Assessment of microbial biota associated with rhizosphere of wheat (Triticum aestivum) during flowering stage and their plant growth promoting traits.

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Citation

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Abstract

Microbial biota associated with wheat rhizosphere during flowering-stage and their plant growth promoting traits was investigated. 16S rRNA gene sequencing was performed of the isolates, which were obtained on selective media. Isolates belonged to: alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria; Actinobacteria; Bacteroidetes and Firmicutes. Bacillus was the most dominant genus (34.7%) followed by Pseudomonas (14.4%). Among diazotrophs, Arthrobacter sp. (n=3), Cupriavidus sp. (n=3) and Stenotrophomonas sp. (n=4) occurred more frequently. Most of the isolates produced indole acetic acid. Ac.
baumannii
, Ps. lini, Ser. marcescens, C. respiraculi and Ag.tumfaciens solubilized phosphate. Two Acinetobacter strains, five Pseudomonas srains and four Bacillus strains produced siderophore. Strains of Ps. aeruginosa, Ps. lini and Ps. thivervalensis exhibited in vitro fungal growth inhibition. Arthrobacter globiformis Y2S3 exhibited indole acetic acid production, siderophore production and antifungal activity.
Plant growth promoting traits of these rhizobacteria indicated beneficial relationship between rhizobacteria and wheat plants. Several of the strains could be further developed as an effective bio-inoculant.

INTRODUCTION

Rhizosphere soil is a “hot-spot” for microbial growth and major microbial activities. The growth of many microorganisms in the rhizospheric region depends on the root exudates released by the plants (Bais et al. 2006). Interactions between plant and microbes are intensely studied and especially those that benefit plant growth. Such free-living soil bacteria isolated from the rhizosphere of plants, which have been shown to be beneficial for plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (Klopper et al. 1980). It has been well established that PGPR enhance plant growth by direct or indirect means. The direct means may includes : Fixation of atmospheric nitrogen (Zehr et al. 2003; Dixon 1984), production of siderophores (Machuca and Milagres 2003; Schwyn and Neilands 1986), solubilization of minerals like phosphorus (Tilak et al. 2005) and synthesis of phytohormones like indole acetic acid (IAA) (Chung and Tzeng 2004; Huddedar et al. 2002). Indirect mechanisms include inhibition of phytopathogens and thus promoting plant growth (Compant et al. 2005; Padalalu and Chopade 2006).

Among all the plant growth promoting properties, N_2 fixation is of prime importance for plant growth. N_2 fixers, also called ‘diazotrophs’ play a critical role in the plant ecosystem by reducing dinitrogen (N_2) to ammonia (NH_3) (Dilworth 1974). N_2 fixation is carried out by a diverse group of prokaryotes, Bacteria and Archaea (Zehr et al. 2003). These include symbiotic nitrogen fixing forms; Rhizobium, the obligate symbionts in leguminous plants and Frankia in non-leguminous trees, associative nitrogen fixers which adhere to the root surfaces (e.g. Achromobacter, Enterobacter etc.) and non-symbiotic (free living) forms such as Azospirillum, Azotobacter, Acetobacter diazotrophicus, cyanobacteria etc. Diazotrophs have been reported to exert a positive effect on plant growth when they, synthesize phytohormones and vitamins, improve nutrient uptake, enhance stress resistance and solubilize inorganic phosphate. Indirectly, diazotrophs are able to prevent the deleterious effects of pathogenic microorganisms, mostly

through the synthesis of antibiotics and/or fungicidal compounds, through competition for nutrients by siderophore production or by the induction of systemic resistance to pathogens (Dobbelaere et al. 2003).

Wheat is one of the major crops cultivated in India and all over the world. The different stages of life cycle of wheat consist of elongation (30days), flowering stage (45days), fruiting stage (60days) and ripened fruiting stage (75days) (Huddedar and Chopade 2000; Huddedar et al. 2002). It is found that rate of root exudates released by the roots of the wheat at flowering stage is higher as compared to other stages, hence greater microbial biota and activity is expected during this stage (Huddedar and Chopade 2000). Thus present study was done to investigate the microbial biota, with special emphasis on diazotrophs, associated with rhizosphere of wheat variety Lokwan during flowering stage and in vitro plant growth promoting traits of these bacteria were also investigated to understand the beneficial role of these bacteria in the same niche.

MATERIALS AND METHODS

COLLECTION OF SOIL SAMPLES

The rhizosphere soil samples of the wheat during the flowering stage were collected from three agricultural fields in triplicates in the winter season. The wheat plants were uprooted from the agricultural fields and the rhizosphere soil was pooled together and immediately microbiological processing was carried out.

PROCESSING OF SOIL SAMPLES

Soil samples were processed within 1-2 h of sampling as follows: 10 g of rhizosphere soil was weighed aseptically and added into 100 ml of sterile phosphate buffer saline (PBS), pH 7.0 in a 250 ml flask. Flask was kept in shaking condition at 200 rpm for 15 min and 1 ml of the suspension was diluted up to 10^{-7} in 9 ml sterile PBS in tubes. 100 µl of each dilutions, 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} were spread on different nitrogen free media like Burk's medium, Jensen's medium, yeast extract mannitol agar, Acinetobacter minimal medium (AMM) (Juni, 1972), Holton's medium (Holton, 1983), Pseudomonas agar base + 1 % (v/v) glycerol, Violet red bile (VRB) agar and Standard plate count (SPC) agar media. All media were supplemented with antifungal agent amphotericin B (40µg/ml) to prevent fungal growth. Except AMM and Holton's medium, all other media were obtained from HiMedia, Mumbai, India. Plates were incubated at 28°C up to 7 days. Colonies having different morphology were picked randomly after 48 and 96 h of incubation and cultured on respective media for further studies. The bacterial isolates were preserved at -80°C in LB medium containing glycerol (25 % v/v) as a cryopreservative.

IDENTIFICATION OF THE BACTERIAL ISOLATES BY 16S RRNA GENE SEQUENCING

Genomic DNA was extracted from the isolates by the standard method (Sambrook et al. 1989). The genomic DNA pellet was resuspended in 50µl of nuclease-free water and detection was done by agarose gel electrophoresis (Sambrook et al. 1989). The partial 16S rRNA genes were amplified from genomic DNA by PCR using the universal bacterial primers, corresponding to E. coli position 27f and 1525r (Rainey et al. 1996). Amplification was performed in GeneAmp PCR System 9700 (Applied Biosystems, USA) in 25 µl reaction volume containing, 20 to 50 g of DNA template, 0.2mM each of dATP, dCTP, dGTP, and dTTP, 12.5 pM of each primer, 1X PCR buffer (Bangalore Genei, India) and 0.6U of Taq DNA polymerase (Bangalore Genei, India). The final volume of the PCR mixture was adjusted by adding milli Q water. A reagent blank containing all components of the reaction mixture except template DNA, was included in every PCR procedure. The thermal cycling was performed as follows: initial denaturation at 95°C for 5min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products (expected size about 1500 bp) were analyzed by running 3 µl aliquots of the reaction mixtures in 1 % (w/v) agarose gels along with 1kb Plus DNA ladder (Invitrogen Corporation, California).

PCR products were purified using the QIAquick PCR purification kit (Qiagen, USA) according to the manufacturer's suggested protocol and nearly complete sequences of 16S rRNA genes were obtained using the primers 343r, 27f, 1525r (Rainey et al. 1996), 530f (Gee et al. 2004), 946f (Moreno et al. 2002), 704f, 685r and 907r (Jang et al. 2005). Cycling sequencing was performed using the Big Dye Terminator Cycle Sequencing Kit (v3.1, Applied Biosystems), according to the manufacturer's protocol, and analysed in an Applied Biosystems 3730 DNA Analyzer.

SEQUENCE SUBMISSION

The partial16S rRNA gene nucleotide sequences of all the isolates determined in this study have been deposited in GenBank database

(http://www.ncbi.nlm.nih.gov/GenBank/index .html) under accession numbers as mentioned in Table 1.

SCREENING OF PLANT GROWTH PROMOTING TRAITS

NITROGEN FIXATION

The isolates were grown on Jensen's nitrogen free medium (Himedia, Mumbai, India) for 48 h. The cotton plug was then replaced with gas tight septa and 2 ml of air was replaced with acetylene gas (10%). The tubes were incubated for 24 h and the production of ethylene was detected using gas chromatography with FID detector (Hardy et al. 1989). The isolates which showed presence of ethylene were considered as nitrogen fixers.

INDOLE ACETIC ACID (IAA) PRODUCTION

Isolates were grown at 28°C in LB broth supplemented with 1mg/ml of tryptophan (LBT). After 48 h of incubation at 150 rpm, cells were harvested by centrifugation at 10000 rpm for 10 min at 4°C. 1 ml of supernatant and 4 ml of Salkowski reagent were mixed and allowed to react in dark at room temperature for 20 min. Development of pink to red color formation was considered positive evidence for IAA production (Huddedar et al. 2002).

PHOSPHATE SOLUBILIZATION

10 µl of overnight grown isolates in LB broth were spot inoculated on Pikovskaya's agar (Pikovskaya 1948) and incubated at 28°C up to 5 days. The bacterial isolates forming clear halos were considered positive for phosphate solubilization.

SIDEROPHORES PRODUCTION

10 µl of overnight grown isolates in LB broth were spot inoculated on chrome azurol S agar plates and incubated at 28°C up to 5 days. Microorganisms exhibiting an orange halo were considered positive for production of siderophores (Schwyn and Neilands 1987).

ANTIFUNGAL ACTIVITY

Seven fungal phytopathogens viz; Aspergillus phoenicus, Fusarium moniliformis, Bipolaris tetramera, Rhizopus stolonifer, Sclerotium oryzae, Alternaria tritiana and Cephalosporum maydis obtained from Indian Type Culture Collection, Indian Agriculture Research Institute (IARI), New Delhi were used. Fungal spore suspension containing 10^6 spores was prepared in 0.2% Tween 80 and were spread on potato dextrose agar (PDA) (HiMedia, Mumbai, India) plates to obtain mat growth. 10 µl overnight grown bacterial isolates in LB broth were spot inoculated on the above PDA plates and this was done in triplicates. The plates were incubated at 28°C up to 7 days. A test was considered positive when a clear inhibition zone was observed around the bacterial growth in the three replications (Sathe et al. 2007).

RESULTS AND DISCUSSION

Rhizosphere is a rich habitat of microbes and should be explored for obtaining potential PGPR, which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants. Enumeration of total bacteria population was determined on standard plate count (SPC) agar. Gram-negative bacterial populations were enumerated on violet bile red (VRB) agar. Count of Gram negative population in the rhizosphere during flowering stage was found to be about ca. 10^7 per g of soil. A total of 69 strains were isolated in this study from rhizosphere of wheat at flowering stage. 16S rRNA gene of these isolates was sequenced using internal primers (≥90% of the E. coli sequence). These sequences determined were compared with previously reported type strain sequences in the GenBank database using the EzTaxon server (Chun et al. 2007). 16S rRNA gene sequence similarity of 98% was used as the cut-off for positive identification of taxa. The nomenclature used was that described by (Garrity et al. 2001) for Bergey's Manual of Systematic Bacteriology, (2001). All the isolates were successfully identified up to the species level and the shown affiliation with six major bacterial lineages: alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria; Actinobacteria; Bacteroidetes and Firmicutes. Firmicutes constituted 40% and were the most dominant lineage followed by gamma-proteobacteria (28.9%), alpha-proteobacteria (7.2%), beta-proteobacteria (7.2%), Actinobacteria (11.5%) and Bacteroidetes (2.8%) (Table 1). The bacterial diversity associated with rhizosphere of wheat as observed in this study was significantly higher as compared to the previous reports (Guemouri-Athmani et al. 2000; Smit et al. 2001; Mavingui et al. 1992; Mittal 2004).

The organisms were identified as Acinetobacter sp. (n=3), Arthrobacter sp. (n=3), Achromobacter sp. (n=1), Agromyces sp. (n=1), Bacillus sp. (n=24), Brevibacillus sp. (n=1), Cupriavidus sp. (n=3), Chryseobacterium sp. (n=1), Ensifer sp. (n=1), Enterobacter sp. (n=1), Flavobacterium sp. (n=1), Massilia sp. (n=1), Microbacterium sp. (n=1), Pseudomonas sp. (n=11), Agrobacterium sp. (n=3),

Sinorhizobium sp. (n=1), Stenotrophomonas sp. (n=4), Salana sp. (n=1), Serratia sp. (n=1), Streptomyces sp. (n=1) and Staphylococcus sp. (n=4). Bacillus was the most dominant genus (34.7%) followed by Pseudomonas (14.4%). Pseudomonas species were isolated from the rhizosphere of wheat included; Ps. aeruginosa, Ps. thivervalensis, Ps. lini and Ps. flavescens. There are many reports on the occurrence of Pseudomonas sp. in the rhizosphere of wheat (Mavrodi et al. 2006; Germida and Siciliano 2001; Turnbull et al. 2001; Wang et al. 2001; Whipps 2001, Smit et al. 2001; van Overbeek and van Elsas 1995; Roberts and Brewster 1991; Parke et al. 1986).

To best of our knowledge the following are the nitrogen fixers reported from the rhizopshere of wheat : Paenibacillus polymyxa (Guemouri-Athmani et al. 2000), Arthrobacter sp., Bacillus sp. (Smit et al. 2001; Mavingui et al. 1992) and organisms belonging to gamma-proteobacteria like Pseudomonas sp., Stenotrophomonas sp., Enterobacter sp. (Mittal 2004). In the present study, several diazotrophs was obtained from the rhizosphere of wheat during flowering stage (Table 1). Among diazotrophs, Arthrobacter sp., Cupriavidus sp. and Stenotrophomonas sp. occurred more frequently than others. Species belonging to genus Salana, has not been previously isolated from rhizosphere of wheat and our work constitutes its first report from rhizosphere of wheat. Among the strains isolated in the present study Acinetobacter sp., Arthrobacter sp., Achromobacter sp., Bacillus sp., Cupriavidus sp., Ensifer sp., Stenotrophomonas sp., Agrobacterium sp., Enterobacter sp., and Serratia sp. were previously reported as dinitrogen fixers (Liba et al. 2006; Mansoor and Gray 1995; Smit et al. 2001; Barret and Parker 2006; Willems et al. 2003; Mittal 2004; Kanvinde and Sastry 1990; Requena et al. 1997; Pedersen et al. 1978). Streptomyces venezualae, one of the actinomycetes isolated in the present study was also a reported nitrogen fixer (Nandi and Sen 1981). Interestingly, the symbiotic nitrogen fixers such as Ensifer sp. and Agrobacterium sp. which nodulate leguminous plants were isolated from the rhizosphere of wheat (Willems et al. 2003).

Figure 1

Table 1. Microbial biota associated with rhizosphere of wheat at flowering stage along with their plant growth promoting traits

Strain designation	Identification of isolates by 16S rRNA gene sequencing	NCBI Accession Number	Nitrogen fixation	IAA production	Phosphate solubilisation	Siderophore production	Antifungal activity (Fungi inhibited)
	Firmicutes						
B2S2	*B. badius*	EU221363	-	-	-	-	-
B2P1	*B. flexus*	EU221369	-	-	-	-	-
B2S1	*B. licheniformis**	EU221362	-	-	-	-	-
B2S9	*B. megaterium*	EU221368	-	-	+	+	-
B2P2	*B. megaterium*	EU221370	-	+	-	-	-
J2S5	*B. niacini*	EU221359	-	-	-	-	-
J2S7	*B. niacini*	EU221360	-	-	-	-	-
J2S3	*B. simplex*	EU221357	-	-	-	-	+ (*A. phoenicis; C. maydis*)
B2P6	*B. vireti*	EU221371	-	-	-	-	-
N2P1	*B. flexus*	EU221353	-	+	-	-	-
P2P3	*B. luini*	EU221387	-	-	-	-	-
P2P5	*B. megaterium*	EU221388	-	+	-	-	-
Y2S4	*B. niacini*	EU221374	-	-	-	-	-
Y2S5	*B. niacini*	EU221375	-	-	-	-	-
Y2S2	*B. vireti*	EU221372	-	-	-	-	+ (*B. tetramera; S. oryzae*)
L2S7	*B. endophyticus*	EU221417	-	+	-	+	+ (*B. tetramera*)
A2P5	*B. flexus*	EU221411	-	+	-	-	-
L2S2	*B. flexus*	EU221413	-	-	-	+	-
A2P2	*B. megaterium*	EU221408	-	-	-	-	-
A2P4	*B. megaterium*	EU221410	-	+	-	-	-
L2S3	*B. megaterium*	EU221414	-	+	-	-	-
L2S5	*B. mycoides*	EU221416	-	-	-	+	-
L2S8	*B. mycoides*	EU221418	-	-	-	-	+ (*B. tetramera*)
L2S1	*B. subtilis*	EU221412	-	-	-	-	+ (*B. tetramera*)
B2S6	*Staph. aquorum*	EU221366	-	-	-	-	-
B2S7	*Staph. aquorum*	EU221367	-	-	-	-	-
B2S4	*Staph. arlettae*	EU221364	-	-	-	-	-
P2S4	*Staph. arlettae*	EU221385	-	+	-	-	-
Y2S9	*Brevibacillus laterosporus*	EU221377	-	-	-	-	-

Figure 2

Strain designation	Identification of isolates by 16S rDNA sequencing	NCBI Accession Number	Nitrogen fixation	IAA production	Phosphate solubilisation	Siderophore production	Antifungal activity (Fungi inhibited)
	Actinobacteria						
N2S6	*Micrococcus paroxydans*	EU221351	-	+	-	+	-
B2S5	*Arthrobacter globiformis**	EU221365	+	+	-	-	-
N2P4	*Arthrobacter globiformis**	EU221355	+	+	-	-	-
N2S8	*S. venezuelae**	EU221352	+	+	-	-	-
Y2S3	*Arthrobacter globiformis**	EU221373	+	+	-	+	+ (*B. tetramera*)
Y2S10	*Agromyces ulmi*	EU221378	-	-	-	-	-
Y2S8	*Salana multivorans*	EU221376	-	-	-	-	-
A2S3	*Arthrobacter globiformis**	EU221407	+	+	-	-	+ (*C. maydis; S.oryzae*)
	Bacteriodates						
H2S10	*Chryseobacterium indologenes*	EU221399	-	+	-	-	-
H2P6	*Fl. johnsoniae*	EU221404	-	-	-	-	-
	Alpha proteobacteria						
N2P3	*Ag. tumefaciens**	EU221354	-	+	-	-	-
H2P4	*Ag. tumefaciens**	EU221403	-	+	+	-	-
A2P3	*Ag. tumefaciens**	EU221409	-	+	-	-	-
J2S2	*Ensifer adhaerens**	EU221356	-	+	-	-	-
H2P7	*Sinorhizobium meliloti**	EU221405	-	-	-	-	-
	Beta proteobacteria						
H2S5	*Cupriavidus respiraculi**	EU221394	+	-	-	-	-
H2S6	*Cupriavidus respiraculi**	EU221395	+	-	+	-	-
H2S2	*Cupriavidus respiraculi**	EU221391	+	+	-	-	-
H2P8	*Massilia timonae*	EU221406	-	+	-	-	-
Y2P1	*Achromobacter insolitus**	EU221379	+	-	-	-	-

Figure 3

Strain designation	Identification of isolates by 16S rDNA sequencing	NCBI Accession Number	Nitrogen fixation	IAA production	Phosphate solubilisation	Siderophore production	Antifungal activity (Fungi inhibited)
	Gamma proteobacteria						
N2S4	*Ac. baumannii*	EU221350	+	-	+	-	-
P2P8	*Ac. baumannii*	EU221389	-	-	+	+	-
P2S6	*Ac. calcoaceticus*	EU221386	-	-	-	+	-
J2S4	*Enterobacter asburiae**	EU221358	+	-	-	-	-
Y2P3	*Ps. aeruginosa*	EU221381	-	-	-	+	+ (*A. phoenicus; C. maydis*)
Y2P5	*Ps. aeruginosa*	EU221382	-	-	-	+	+ (*A. phoenicus; C. maydis*)
Y2P7	*Ps. aeruginosa*	EU221383	-			+	+ (*C. maydis*)
Y2P8	*Ps. aeruginosa*	EU221384	-			+	+ (*A. phoenicus; C. maydis*)
H2S3	*Ps. thivervalensis*	EU221392	-	+	-	-	-
Y2P2	*Ps. aeruginosa*	EU221380	-			+	+ (*A. phoenicus; C. maydis*)
H2P2	*Ps. lini*	EU221401	-	-	+	-	+ (*A. phoenicus; C. maydis; R. stolonifer; S.oryzae*)
H2P1	*Ps. thivervalensis*	EU221400	-	-	-	-	+ (*A. phoenicus; C. maydis; R. stolonifer; S.oryzae*)
H2P3	*Ps. thivervalensis*	EU221402	-	+	-	-	-
L2S4	*Ps. thivervalensis*	EU221415	-	+	-	-	+ (*S. oryzae*)
H2S9	*Ps. flavescens*	EU221398	-	+	-	-	-
J2P3	*Ser. marcescens**	EU221361	+	+	+	-	-
H2S4	*Stenotrophomonas maltophilia**	EU221393	+	+	-	-	-
H2S7	*Stenotrophomonas maltophilia**	EU221396	-	+	-	-	-
H2S1	*Stenotrophomonas maltophilia**	EU221390	-	+	-	-	-
H2S8	*Stenotrophomonas maltophilia**	EU221397	-	-	-	-	-

*: Reported nitrogen fixers.
+: Presence of the trait; -: Absence of the trait

Each of the isolates was investigated for the plant growth promoting traits such as production of IAA and siderophore, as well as ability to inhibit fungal growth and solubilize phosphate. The PGP properties of each isolate tested are mentioned in Table 1. It was observed that 31 isolates from rhizosphere of wheat produced plant growth promoting hormone IAA. Production of IAA by Acinetobacter sp. isolated from rhizosphere of wheat has been reported previously (Huddedar et al. 2002). However, in present studies IAA production was not detected in Acinetobacter species isolated from rhizosphere of wheat. Certain species of Pseudomonas previously obtained from rhizosphere of wheat are known to produce IAA (Pedraza et al. 2004). In our study, out of four Pseudomonas sp. isolated from rhizosphere of wheat, Ps. flavescens and Ps. thivervalensis showed IAA. Among diazotrophs obtained in our study, Arthrobacter globiformis, Agrobacterium tumfaciens, Serratia marcescens, Streptomyces venezualae, Ensifer adhaerens, Stenotrophomonas maltophilia and Cupriavidus respiraculi showed production of IAA. These nitrogen fixing species are known to produce IAA (Dobbelaere et al. 2003).

Seven isolates from rhizosphere demonstrated in vitro solubilization of phosphate. Ac. baumannii strains found to be the present study solubilized phosphate. Ability of Acinetobacter sp. to solubilize phosphate has been reported earlier (Huddedar and Chopade 2000; Liba et al. 2006). Amongst the four Pseudomonas spp. isolated, only one species Ps. lini could solubilize phosphate. Ps. aeruginosa, Ps. thivervalensis and Ps. flavescens did not show phosphate solubilization. There are reports on solubilisation of phosphate by Ps. aeruginosa and other Pseudomonas spp. (Nair and Rao 1977; Bardiya and Gaur 1974). Ser. marcescens, strains of C. respiraculi and Ag. tumfaciens could solubilize phosphate. Interestingly, Ser. marcescens and Ag. tumfaciens, apart from being nitrogen fixers, were able to solubilize phosphate as well as produce IAA, thus can act as potent PGPR individually or in combination with other bacteria. It should be noted that some nitrogen fixers like B. licheniformis and Serratia sp. are known to solubilize phosphate (Pal et al. 2000). However, in our studies, B. licheniformis did not show phosphate solubilization.

Eleven strains isolated from rhizosphere of wheat were able to produce siderophore. Ac. baumannii and Ac. calcoaceticus obtained in this study were positive for siderophore production. Acinetobacter spp. have been reported for production of siderophores (Corsa and Walsh 2002; Dorsey et al. 2003). Amongst the four Pseudomonas spp. isolated, all the strains of Ps. aeruginosa showed siderophore production. Ps. aeruginosa is reported to produce siderophores like pyoverdine and pyochelin (Wendenbaum et al. 1983; Cox et al. 1981). Nitrogen fixer obtained in our studies, which showed siderophore production was A. globiformis, while others did not show siderophore production. However, there are reports on nitrogen fixers like Enterobacter sp., Arthrobacter sp., Rhizobium sp. Erwinia sp., and Azospirillum sp. for siderophore production (Lochhead et al. 1984; Smith et al. 1985; Expert and Toussaint 1985; Shah et al. 1993).

Fifteen strains isolated from rhizosphere exhibited in vitro antifungal activity against multiple plant pathogenic fungi viz; A.phoenicus, F.moniliformis, C. maydis, B. tetramera, S. oryzae and R. stolonifer. None of the Acinetobacter isolates in this study showed antifungal activity against tested fungal cultures. There is a report on Ac. baumannii producing iturin an antifungal compound (Liu et al. 2007). There are many reports on production of antifungal compounds by Pseudomonas sp. (Weller 1988). A variety of antibiotics are identified including compounds such as amphisin, 2,4-diacetylphloroglucinol (DAPG), HCN, oomycin A, phenazine, pyoluteorin, tensin and pyrrolnitrin (Mavrodi et al. 2006; Wang et al. 2001). The antibiotic

phenazine-1-carboxylic acid produced by fluorescent pseudomonads isolated from rhizosphere of wheat previously has been also reported (Thomashow et al. 1990). In our study, most of the isolates exhibiting antifungal activity belonged to species of Bacillus and Pseudomonas. Ps. aeruginosa showed inhibition against Aspergillus phoenicus and Cephalosporum maydis. Ps. thivervalensis inhibited growth of Sclerotium oryzae and A. phoenicus, while Ps. lini inhibited only A. phoenicus growth. Growth of Fusarium moniliformis and Alternaria tritiana were not inhibited by any of the isolates. Nitrogen fixers that exhibited antifungal activity were A. globiformis Y2S3 and A. globiformis A2S3. It showed activity against B. tetramera, Sc. oryzae and C. maydis. None of the other nitrogen fixers showed antifungal activities against any of the fungal cultures.

Arthrobacter globiformis Y2S3 exhibited IAA production, as well as siderophore production and demonstrated antifungal activity against Bipolaris tetramera, causative agent of leaf spot in turf grasses. Thus this strain of Arthrobacter globiformis is an efficient PGPR and can be used for development of bio-inoculant. This study has given an insight into the microbial biota associated with rhizosphere of wheat during flowering stage and the plant growth promoting traits of the strains isolated from the same niche. These strains could thus be explored further to develop an effective plant growth promoting bio-inoculants to improve the growth and health of wheat crop in economically as well as environmentally feasible way.

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