Insight Microbiology 1 (1): 8-19, 2011
ISSN 2041-0387

Assessment of Pesticide-Tolerance and Functional Diversity of Bacterial Strains Isolated from Rhizospheres of Different Crops

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Abstract: Background: This study was designed to assess the pesticide-tolerance and the functional diversity of both intracellular and extracellular plant growth promoting rhizobacteria recovered from the rhizospheres of four crops (mustard, chickpea, pea, greengram, and lentil) rhizospheres. **Results:** A total of 53 rhizobacterial isolates (11, 7, 9, 8 and 18 from chickpea, pea, greengram, lentil nodules and mustard rhizosphere, respectively) were characterized on the basis of morphological, biochemical and host specificity tests and were found to belong to genera *Mesorhizobium*, *Rhizobium* (pea), *Bradyrhizobium*, *Rhizobium* (lentil), *Pseudomonas*, *Bacillus*, *Enterobacter* and *Klebsiella*. Most promising rhizobacterial strains (*Pseudomonas*, *Enterobacter* and *Klebsiella*) were identified based on 16S rDNA sequencing. All the rhizobacterial strains generally, tolerated the selected 12 pesticides [herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl, and kitazin)] in a range of 400 to 3200 μg mL^{-1}. Most of the strains produced substantial amount of one or more Plant Growth Promoting (PGP) substances like indole-3-acetic acid, siderophores, exo-polysaccharides, hydrogen cyanide, ammonia and phosphate solubilization. **Conclusions:** Substantial variation was observed among the bacterial strains of each rhizosphere taking into consideration the amount and type of PGP activity. Generally, the mustard rhizosphere exhibited more functionally diverse rhizobacteria than that of the selected legumes.

Key words: Pesticide, rhizobacteria, resistance, rhizosphere, functional diversity

INTRODUCTION

The soil ecosystem is supported by the several interactions among its physical, chemical and biological components (Buscot, 2005). Principally, the genetic and the functional activities of the heterogeneous microorganisms have a crucial effect on soil functions (Nannipieri *et al*., 2003). Microbial communities of soils also interact with plant roots and soil constituents at the root–soil interface (Khan *et al*., 2009; Attitalla *et al*., 2011). Due to abundantly available nutrients on the root surface, the population and activities of microbes in this region is higher than that of the region away from it. This nutrient-rich region having high microbial activity surrounding the plant root is called rhizosphere (Dessaux *et al*., 2009) and the bacteria inhabiting rhizosphere are called rhizobacteria (Yasmin *et al*., 2007; Zaidi *et al*., 2009). Moreover, plant growth-promoting rhizobacteria (PGPR) are free-living (outside root cells/ extracellular/ePGPR) or nodular (inside root cells/ intracellular/iPGPR), soil-borne bacteria which, when applied to seeds/soils or crops, enhance the growth of the plant directly by providing nutrients to plants or indirectly by reducing the damage from soil-borne plant pathogens (Mia and Shamsuddin, 2009; Wani and Khan, 2010; Verma *et al*., 2010a, b).

In current agricultural practices, farmers apply pesticides (including herbicides, insecticides and fungicides) of various chemical groups indiscriminately to control the pests and phytopathogens which are detrimental for crop productivity. Consequently, a large amount of pesticides reach the soils and persists for long periods and destabilizes the soil-ecosystem (Ahemad *et al*., 2009) causing harm to PGPR and eventually the plant growth (Guo *et al*., 2007; Fox *et al*., 2007). In addition, organic pesticides applied to soil may be used as substrates by the tolerant microorganisms and undergo degradation, resulting in the formation of new compounds which may be far more deleterious to the growing plants than the parent molecule (Ahemad and Khan, 2010). Pesticides and their degraded products in soils interact with PGPR including rhizobia and cause DNA, protein, oxidative or membrane damage (Pham *et al*., 2004).

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Furthermore, these plant protection agents have been shown to adversely affect N_2-fixation b slowing down the rhizobial growth and metabolism (Ahemad and Khan, 2010).

Pulses are important source of dietary proteins, and have unique property of maintaining and restoring soil fertility through biological N_2 fixation as well as conserving and improving physical properties of soil by virtue of their deep root system and leaf fall. Pulse crops add a reasonable quantity of nitrogen (upto 30 kg^{-1} N ha^{-1}) to soils (ICAR, 2006). Of the different legumes grown around the world, chickpea, pea, greengram and lentil are the most widely grown legumes. In addition, mustard is one the important oil crop which is globally cultivated. Most of the earlier studies of rhizospheres are mainly concentrated on the diversity of rhizobacteria (in terms of the number) generally, restricted to any single crop and comprehensive data assessing the functional diversity along with pesticide-tolerance levels of rhizobacteria are rare. An understanding of functional diversity of rhizobacteria is essential in order to harness the full potentials of these microbes for the sustained growth of crops in different agro-ecosystems. The present study was therefore, designed to evaluate the pattern of pesticide-tolerance and plant growth promoting (PGP) activities of PGPR from rhizospheres of five different crops so that a firm conclusion can be drawn about the pesticide-tolerance vis-à-vis the functional diversity among rhizobacteria.

MATERIALS AND METHODS

Isolation of nitrogen fixing bacteria: The nitrogen fixing bacteria were isolated from the nodules of chickpeas (*Cicer arietinum* L.), pea (*Pisum sativum*), greengram (*Vigna radiata* L. Wiclzek) and lentil (*Lens esculentus*) grown at the experimental fields (sandy clay loam, organic C 0.4%, Kjeldahl N 0.75 g kg^{-1}, Olsen P 16 mg kg^{-1}, pH 7.2 and water holding capacity 0.44 mL g^{-1}, cation exchange capacity 11.7 and 5.1 cmol kg^{-1} anion exchange capacity) of Faculty of Agricultural Sciences, Aligarh Muslim University Aligarh (27° 29' latitude and 72° 29' longitude), U.P., India, using standard method (Vincent, 1970). The nodules removed from the root system of each legume plant were surface sterilized with 2.5% sodium hypochlorite for 2 min, following a rinse in 95% ethanol (v/v) and washing six times with sterile water and squashed in normal saline solution. Nodule suspensions were diluted in normal saline solution and 10 µL of each suspension was spread plated on solid yeast extract mannitol (YEM) medium (g L^{-1}: mannitol 10; K_2HPO_4 0.5; $MgSO_4.7H_2O$ 0.2; NaCl 0.1; yeast extract 1.0; $CaCO_3$ 1.0; pH 7) supplemented with 2.5% Congo red indicator. The plates were incubated at 28±2°C for five days. The single colony was picked and streaked four times on the same medium to affirm the purity of the cultures. In this way, 11, 7, 9, 8 bacterial colonies exhibiting luxurious growth were selected and purified from the chickpeas, pea, greengram and lentil nodules, respectively. Isolated colonies were maintained on the YEM agar medium in slants at 4±1°C for further experimentation.

Isolation of phosphate solubilizing bacteria: The phosphate solubilizing microorganisms were isolated from the rhizospheric soils of mustard (*Brassica compestris*), grown at the experimental fields of Faculty of Agricultural Sciences, A. M. U., Aligarh, using Pikovskaya agar (gL^{-1}: glucose 10; $Ca_3(PO_4)_2$ 5; $(NH_4)_2SO_4$ 0.5; NaCl 0.2; $MgSO_4.7H_2O$ 0.1; KCl 0.1; yeast extract 0.5; $MnSO_4$ and $FeSO_4$ trace; agar 15; pH 7.0) medium (Pikovskaya, 1948) by spread plate method. A 10 µL of serially diluted suspension was spread plated on solid Pikovskaya medium. Plates were incubated at 28±2°C for seven days. The isolates showing clear halo formed within seven days around bacterial colonies were considered as phosphate solubilizers. A total of 18 phosphate solubilizing isolates with maximum halo sizes and different pigmentations and morphological parameters were selected. The phosphate solubilizers were maintained on solid Pikovskaya agar medium until use.

Characterization and molecular identification: A total number of 53 rhizobacteria from mustard (18), chickpea (11), pea (7), greengram (9) and lentil (8) rhizospheres PS19 were characterized and identified by using the standard morphological, physiological and biochemical tests (Holt *et al*., 1994). Based on their characteristics, the rhizobacteria were identified as *Pseudomonas* (PS1 and PS9), *Enterobacter* (PS2), *Bacillus* (PS3, PS4, PS5, PS6, PS7, PS10, PS12, PS14, PS16, PS17, PS20, PS21, PS22, and PS23), *Klebsiella* (PS19) and *Rhizobium* (MRC1, MRC3, MRC4, MRC5, MRC6, MRC7, MRC9, MRC10, MRC11, MRC12, MRC14, MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, MRP7, MRM1, MRM2, MRM3, MRM4, MRM5, MRM6, MRM7, MRM8, MRM9, MRL1, MRL2, MRL3, MRL4, MRL5, MRL6, MRL7, and MRL8). Moreover, morphologically and biochemically identified rhizobia from chickpea, pea, greengram, and lentil nodules were tested for their host-specificity in sterile soils (Somasegaran and Hoben, 1994) and referred to as *Mesorhizobium*, *Rhizobium*, *Bradyrhizobium* and *Rhizobium*, respectively.

Among all the rhizobacterial strains, *Pseudomonas* strain PS1, *Enterobacter* strain PS2, *Pseudomonas* strain PS9 and *Klebsiella* strain PS19 were proved to be most promising. To further consolidate the identity of the strain

PS1, PS2, PS9 and PS19, 16S rDNA sequence analysis these strains was performed commercially at Macrogen Inc., Seoul, South Korea using universal primers, 518F (5'CCAGCAGCCGCGGTAATACG3') and 800R (5'TACCAGGGTATCTAATCC3'). The 16S rDNA of PS1, PS2, PS9 and PS19 were found to be approximately 950 bp, 947 bp, 845 bp and 944 bp, respectively in size and the sequences of 16S rDNA of these strains were submitted to Gen-Bank (Gene Bank accession numbers: FJ705886; FJ705887; FJ705888 and FJ705889, respectively). A similar search was performed by using the BLAST program that indicated the strains PS1, PS2, PS9 and PS19 shared a close relationship with the DNA sequence of *Pseudomonas aeruginosa* strain MW3AC (16S: 99% similarity with the reference strain GQ180118), *Enterobacter asburiae* strain J2S4 (16S: 99% similarity with the reference strain EU221358), *Pseudomonas putida* strain ATCC 17514 (16S: 99% similarity with the reference strain AF094741), and *Klebsiella* sp. WW2 (16S: 98% similarity with the reference strain EF433545), respectively in NCBI database. Such high similar values confirmed the strain PS1, PS2, PS9 and PS19 to be *Pseudomonas aeruginosa*, *Enterobacter asburiae*, *Pseudomonas putida* and *Klebsiella* sp., respectively.

Evaluation of rhizobacterial strains for pesticide tolerance: Herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin) (Table 1) were specifically selected for this study due to their extensive application. Concentrations of these pesticides were calculated on the basis of the percentage of the active ingredients in the formulations. Stock solutions of these pesticides were prepared in an appropriate solvent and to prevent pesticide degradation, stock solutions were prepared just prior to each experiment. The rhizobacterial strains were tested for the tolerance against 12 pesticides by agar plate dilution method using minimal salt agar medium (g L^{-1}: KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $MgSO_4.7H_2O$ 0.2; $CaCl_2.2H_2O$ 0.02; $FeSO_4.7H2O$ 0.01; agar 15; pH 6.5). The freshly prepared agar plates amended separately with increasing concentration (0 to 3200 μg mL^{-1}; at a two fold dilution interval) of herbicides, insecticides and fungicides were spot inoculated with 10 μL^{-1} of 10^8 cells mL^{-1} of each rhizobacterial strain. Plates were incubated at 28±2°C for five days and the highest concentration of pesticides supporting rhizobacterial growth was defined as the maximum tolerance level (MTL). Each experiment was conducted in triplicate.

Bioassay of plant growth promoting activities: Plant growth promoting activities [e.g., indole-3-acetic acid (IAA), siderophore, phosphate (P) solubilization, hydrogen cyanide and ammonia] of rhizobacteria were assessed under *in vitro* conditions. Indole-3-acetic acid synthesized by rhizobacterial strains was quantitatively

Table 1: Agrochemicals used in the present study

Common name		Grade (purity)	Chemical name	Chemical family	Source
Herbicides	Quizalafop-p-ethyl	Technical (98% w/w)[a]	(*RS*)-2-[4-(6-chloroquinoxalin-2-yloxy) phenoxy]propionic acid	Aryloxyphenoxy	Parijat Agrochemicals, New Delhi, India
	Clodinafop	Technical (98% w/w)	(*R*)-2-[4-(5-chloro-3-fluoro-2-pyridyloxy) phenoxy]propionic acid	Aryloxyphenoxy	Parijat Agrochemicals, New Delhi, India
	Metribuzin	Commercial (70% w/w)	4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one	Triazinone	Singhal Pesticides, Mumbai, India
	Glyphosate	Commercial (71% w/w)	*N*-(phosphonomethyl)glycine	Organophosphorus	Excel Crop Core LTD., Mumbai, India
Insecticides	Fipronil	Technical (98% w/w)	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-toly l)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile	Phenylpyrazole	Parijat Agrochemicals, New Delhi, India
	Pyriproxyfen	Technical (98% w/w)	4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether	Juvenile hormone mimics	Parijat Agrochemicals, New Delhi, India
	Imidacloprid	Technical (100% EC)[b]	(*E*)-1-(6-chloro-3-pyridylmethyl) -*N*-nitroimidazolidin-2-ylideneamine	Pyridylmethylamine	Parijat Agrochemicals, New Delhi, India
	Thiamethoxam	Technical (100% w/w)	(*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl) -5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro)amine	Thiazole	Parijat Agrochemicals, New Delhi, India
Fungicides	Tebuconazole	Technical (100% w/w)	(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol	Conazole	Parijat Agrochemicals, New Delhi, India
	Hexaconazole	Technical (100% w/w)	(*RS*)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)hexan-2-ol	Conazole	Parijat Agrochemicals, New Delhi, India
	Metalaxyl	Commercial (35% w/w)	methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate	Anilide	Tropical Agrosystem LTD., Chennai, India
	Ketazin	Commercial (48% EC)	O,O-Bis(1-methylethyl) S-phenylmethyl phosphorothioate	Organophosphate	P.I. Industries LTD., Rajasthan, India

[a]Weight/Weight; [b]Emulsified concentration

evaluated by the method of Gordon and Weber (1951) and Bric *et al*. (1991). The rhizobacterial strains were further tested for siderophore production using Chrome Azurol S (CAS) agar medium following the method of Alexander and Zuberer (1991). Catechol type phenolates [salicylic acid (SA) and 2,3-dihydroxybenzoic acid (DHBA)] were measured on ethyl acetate extracts of the culture supernatant using the ferric chloride-ferricyanide reagent of Hathway (Reeves *et al*., 1983). The exo-polysaccharide (EPS) produced by the rhizobacterial strains was determined under *in vitro* conditions as suggested by Mody *et al*. (1989).

The rhizobacterial strains showing phosphate (P) solubilizing activity during screening process were inoculated into Pikovskaya agar medium and incubated at 28±2°C for seven days and observed for halo formation. The solubilization index (SI) of the P- solubilizing organism was calculated as: zone size including colony diameter - colony diameter/ colony diameter. The colony forming clear halo around bacterial growth was measured and the amount of water soluble P released into the culture supernatant was estimated by the chlorostannous-reduced molybdophosphoric acid blue method (King, 1932; Jackson, 1967). Hydrogen cyanide (HCN) and ammonia production by rhizobacterial strains was detected by the method of Bakker and Schippers (1987) and Dye (1962), respectively.

A control without organisms was also run parallel for each experiment. Each independent experiment was repeated three times after several sub-cultures.

Statistical analysis: The experiments were conducted in three replicates using the same treatments. The difference among treatment means was compared by high range statistical domain (HSD) with Tukey test at 5% probability level using software, SPSS 10. The correlation (r) between data was evaluated through Sigma Plot 10.

RESULTS

Tolerance of the rhizobacterial strains against pesticides: All the rhizobacterial strains were tested for their tolerance against 12 pesticides [herbicides (quizalafop-p-ethyl, clodinafop, metribuzin and glyphosate), insecticides (fipronil, pyriproxyfen, imidacloprid and thiamethoxam) and fungicides (tebuconazole, hexaconazole, metalaxyl and kitazin). Generally, the rhizobacterial strains tolerated all the pesticides substantially. Among herbicides, the rhizobacterial strains tolerated concentrations up to 3200 μg of metribuzin mL^{-1}, 3000 μg of glyphosate mL^{-1}, 2400 μg of clodinafop mL^{-} and 1600 μg of quizalafop-p-ethyl mL^{-1} amended in minimal salt agar medium. Of insecticides, the rhizobacterial strains however, tolerated concentrations up to 2800 μg of fipronil mL^{-1}, 2800 μg of thiamethoxam mL^{-1}, 2400 μg of imidacloprid mL^{-1} and 1800 μg of pyriproxyfen mL^{-1}. Moreover, the selected rhizobacteria tolerated up to 3200, 2800, 2400, 1800 μg mL^{-1} of ketazin, metalaxyl, hexaconazole and tebuconazole, respectively among fungicides (Table 2).

Functional diversity among plant growth promoting rhizobacteria: A total of 53 rhizobacterial strains were screened for their multiple PGP traits. Based on the PGP activity expressed by the rhizobacterial strains under *in vitro* conditions, these strains were grouped into 10 PGP groups (Table 3). The PGP group I included 7 strains which showed 6 PGP traits like phosphate solubilization,

Table 2: Pesticide-tolerance in rhizobacterial strains[1]

	Herbicides				Insecticides				Fungicides			
MTL (μg mL^{-1})	Quizalafop -p-ethyl	Clodinafop	Metribuzin	Glyphosate	Fipronil	Pyriproxyfen	Imidacloprid	Thiamethoxam	Tebuconazole	Hexaconazole	Metalaxyl	Ketazin
100	-	-	-	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	-
400	-	-	-	-	-	-	-	-	1 (1.9)	-	-	-
600	-	-	-	-	1 (1.9)	3 (5.7)	-	-	1 (1.9)	-	-	-
800	8 (15)	2 (3.8)	-	-	1 (1.9)	7 (13.2)	-	-	3 (5.7)	-	-	-
1000	9 (17)	4 (7.5)	-	-	6 (11.3)	11 (20.8)	2 (3.8)	2 (3.8)	7 (13.2)	1 (1.9)	-	-
1200	13 (24.5)	10 (18.9)	-	-	14 (26.4)	15 (28.3)	10 (18.9)	1 (1.9)	11 (20.8)	8 (15)	1 (1.9)	-
1400	14 (26.4)	14 (26.4)	-	2 (3.8)	13 (24.5)	10 (18.9)	11 (20.8)	2 (3.8)	15 (28.3)	12 (22.6)	2 (3.8)	-
1600	9 (17)	18 (34)	-	3 (5.7)	13 (24.5)	6 (11.3)	10 (18.9)	13 (24.5)	10 (18.9)	12 (22.6)	6 (11.3)	3 (5.7)
1800	-	2 (3.8)	1 (1.9)	5 (9.4)	1 (1.9)	1 (1.9)	6 (11.3)	10 (18.9)	5 (9.4)	9 (17)	12 (22.6)	4 (7.5)
2000	-	1 (1.9)	4 (7.5)	15 (28.3)	-	-	8 (15)	10 (18.9)	-	6 (11.3)	8 (15)	10 (18.9)
2200	-	1 (1.9)	8 (15)	6 (11.3)	-	-	3 (5.7)	5 (9.4)	-	3 (5.7)	11(20.8)	8 (15)
2400	-	1 (1.9)	12 (22.6)	9 (17)	1 (1.9)	-	3 (5.7)	4 (7.5)	-	2 (3.8)	6 (11.3)	7 (13.2)
2600	-	-	10 (18.9)	4 (7.5)	2 (3.8)	-	-	5 (9.4)	-	-	4 (7.5)	6 (11.3)
2800	-	-	7 (13.2)	7 (13.2)	1 (1.9)	-	-	1 (1.9)	-	-	3 (5.7)	7 (13.2)
3000	-	-	5 (9.4)	2 (3.8)	-	-	-	-	-	-	-	5 (9.4)
3200	-	-	6 (11.3)	-	-	-	-	-	-	-	-	3 (5.7)

Values in parentheses indicate the percentage of the total strains. [1]Total number of strains = 53 -: Not detected

Table 3: Plant growth promoting activity based typing of rhizobacterial strains [1]

PGP group	Strains	Plant growth promoting traits: P- solubilization	Siderophores	IAA[A]	EPS[B]	Ammonia	HCN[C]	Activity profile
I	PS1, PS2, PS9, PS19	4 (7.5)	4 (7.5)	4 (7.5)	4 (7.5)	4 (7.5)	4 (7.5)	PS[D], S[E], IAA, EPS, A[F], HCN
II	PS5, PS7, PS12, PS14, PS22, PS23	6 (11.3)	6 (11.3)	6 (11.3)	6 (11.3)	6 (11.3)	-	PS, S, IAA, EPS, A
III	PS3, PS4, PS10, PS16, PS20	5 (9.4)	-	5 (9.4)	5 (9.4)	5 (9.4)	5 (9.4)	PS, IAA, EPS, A, HCN
IV	MRC1, MRC4, MRC7, MRC10, MRP1, MRP4 MRP7, MRM3, MRM6, MRM8, MRL1, MRL3, MRL6	-	13 (24.5)	13 (24.5)	13 (24.5)	13 (24.5)	13 (24.5)	S, IAA, EPS, A, HCN
V	PS6, PS17, PS21	3 (5.7)	-	3 (5.7)	3 (5.7)	3 (5.7)	-	PS, IAA, EPS, A
VI	MRC3, MRC9, MRC12, MRP3, MRP5, MRP6, MRL2, MRL4, MRL8	-	-	9 (17)	-	9 (17)	9 (17)	IAA, A, HCN
VII	MRRM1, MRRM2, MRM5, MRM7, MRM9, MRL7	-	6 (11.3)	6 (11.3)	-	6 (11.3)	-	S, IAA, A
VIII	MRC5, MRC6, MRC11, MRM4, MRL5	-	-	5 (9.4)	-	5 (9.4)	-	IAA, A
IX	MRP2	-	-	1 (1.9)	-	-	1 (1.9)	IAA, HCN
X	MRC14	-	-	1 (1.9)	-	-	-	IAA
Total PGP strains		18 (33)	28 (53)	53 (100)	31(58)	51 (96)	32 (60)	

Values in parentheses indicate the percentage of the total strains. [A]Indole-3-acetic acid, [B]exopolysaccharides, [C]Hydrogen cyanide, [D]Phosphate solubilization, [E]siderophore, [F]Ammonia. [1]Total number of strains = 53 -: Not detected

production of ammonia, HCN, siderophore, IAA and EPS followed by PGP group II which had 6 strains positive to 5 PGP traits: phosphate solubilization, ammonia, siderophore, IAA and EPS). In PGP group III, 5 strains exhibited a positive reaction to 5 PGP traits such as phosphate solubilization, ammonia, HCN, IAA and EPS production, whereas PGP group IV included 13 rhizobacterial strains which showed 5 PGP traits: the synthesis of ammonia, HCN, siderophore, IAA and EPS. Moreover, PGP group V having 3 rhizobacteria expressed 4 PGP traits such as phosphate solubilization, ammonia, siderophore, IAA and EPS production. Both PGP group VI (9 rhizobacterial strains) and VII (6 rhizobacterial strains) displayed 3 PGP traits [(IAA, ammonia, and HCN) and (siderophores, IAA and ammonia production) respectively]. The PGP group VIII had 5 rhizobacterial strains with 2 PGP traits like, synthesis of IAA and ammonia. In contrast, each PGP group IX and X exhibiting only one rhizobacterial strain showed 2 (IAA and HCN) and 1 (IAA) PGP property, respectively. Overall, 33% of rhizobacterial strains had phosphate solubilizing potential while 53, 100, 58, 96 and 60% of rhizobacterial strains released siderophores, IAA, EPS, ammonia and HCN, respectively (Table 3).

Plant growth promoting activities: The plant growth promoting substances like IAA, phosphate solubilization, siderophore, HCN and ammonia synthesized by the rhizobacterial strains were determined both qualitatively and quantitatively under *in vitro* conditions.

Indole-3-acetic acid production: The production of IAA by the selected rhizobacterial strains namely, *Mesorhizobium* spp. (n = 11), *Rhizobium* spp. (pea, n = 7), *Bradyrhizobium* spp. (n = 9), *Rhizobium* spp. (lentil, n = 8) and PSB (n = 18) was assayed in LB broth supplemented with a concentration of (100 μg mL^{-1}) tryptophan. The *Mesorhizobium* spp. exhibited a substantial production of IAA after seven days of incubation. Moreover, a wide range of variability in the secreted amount of IAA was observed among rhizobial strains. Of the mesorhizobial strains, strain MRC4 produced a maximum amount (44 μg mL^{-1}) of IAA and was followed by strain MRC 5, which produced 43 μg IAA mL^{-1} in LB broth. Generally, the amount of IAA synthesized by mesorhizobial strains varied between 14 (MRC10) to 44 μg mL^{-1} (MRC4). The percent increase in IAA synthesized by MRC4 over other mesorhizobial strains ranged between 2 (MRC5) to 68 (MRC10) (Table 4). Among the pea specific *Rhizobium* strains, strain MRP1 p roduced a detectable amount (32 μg mL^{-1}) of IAA in LB broth. This was followed by strain MRP3 which produced 28 μg IAA mL^{-1} in LB broth. In general, the amount of IAA synthesized by rhizobial strains varied between 17 (MRP4) to 32 μg mL^{-1} (MRP1). The percent increase in IAA synthesized by MRP1 over other rhizobial strains ranged between 12 (MRP3) to 47 (MRP4) (Table 5). *Bradyrhizobium* strains also produced a significant amount of IAA, maximum being 38 μg mL^{-1} IAA by the strain MRM6 followed by MRM5 (34 μg mL^{-1}). Indole acetic acid synthesized by bradyrhizobial strains varied between 15 (MRM7) to 38 μg mL^{-1} (MRM6). The increase in IAA synthesized by MRM6 over other rhizobial strains ranged between 11% (MRM5) to 61% (MRM7) (Table 6). In comparison, *Rhizobium* strains isolated from lentil nodules showed a substantial production of IAA. For instance, strain MRL3 secreted highest amounts (37 μg mL^{-1}) of IAA and was followed by strain MRL5 that produced 30 μg mL^{-1} IAA. Generally, IAA production by rhizobial strains varied between 15 (MRL2, MRL7) to 37 μg mL^{-1} (MRL3).

Table 4: Plant growth promoting activities of *Mesorhizobium* species (n = 11) isolated from chickpea nodules

Rhizobacterial strains	Plant growth promoting activities: Siderophores: Zone[a] (mm)	SA[b] (µg mL^{-1})	DHBA[c] (µg mL^{-1})	IAA[d] (µg mL^{-1}) 100T[e]	EPS[f] (µg mL^{-1})	Ammonia	HCN[g]
MRC1	11±1b	30±1.7b	17±1.3c	18.±1.5d	16±1.5b	+	+
MRC3	-	-	-	15±2.2g	-	+	+
MRC4	12±1a	35±1.5a	19±1.7a	44±2.4a	21±2.3a	+	+
MRC5	-	-	-	43±2.5ab	-	+	-
MRC6	-	-	-	31±2.3c	-	+	-
MRC7	10±1c	25±1.0c	18±1.4b	18±1.3d	10±1.1c	+	+
MRC9	-	-	-	16±2.4f	-	+	+
MRC10	9±1d	21±1.2d	17±1.1c	14±2.5h	8±0.7d	+	+
MRC11	-	-	-	17±1.9e	-	+	-
MRC12	-	-	-	17±1.7e	-	+	+
MRC14	-	-	-	15±1.5g	-	-	-
F-value	14.5	144.2	78.5	158.4	41.7		

Values indicate Mean±SD of three replicates. Mean values (±SD) followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test. [a]Chrome azurol S (CAS) agar, [b]Salicylic acid, [c]2,3 Dihydroxy benzoic acid, [d]Indole-3-acetic acid, [e]Tryptophan concentration (µg ml^{-1}), [f]Exo-polysaccharides, gHydrogen cyanide, indicates positive reaction, indicates no reaction

Table 5: Plant growth promoting activities of *Rhizobium* species (n = 7) isolated from pea nodules

Rhizobacterial strains	Plant growth promoting activities: Siderophores: Zone[a] (mm)	SA[b] (µg mL^{-1})	DHBA[c] (µg mL^{-1})	IAA[d] (µg mL^{-1}) 100T[e]	EPS[f] (µg mL^{-1})	Ammonia	HCN[g]
MRP1	11±1a	32±1.5a	22±1.1a	32±1.6a	20±1.3a	+	+
MRP2	-	-	-	19±2.1f	-	-	+
MRP3	-	-	-	28±2.3b	-	+	+
MRP4	10±1b	29±1.9b	18±0.9b	17±0.8g	15±1.7c	+	+
MRP5	-	-	-	27±1.3c	-	+	+
MRP6	-	-	-	25±2.6d	-	+	+
MRP7	11±2a	25±1.2c	14±1.3c	23±1.4e	18±1.6b	+	+
F value	21.7	29.5	35.2	22.4	32.5		

Values indicate Mean±SD of three replicates. Mean values (±SD) followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test. [a]Chrome azurol S (CAS) agar, [b]Salicylic acid, [c]2,3 Dihydroxy benzoic acid, [d]Indole-3-acetic acid, [e]Tryptophan concentration (µg mL^{-1}), [f]Exo-polysaccharides, [g]Hydrogen cyanide, Indicates positive reaction, Indicates no reaction

Table 6: Plant growth promoting activities of *Bradyrhizobium* species (n = 9) isolated from nodules of greengram plants

Rhizobacterial strains	Plant growth promoting activities: Siderophores: Zone[a] (mm)	SA[b] (µg mL^{-1})	DHBA[c] (µg mL^{-1})	IAA[d] (µg mL^{-1}) 100T[e]	EPS[f] (µg mL^{-1})	Ammonia	HCN[g]
MRM1	-	-	-	17±2.2g	-	+	-
MRM2	-	-	-	29±1.1c	-	+	-
MRM3	10±1c	30±2.3b	15±1.2c	17±1.3g	13±1.4c	+	+
MRM4	-	-	-	23±1.4d	-	+	-
MRM5	-	-	-	34±1.7b	-	+	-
MRM6	13±1a	32±1.8a	18±1.3a	38±1.8a	21±1.6a	+	+
MRM7	-	-	-	15±2.4h	-	+	-
MRM8	11±1b	28±1.5c	16±2.1b	18±1.2f	14±1.3b	+	+
MRM9	-	-	-	21±1.6e	-	+	-
F value	34.6	12.0	41.3	38.5	17.7		

Values indicate Mean±SD of three replicates. Mean values (±SD) followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test. [a]Chrome azurol S (CAS) agar, [b]Salicylic acid, [c]2,3 Dihydroxy benzoic acid, [d]Indole-3-acetic acid, [e]Tryptophan concentration (µg mL^{-1}), [f]Exo-polysaccharides, [g]Hydrogen cyanide, Indicates positive reaction, Indicates no reaction

The increase in IAA secreted by MRL3 relative to other rhizobial strains ranged between 19 (MRL5) to 59% (MRL2, MRL7) (Table 7). Similarly, the IAA production by phosphate solubilizing bacteria (n=18) was also assayed in this study (Table 8). Of these, *Klebsiella* sp. PS19 was most effective and produced a highest amount of IAA (42 µg mL^{-1}) followed by *Pseudomonas aeruginosa* PS1 (39 µg mL^{-1}), *Pseudomonas putida* PS9 (34 µg mL^{-1}) and *Enterobacter asburiae* PS2 (32 µg mL^{-1}) under normal conditions. Summarily, IAA synthesis by PSB strains varied between 9 (*Bacillus* sp. PS23) to 42 µg mL^{-1} (*Klebsiella* sp. PS19). The percent

Table 7: Plant growth promoting activities of *Rhizobium* species (n = 8) from lentil-nodules

Rhizobacterial strains	Plant growth promoting activities: Siderophores: Zone[a] (mm)	SA[b] (μg mL^{-1})	DHBA[c] (μg mL^{-1})	IAA[d] (μg mL^{-1}) 100T[e]	EPS[f] (μg mL^{-1})	Ammonia	HCN[g]
MRL1	10±2b	26±2.4c	18±1.2b	21±1.5d	13±0.8b	+	+
MRL2	-	-	-	15±2.3g	-	+	+
MRL3	12±1a	29±1.6a	21±1.4a	37±1.7a	18±1.6a	+	+
MRL4	-	-	-	23±1.6c	-	+	+
MRL5	-	-	-	30±1.1b	-	+	-
MRL6	10±1b	27±1.2b	17±1.5c	18±2.2e	11±1.0c	+	+
MRL7	10±2b	25±1.7d	15±1.4d	15±1.3g	-	+	-
MRL8	-	-	-	17±1.4f	-	+	+
F value	81.7	48.2	105.5	165.9	29.6		

Values indicate Mean±SD of three replicates. Mean values (±SD) followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test. [a]Chrome azurol S (CAS) agar, [b]Salicylic acid, c2,3 Dihydroxy benzoic acid, [d]Indole-3-acetic acid, [e]Tryptophan concentration (μg mL^{-1}), [f]Exo-polysaccharides, [g]Hydrogen cyanide, Indicates positive reaction, Indicates no reaction

enhancement in IAA synthesis by *Klebsiella* sp. PS19 over other phosphate solubilizer ranged between 7 (*Pseudomonas aeruginosa* PS1) to 79 (*Bacillus* sp. PS23).

Siderophore production: In the present investigation, the production of siderophores was assayed qualitatively as well as quantitatively using CAS agar and ethyl acetate extraction method, respectively. On the CAS agar plates, a total of 36% of the *Mesorhizobium* strains produced siderophores. The siderophore as detected by the formation of orange yellow halo on CAS agar plates after five days of incubation varied between 9 (MRC10) to 12 mm (MRC4). Further, the ethyl acetate extraction from culture supernatant of *Mesorhizobium* strain MRC4 yielded the highest amount of siderophores (35 μg mL^{-1} SA and 19 μg mL^{-1} DHBA) among all mesorhizobial strains. The strain MRC4 displayed a substantial increase in SA (34%) and DHBA (12%) over the lowest siderophore producing bacterial strain (MRC10) (Table 4). Among the *Rhizobium* species isolated from pea nodules, only three strains were positive for siderophore activity where strain MRP1, MRP4 and MRP7 demonstrated 11, 10 and 11 mm orange yellow colored zone on CAS plates after five days of incubation (Table 5). Further, these strains produced 32 and 22 (strain MRP1), 29 and 18 (MRP4) and 25 and 14 (MRP7) μg mL^{-1} SA and DHBA, respectively. Of all the siderophore producing rhizobial strains, strain MRP1 showed a considerable increase in SA (22%) and DHBA (36%) compared to the lowest siderophore producing rhizobial strain (MRP7) (Table 5). Strains MRM3, MRM6 and MRM8 of *Bradyrhizobium* species showed 10, 13 and 11 mm orange yellow colored zone, respectively, on CAS agar plates after five days of incubation and produced 30 and 15 (strain MRM3), 32 and 18 (MRM6) and 28 and 16 (MRM8) μg mL^{-1} SA and DHBA, respectively. Among all the siderophore producing bradyrhizobial strains, strain MRM6 displayed a substantial augmentation in SA (13%) and DHBA (11%) relative to the lowest siderophore producing strain MRM8 (Table 6). Furthermore, among the *Rhizobium* species isolated from lentil nodules, four rhizobial strains showed a positive reaction to siderophores both on CAS agar plates and in liquid culture medium. The siderophore halo size produced by such strains ranged between 10 (strain MRL1, MRL3, MRL7) to 12 mm (MRL6). Among all the siderophore producing rhizobial strains, strain MRL3 displayed a substantial increase in SA (14%) and DHBA (29%) over the lowest siderophore producing rhizobial strain (MRL7) (Table 7). The phosphate solubilizing bacteria (*Pseudomonas*, *Bacillus*, *Enterobacter* and *Klebsiella*) were also analyzed for siderophore production (Table 8). A total of 55% strains of the selected phosphate solubilizing bacteria displayed the siderophore activity on CAS agar plates and also in liquid culture medium. Among the phosphate solubilizers, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 had 15, 13, 14 and 14 mm colored zone, respectively on CAS plates. In liquid culture medium, *Pseudomonas aeruginosa* PS1 showed 41 and 21 μg mL^{-1} of SA and DHBA production. *Enterobacter asburiae* PS2 produced 24 and 9, *Pseudomonas putida* PS9 produced 41 and 17 and *Klebsiella* sp. PS19 produced 47 and 10 μg mL^{-1} of SA and DHBA, respectively. Of the siderophore producing phosphate solubilizing bacterial strains, *Pseudomonas aeruginosa* PS1 displayed considerable increase in SA (73%) and DHBA (76%) compared to the lowest siderophore producing bacterial strain (*Bacillus* sp. PS5) (Table 8).

Exo-polysaccharidesproduction: Theexo-polysaccharides secretion by the pesticide tolerant bacterial strains was determined in culture supernatant (Table 4-8). Generally,

Table 8: Plant growth promoting potentials of rhizobacteria (n = 18) isolated from mustard rhizosphere

Rhizobacterial strains	Plant growth promoting activities: Siderophores: Zone[a] (mm)	SA[b] (μg mL^{-1})	DHBA[c] (μg mL^{-1})	IAA[d] (μg mL^{-1}): 100T[e]	EPS[f] (μg mL^{-1})	Ammonia	HCN[g]
PS1	15±0.8a	41±1.2b	21±0.3a	39±2.6b	18±2.1a	+	+
PS2	13±0.6c	24±1.1c	9±0.2d	32±2.1d	16±1.4b	+	+
PS3	-	-	-	21±1.6i	8±0.5fg	+	+
PS4	-	-	-	17±1.3	9±0.3e	+	+
PS5	12±1.0d	11±0.9i	5±0.3f	22±1.4h	13±1.2c	+	-
PS6	-	-	-	20±1.5j	8±0.3fg	+	-
PS7	11±0.7e	21±0.7e	6±0.9e	26±2.1f	7±0.2g	+	-
PS9	14±0.8b	41±0.4b	17±1.2b	34±1.7c	17±1.1ab	+	+
PS10	-	-	-	14±0.9k	16±1.6b	+	+
PS12	9±0.6f	23±0.6d	9±0.5d	11±1.2m	10±1.2de	+	-
PS14	8±0.8g	20±0.3f	4±0.3g	15±1.3k	12±1.4cd	+	-
PS16	-	-	-	23±2.4g	8±0.8fg	+	+
PS17	-	-	-	23±1.7g	7±0.6g	+	-
PS19	14±0.7b	47±0.5a	10±0.3c	42±2.7a	18±1.4a	+	+
PS20	-	-	-	27±1.5e	12±1.3cd	+	+
PS21	-	-	-	13±1.1l	12±0.8cd	+	-
PS22	8±0.5g	14±0.4h	6±0.5e	12±1.3lm	9±1.1e	+	-
PS23	10±0.9ef	15±0.6g	4±0.4g	9±0.8n	9±0.9e	+	-
F-value	23.5	209.6	154.5	259.7	145.2		

Values indicate Mean±SD of three replicates. Mean values (±SD) followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test. [a]Chrome azurol S (CAS) agar, [b]Salicylic acid, [c]2,3 Dihydroxy benzoic acid, [d]Indole-3-acetic acid, [e]Tryptophan concentration (μg mL^{-1}), [f]Exo-polysaccharides, [g]Hydrogen cyanide, Indicates positive reaction, Indicates no reaction

the amount of EPS released by rhizobacteria varied considerably among bacterial species. Of the mesorhizobial strains, strain MRC4 produced a maximum amount (21 μg mL^{-1}) of EPS and was followed by strain MRC1 (16 μg mL^{-1}) (Table 4). Among *Rhizobium* strains isolated from pea nodules, only three strains secreted EPS in the culture medium wherein strain MRP1 produced a maximum amount (20 μg mL^{-1}) of EPS. However, strain MRP4 exhibited the lowest production of EPS (15 μg mL^{-1}) in broth (Table 5). *Bradyrhizobium* strains also produced a significant amount of EPS, maximum being 21 μg mL^{-1} EPS observed for the strain MRM6 followed by MRM8 which produced 14 μg mL^{-1} EPS (Table 6). Rhizobial strains specific to lentil plants also produced adequate amount of EPS. For example, strain MRL3 synthesized highest amount (18 μg mL^{-1}) of EPS which was followed by strain MRL1 (13 μg mL^{-1}) (Table 7). The EPS production by phosphate solubilizing bacteria ranged between 7 (*Bacillus* PS7 and *Bacillus* PS17) to 18 μg mL^{-1} (*Pseudomonas aeruginosa* PS1 and *Klebsiella* sp. PS19). Among all phosphate solubilizers, both *Pseudomonas aeruginosa* PS1 and *Klebsiella* sp. PS19 produced maximum amount of EPS (18 μg mL^{-1}) followed by *Pseudomonas putida* PS9 and *Enterobacter asburiae* PS2 that produced 17 and 16 μg mL^{-1} EPS, respectively (Table 8).

Qualitative and quantitative assay of phosphorus: The rhizobacteria were further evaluated for phosphate solubilizing potential, both on solid and in liquid Pikovskaya medium supplemented with 5 g L^{-1} Tri-Calcium Phosphate (TCP). In the present study, a total of 34% rhizobacterial strains showed phosphate solubilizing activity and formed a clear halo around bacterial growth. Generally, the size of phosphate solubilizing zone (halo) on solid Pikovskaya ranged from 4 (*Bacillus* sp. PS4) to 14 mm (*Klebsiella* sp. PS19). Among the phosphate solubilizing PGPR strains, *Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2, *Bacillus* sp. PS3, *Pseudomonas putida* PS9 and *Klebsiella* sp. PS19 displayed the largest zone of P solubilization on solid Pikovskaya medium after seven days of incubation. The percent increase in PS zone by the highest P activity showing *Klebsiella* sp. strain PS19 over other bacterial strains varied between 14% (*Pseudomonas aeruginosa* PS1, *Enterobacter asburiae* PS2 and *Bacillus* sp. PS3) to 71% (*Bacillus* sp. PS4) (Fig. 1). The solubilization index (SI) calculated based on size of bacterial colony and zone diameter ranged between 0.5 (*Bacillus* PS 4) to 2.5 (*Klebsiella* PS 19) (Fig. 1). Similarly, a considerable amount of Tri-Calcium Phosphate (TCP) was solubilized in liquid culture by *Pseudomonas* aeruginosa PS1 (345 μg mL^{-1}), *Enterobacter asburiae* PS2 (258 μg mL^{-1}), *Pseudomonas putida* PS9 (298 μg mL^{-1}) and *Klebsiella* sp. PS19 (294 μg mL^{-1}). The percent increase in P solubilizing activity of *Pseudomonas aeruginosa* strain PS1, solubilizing maximum TCP in broth over other bacterial strains varied between 14% (*Pseudomonas putida* PS9) to 82% (*Bacillus* sp. PS23) (Fig. 1). The solubilization of TCP was accompanied by decrease in pH of the medium and a maximum decrease

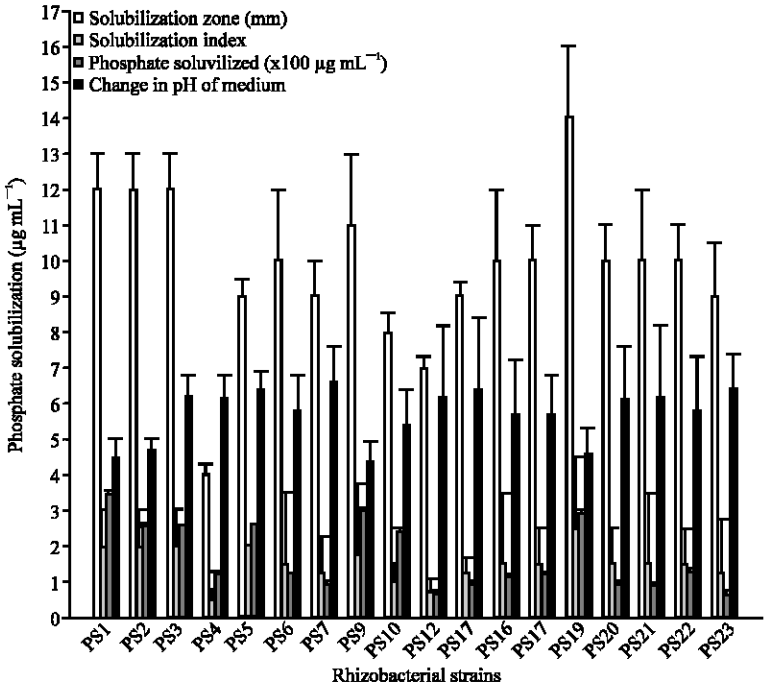

Fig. 1: Solubilization zone, solubilization index, solubilization of tri-calcium phosphate and change in pH in Pikovskaya broth by rhizobacterial strains (from mustard rhizosphere) after seven days of incubation

(35%) in pH was observed for strain PS9 compared to those observed for uninoculated control. The decrease in pH of the medium varied from strain to strain (Fig. 1). Generally, no relationship between amounts of solubilized P and change in pH was observed.

***In vitro* assay of ammonia and HCN:** The rhizobacterial strains were tested further for the synthesis of ammonia and HCN using peptone water and HCN induction medium, respectively. All the rhizobacterial strains of *Bradyrhizobium*, *Rhizobium* (lentil) and phosphate solubilizers showed a positive reaction for ammonia (Table 6-8). In contrast, 10 of *Mesorhizobium* and 6 of *Rhizobium* (pea) were positive for ammonia production as shown in Table 4-5. Furthermore, 63% *Mesorhizobium*, 100% *Rhizobium* (pea), 33% *Bradyrhizobium*, 75% *Rhizobium* (lentil) and 50% phosphate solubilizing strains were found to be positive for HCN production (Table 4-8).

DISCUSSION

Pesticide-tolerance: Both the genetics and the physiologies of microorganisms are involved to make them tolerant/resistant against one or several pesticides. It is observed that the tolerant microorganisms to any specific pesticide generally, have biodegrading potential to breakdown it into smaller products which are later utilized by these organisms as Carbon (C) and Nitrogen (N) sources. Moreover, a pesticide induces the microorganisms for the formation of a new metabolic pathway to bypass a biochemical reaction inhibited by the very pesticide (Bellinaso *et al*., 2003). Permanent resistance, on the other hand, depends upon genetic modifications, inherited by the subsequent generation of microbes (Johnsen *et al*., 2001; Herman *et al*., 2005). Present study however, documented abnormally higher tolerance levels of the rhizobacterial strains of rhizospheres of mustard, chickpea, pea, greengram, and lentil plants against the selected pesticides amended in minimal salt agar medium. Since the medium used to assess the MTL values of the rhizobacterial strains did not contain any C and N sources except the tested `pesticides, the rhizobacterial strains might have utilized them as the only energy sources.

In similar studies, Gram negative bacteria have also shown resistance to other pesticides. For instance, the maximum tolerant concentrations of different organophosphorus pesticides for both resistant strains of *Pseudomonas* and *Flavobacterium* species isolated from polluted sites were 250, 4000 and 8000 µg mL^{-1} of guthion, methyl parathion and dimethoate, respectively (Nazarian and Mousawi, 2005). Likewise, *Rhizobium* sp. specific to chickpea tolerated aldrin upto 2000 µg mL^{-1} (Juneja and

Dogra, 1978). Moreover, Boldt and Jacobsen (1998) also reported a variation in the MTLs of *Pseudomonas* strains to sulfonylurea herbicides (e.g. metsulfuron methyl, chlorsulfuron and thifensulfuron methyl). Among the herbicides, metsulfuron methyl was more toxic compared to other herbicides and order of toxicity was: metsulfuron methyl>chlorsulfuron>thifensulfuron methyl. The variation in tolerance against pesticides by PGPR could probably be due to the fact that rhizobacteria adopt different strategies to overcome the toxic effects of pesticides and such mechanisms include: biodegradation (Yang and Lee, 2008) and enzymatic hydrolysis (Dumas *et al*., 1989) of pesticide. Present study however, showed that the tolerance levels of the selected rhizobacterial strains were considerably high against the pesticides.

Plant growth promoting activities of rhizobacteria: Among the phytohormones, indole acetic acid (IAA) and its analogues, synthesized from tryptophan are the main auxin controlling many important physiological processes of plants including cell enlargement and division, tissue differentiation, root initiation, root growth inhibition, increased growth rate, phototropism, geotropism and apical dominance (Khan *et al*., 2009). Bacterial IAA has the potential to interfere with any of these processes by input of IAA into the plant's auxin pool. Effect of IAA (both bacterial and plant origin) on plants however, depends upon the amount of IAA produced and the sensitivity of the plant tissue to changes in IAA concentration. In this study, all the rhizobacterial strains of *Mesorhizobium* (n=11), *Bradyrhizobium* (n=9), *Rhizobium* (n=7) isolated from pea and *Rhizobium* (n= 8) from lentil nodules and a total of 18 phosphate solubilizers (*Pseudomonas*, *Bacillus*, *Enterobacter* and *Klebsiella*) produced a substantial amount of IAA in LB broth (Table 4-8). Differences in the synthesis of IAA among rhizobacterial strains however, can be attributed to the involvement of various biosynthetic pathways, location of the genes involved, regulatory sequences, the presence of enzymes to convert active free IAA into conjugated forms and changing environmental conditions (Patten and Glick, 1996).

Siderophore is yet another important metabolite released by the PGPR strains that indirectly affects the growth of plants. In the present study, among the selected 53 rhizobacterial strains, four strains belonging to genera *Mesorhizobium*, three strains of pea rhizobia and *Bradyrhizobium,* four strains belonging to rhizobia of lentil origin and 10 strains of phosphate solubilizers produced the siderophores on CAS agar plates as well as in liquid culture medium (Table 4-8). Siderophores synthesized by microbial communities of soil supply iron to plants that possess the mechanisms for its uptake under iron-deficient conditions (Indiragandhi *et al*., 2008). Furthermore, siderophores chelate iron and other metals and consequently cause disease suppression by conferring a competitive advantage to biocontrol agents for the limited supply of essential trace minerals in natural habitats (Zaidi *et al*., 2009). Siderophores may also directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to the bacteria and may function in local and systemic host resistance in plants (Sinha and Mukherjee, 2008). The ability of rhizobial strains and phosphate solubilizers to produce siderophore as observed in this study suggests that such strain could also be used as a biological control agent.

The ammonia released by the bacterial strain plays a signaling role in the interaction between plant growth promoting bacteria and plants (Becker *et al*., 2002). The release of HCN by rhizospheric bacteria into the soil can be toxic to subterranean animals and phytopathogenic organisms (Guo *et al*., 2007; Kang *et al*., 2010). Furthermore, four strains of *Mesorhizobium*, three each of *Bradyrhizobium*, pea and lentil specific rhizobia and all of phosphate solubilizers were positive for the EPS secretion. The EPS production is an important trait of bacteria because it provides protection to cells against desiccation, phagocytosis and phage attack and also helps in N_2 fixation by preventing high oxygen tension (Tank and Saraf, 2003). The EPS synthesized by rhizobacterial strains is likely to provide them protective advantage while inhabiting the stressed environment.

In general, the majority of P applied exogenously or present in complex forms in soils is unavailable for uptake by plants due to its rapid rate of fixation/complex formation with other elements of soils (Khan *et al*., 2009). In this study, PGPR possessing phosphate solubilizing activity often termed PSB are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available. The PGPR strains were therefore further screened and evaluated for their P solubilizing potential using both solid and liquid Pikovskaya medium. In the present study, a total of 34% rhizobacterial strains showed the phosphate solubilizing activity on solid Pikovskaya plates as detected by the formation of a clear halo around their growth. The PGPR strains also solubilized an appreciable amount of TCP in liquid Pikovskaya medium with concomitant drop in pH of the culture medium. The solubilization of insoluble P by the rhizosphere microorganisms and concurrently decrease in pH of the medium, has often been due to the secretion of organic acids (Zaidi *et al*., 2009). In addition,

the bacteria producing higher amounts of EPS exhibit a stronger ability of P-solubilization compared to non-EPS producing strains as reported by Yi *et al*. (2008). This inference is further consolidated by the observation that all phosphate solubilizers produced EPS and that *Pseudomonas aeruginosa* strain PS1, *Enterobacter asburiae* strain PS2, *Pseudomonas putida* strain PS9 and *Klebsiella* sp. strain PS19 solubilizing maximum TCP in liquid medium also produced the highest concentration of EPS. In addition, the amount of P solubilized in Pikovskaya medium and EPS secreted in basal medium by all selected P solubilizers was found significantly correlated (r=0.712).

In conclusion, present study demonstrated that the rhizobacterial strains recovered from the mustard, chickpea, pea, greengram, and lentil rhizospheres showed a wide range of tolerance levels against the selected herbicides, insecticides, and fungicides. The high pesticide-tolerance in these rhizobacteria may be important in the decontamination of agricultural soils polluted with pesticides. In addition, a great deal of functional diversity is found among rhizobacteria isolated from different crops. Generally, mustard rhizosphere exhibited more functionally diverse rhizobacteria than those of legumes.

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