05$ . The values indicate the Mean $\pm$ SD of three replicates.

## RESULTS

**Tolerance of plant growth promoting rhizobacteria to metals:** The selected plant growth promoting rhizobacterial strains were tested for their ability to tolerate various concentrations of heavy metals like cadmium, chromium, nickel, lead, zinc and copper using agar plate dilution method. Generally, the Plant Growth Promoting Rhizobacterial (PGPR) strains showed a varied level of tolerance to heavy metals. Among the *Mesorhizobium* strains, strain RC3 showed highest tolerance to most of the metals (Fig. 1). Strain RC3 tolerated a concentration of 400, 500, 500, 1500, 1500 and 400  $\mu\text{g mL}^{-1}$  of cadmium, chromium, nickel, lead, zinc and copper, respectively, amended in agar plates whereas strain RC4 showed a tolerance level of 400, 400, 400, 1400, 1400 and 300  $\mu\text{g mL}^{-1}$  to cadmium, chromium, nickel, lead, zinc and copper, respectively, added to solid plates.

**Antibiotic Resistance of plant growth promoting rhizobacteria:** Resistance to antibiotics among metal tolerant rhizobacterial strains differed considerably (Table 1). Among *Mesorhizobium* spp., 70% of strains were resistant to nalidixic acid, 60% to tetracycline and nitrofurantoin while 30% were resistant to each streptomycin and methicillin. No strain was found to be resistant to cloxacillin, ciprofloxacin and chloramphenicol.

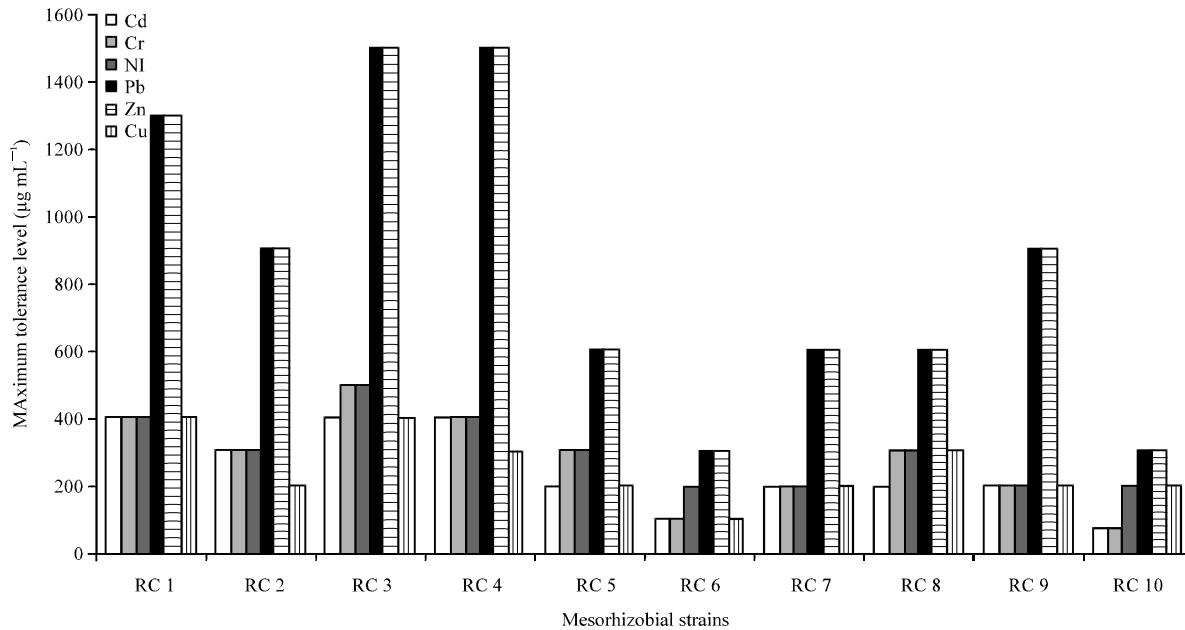


Fig. 1: Maximum tolerance level of *Mesorhizobium* strains to different heavy metals

Table 1: Resistant pattern of *Mesorhizobium* species to various antibiotics

Antibiotics used	Concentrations ( $\mu\text{g disc}^{-1}$ )	No. of resistant isolates (%)
Nalidixic acid	30	7 (70)
Ampicillin	25	1 (10)
Gentamycin	30	1 (10)
Tetracycline	30	6 (60)
Nitrofurantoin	100	6 (60)
Cloxacillin	30	ND
Streptomycin	25	3 (30)
Methicillin	30	3 (30)
Ciprofloxacin	30	ND
Chloramphenicol	25	ND

ND: Not detected, Total number of isolates = 10

**Bioassay of plant growth promoting activities:** The Plant Growth Promoting (PGP) substances like IAA, siderophore, hydrogen cyanide and ammonia synthesized by the metal tolerant PGPR strains were assayed both qualitatively and quantitatively under *in vitro* experiments and are explained as follows:

**Indole acetic acid:** The production of IAA by the metal tolerant bacterial strains was assayed in LB broth supplemented with different concentrations of tryptophan (Table 2). The *Mesorhizobium* spp. exhibited a substantial production of IAA after 24 h of incubation. Moreover, the data revealed a concentration dependent increase in IAA, the maximum being 34.5, 30.6, 27.9, 26.5, 23.5 and 10.9  $\mu\text{g}$  of IAA/mL in LB broth supplemented with 100, 80, 60, 40, 20 and 0 (without tryptophan)  $\mu\text{g}$  tryptophan/mL, respectively, by strain RC3. This was followed by strain RC4 which produced a maximum amount of 34.2, 29, 27.3, 26.1, 23.1 and 10.4  $\mu\text{g}$  IAA/mL in LB broth supplemented

Table 2: Plant growth promoting activities of *Mesorhizobium* species (N = 10) isolated from chickpea nodules

Plant growth promoting activity											
Rhizobial strains	Ammonia		Siderophores			Indole acetic acid ( $\mu\text{g mL}^{-1}$ )					
	HCN <sup>a</sup>	agar <sup>b</sup> (mm)	Zone on CAS		2,3 DHBA <sup>d</sup> ( $\text{mg L}^{-1}$ )	-----					
			Sa <sup>c</sup> ( $\text{mg L}^{-1}$ )	-----		0T <sup>e</sup>	20T	40T	60T	80T	100T
RC1	+	+	7±1	15.5±1.4	20.5±1.6	5.0±0.7	13.9±1.2	16.9±1.3	19.5±1.6	20.8±1.4	26.8±2.2
RC2	+	+	-	-	-	6.4±0.5	13.4±1.0	15.2±1.0	17.7±1.8	20.0±1.0	24.0±2.6
RC3	+	+	11±2	17.0±1.6	24.5±1.5	10.9±0.9	23.5±1.2	26.5±2.5	27.9±2.4	30.6±2.6	34.5±2.1
RC4	+	+	9±1	16.5±1.0	24.0±2.1	10.4±0.8	23.1±1.0	26.1±2.3	27.3±2.0	29.0±2.3	34.2±3.2
RC5	+	+	-	-	-	7.3±0.7	16.6±1.6	18.3±1.5	20.6±2.1	23.1±2.0	28.2±2.8
RC6	+	+	-	-	-	7.9±0.5	17.3±2.0	19.0±1.6	21.5±1.6	24.0±2.1	28.5±3.0
RC7	+	+	-	-	-	6.6±0.4	15.3±2.2	17.2±1.4	19.6±1.4	22.0±1.9	27.3±2.5
RC8	+	+	-	-	-	8.2±0.8	16.6±1.5	20.1±2.1	21.9±1.0	24.8±2.0	30.8±3.2
RC9	+	+	-	-	-	8.6±1.0	20.6±2.1	23.0±2.0	25.0±2.1	28.0±1.8	32.3±2.7
RC10	+	+	-	-	-	8.4±1.1	18.9±1.9	21.5±1.8	24.0±2.5	27.5±1.6	31.5±2.9

<sup>a</sup>: Hydrogen cyanide, <sup>b</sup>: Chrome azurol S agar, <sup>c</sup>: Salicylic acid, <sup>d</sup>: 2,3 Dihydroxy benzoic acid, <sup>e</sup>: Tryptophan concentration ( $\text{mg mL}^{-1}$ ), +: Positive reaction, -: No reaction: The values in this table indicate the Mean±SD of three replicates

with 100, 80, 60, 40, 20 and 0  $\mu\text{g}$  tryptophan/mL, respectively. While comparing the effect of various concentrations of tryptophan on IAA production by the *Mesorhizobial* strains, 100  $\mu\text{g mL}^{-1}$  tryptophan showed a significant ( $p \leq 0.05$ ) increase of 229 and 18% in IAA over 20 and 80  $\mu\text{g mL}^{-1}$  tryptophan, respectively, by the strain RC4.

**Bioassay of siderophore:** Another important trait of plant growth promoting rhizobacteria is the production of siderophores that may indirectly affect the growth of plants. In the present investigation, the metal tolerant PGPR strains were tested for both qualitative and quantitative production of siderophores using CAS agar and ethyl acetate extraction method (Table 2). On CAS agar plates, 30% of the *Mesorhizobium* strains produced siderophore. Of these strains, RC1, RC3 and RC4 displayed 7, 11 and 9 mm colored zone on CAS plates after four days of incubation. Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain RC1 yielded 15.5 and 20.5  $\text{mg mL}^{-1}$  salicylate (SA) and 2,3-dihydroxy benzoic acid (DHBA), strain RC3 produced 17 and 24.5  $\text{mg mL}^{-1}$  of SA and DHBA and strain RC4 yielded 16.5 and 24  $\text{mg mL}^{-1}$  SA and DHBA, respectively (Table 2).

**In vitro assay of ammonia and HCN:** The metal tolerant plant growth promoting rhizobacterial strains were tested further for the synthesis of ammonia and hydrogen cyanide using peptone water and HCN induction medium, respectively. Generally, all PGPR strains were found positive for ammonia and HCN (Table 2).

## DISCUSSION

Deposition of metal into soil over a long period of time results in high concentration of metal in the soil which adversely affects the microflora of the soil (Matsuda *et al.*, 2002). Heavy metals in general show adverse effect on the soil microbial flora by blocking their functional groups or these metals modify the biological molecules in particular their active sites. But these metals when present in low concentrations are important for the microbes as they supply the microorganisms

with the essential co-factors for metallo proteins and enzymes (Nies, 1999). The metal- microbe interaction in natural environment is complex and is influenced by pH or organic matter content (Saeki *et al.*, 2002). The ability to grow at concentration of metals is however, found in many plant growth promoting rhizobacteria including symbiotic nitrogen fixing bacteria (Lakzian *et al.*, 2002) and may be the result of intrinsic or induced mechanism (Giller *et al.*, 1998). There are reports that have shown a high level tolerance to heavy metals by rhizobia (Wani and Khan, 2010). Conflicting reports are, however, available in the literature on the tolerance level of rhizobia which could possibly be due to the variation in the tolerance level of rhizobia and growth conditions employed (Rajkumar *et al.*, 2005). For instance, *Rhizobium leguminosarum* isolated from metal contaminated soil tolerated 92.9  $\mu\text{M}$  of zinc (Delorme *et al.*, 2003) while *Rhizobium* species isolated from nodules of *Trifolium repense* tolerated 300  $\text{mg kg}^{-1}$  nickel and showed an effective symbiosis with its legume host, when grown in nickel amended soils (Smith and Giller, 1992). Similarly Luo *et al.* (2011), isolated *Serratia* sp. LRE07 from cadmium hyperaccumulator *Solanum nigrum* L. was resistant to the toxic effects of heavy metals.

In the present study, *Mesorhizobium* RC3 isolated from chickpea nodules, displayed a high resistance towards cadmium (400  $\mu\text{g mL}^{-1}$ ), Chromium (500  $\mu\text{g mL}^{-1}$ ), nickel (500  $\mu\text{g mL}^{-1}$ ), Lead (1500  $\mu\text{g mL}^{-1}$ ), zinc (1500  $\mu\text{g mL}^{-1}$ ) and copper (400  $\mu\text{g mL}^{-1}$ ) whereas strain RC4 showed a tolerance level of 400, 400, 400, 1400, 1400 and 300  $\mu\text{g mL}^{-1}$  to cadmium, chromium, nickel, lead, zinc and copper, respectively. *Mesorhizobium* strains showed a high tolerance to lead and zinc which was followed for chromium, whereas cadmium was found to be more toxic than the other heavy metals. The metal tolerant strains were characterized by physiological and biochemical methods. In other studies, the Gram-negative bacteria have also shown resistance to cadmium and zinc salts. For instance, cadmium and zinc tolerance by *Protobacteria*, *Actinobacteria* and *Bacteroidetes* were resistant to Zn and Cadmium (Kuffner *et al.*, 2010). Nickel and zinc tolerance by *Rhizobium leguminosarum* biovar *trifolii* isolated from sewage sludge treated soil was also reported by Purchase and Miles (2001), who observed a metal tolerance of 0.24-0.26  $\text{mM Ni}^{2+}$  and 6.0-8.0  $\text{mM Zn}^{2+}$ . Similarly, metal tolerance by *Rhizobium*, *Bradyrhizobium* and *Azotobacter* (Pajuelo *et al.*, 2008) and varying level of resistance among other PGPR (*Bacillus* and *Pseudomonas*) have also been reported (Yilmaz, 2003; Thacker *et al.*, 2007; Wasi *et al.*, 2008).

Bacterial resistance to antibiotics is an emerging problem these days. Resistance to antibiotics is acquired by a change in the genetic make up of microbes which can occur by either a genetic mutation or by transfer of antibiotic resistant genes between organisms in the environment (Spain and Alm, 2003). Furthermore, the increased use of antibiotics in health care as well as in agriculture, is in turn contributing to the growing problems of antibiotic resistant bacteria. Products such as heavy metals used in industry along with antibiotics create a selective pressure in the environment that consequently leads to the mutation in organism that will allow them better to survive and multiply. Clustering of genes on a plasmid, are beneficial to the survival of that organism and its species because those genes are more likely to be transferred together in the event of conjugation. Thus, in an environment with multiple stresses, for example antibiotics and heavy metals, it would be more ecologically favorable in terms of survival for a bacterium to acquire resistance to both stresses. If the resistance is plasmid mediated, bacteria harbouring clustered genes are more likely to pass on those genes to other neighbouring bacteria which would then have a better chance of survival. With these considerations, the antibiotic resistance among PGPR was studied which differed from antibiotic to antibiotic for all the PGPR strains. Multiple antibiotic resistances shown by PGPR strains (e.g., *Mesorhizobium* RC3, RC4) might be associated with a



high degree of tolerance to metals. In many studies, metal tolerance and antibiotic resistance have been reported (Wani *et al.*, 2009; Yilmaz, 2003; Verma *et al.*, 2001). It has been suggested that under environmental conditions of metal stress, metal and antibiotic resistant microorganisms will adapt faster by the spread of R-factors than by mutation and natural selection (Silver and Misra, 1988). Similar observations on antibiotics resistance by PGPR strains have been reported (Thacker *et al.*, 2007). The variation in the resistance to many tested antibacterial drugs (antibiotics) may possibly be due to the differences in growth conditions and exposure of PGPR to stress conditions or toxic substance as well as presence or absence of resistance mechanisms that could be encoded either by chromosome and/or R-plasmid (Spain and Alm, 2003).

In the present study *Mesorhizobium* strains were positive for plant growth promoting activities and produced substantial amount of IAA, siderophore, HCN and ammonia. Bacterial strains produce ammonia; this ammonia plays a signalling role when the plant growth promoting bacteria and plants interact with each other (Becker *et al.*, 2002). Moreover, the ammonia released by the bacterial strains are known to increase the glutamine synthetase activity (Chitra *et al.*, 2002). In addition, ammonium transporters found in several plant growth promoting rhizobacteria are thought to be involved in the reabsorption of  $\text{NH}_4^+$  released as a consequence of  $\text{NH}_3$  diffusion through the bacterial membrane (Van Dommelen *et al.*, 1997). Similarly phytohormone production (Wani and Khan, 2010; Rajkumar *et al.*, 2006; Ahmad *et al.*, 2008) and IAA by rhizobia is reported (Antoun *et al.*, 1998). Siderophores is also known to bind to the available form of iron  $\text{Fe}^{3+}$  in the rhizosphere thus making it unavailable to the phytopathogens and consequently protects the plant health. In other study, the heavy metal resistant *Bacillus* species are also known to produce considerable amount of plant growth promoting substances (Wani *et al.*, 2007).

## CONCLUSION

This study concluded that the *Mesorhizobium* strains not only tolerated heavy metals but also antibiotics. *Mesorhizobium* also produced substantial amount of plant growth promoting substances. Due to multifarious properties expressed by the *Mesorhizobium* strains, these strains could therefore, be used as bioinoculant to increase the performance of crops in soils contaminated with heavy metals.

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