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ORIGINAL PAPER

Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat

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Abstract Development of an effective plant growth promoting rhizobacteria (PGPR) inoculant necessitates the presence of a diverse set of traits that can help its colonization of the rhizosphere and survival under varying environmental conditions. In our investigation, a set of 100 bacterial isolates from the rhizosphere of wheat plants were screened initially on the basis of a seed germination assay; ten bacterial isolates (AW1–AW10) were selected. These isolates were then tested in vitro for specific PGPR traits, such as the production of IAA, siderophore, ammonia, HCN, P solubilization, ACC deaminase activity, acetylene reduction assay and antifungal activity. Of the ten isolates, AW5 was found to be promising for all PGP attributes. An experiment undertaken in the controlled conditions of the National Phytotron Facility revealed the potential of three isolates (AW1, AW5 and AW7) in enhancing the growth parameters of wheat plants. Characterization of these isolates using polyphasic approaches involving both phenotypic and genotypic attributes led to their identification as *Bacillus* sp. (AW1), *Providencia* sp. (AW5), and *Brevundimonas diminuta* (AW7), respectively. These strains could prove effective PGPR inoculants as they possess a number of traits useful for their establishment and proliferation in soil. The genus *Providencia* is reported for the first time for its PGP potential, using cultural as well as functional attributes to show its suitability as an inoculant for wheat crop.

Keywords *Providencia* sp. · Plant growth promoting rhizobacteria · PGPR traits · Wheat rhizosphere · Yield enhancement

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Introduction

Plant growth promoting rhizobacteria (PGPR) are a heterogenous group of bacteria that colonize roots and promote the growth of plants. The term PGPR was first used by Kloepper and Schroth (1978) for the microorganisms closely associated with the rhizosphere region. It is well established that, in the rhizosphere, only 1–2% of bacteria promote plant growth (Antoun and Kloepper 2001). The rhizosphere is a hot-spot of microbial interactions due to the exudates released by plant roots, which constitute the main food source for microorganisms, leading to efficient geochemical cycling of nutrients. Therefore, screening and selection of effective PGPRs and their utilization in integrated practices is of great importance for enhancing the growth and yield of agricultural crops along with maintaining the sustainability of agro-ecosystems.

Wheat, together with rice, represent the most important staple food crops used to sustain humanity, as they provide more calories and proteins in the diet than any other crop. Annually, total wheat production in India is 80.2 million tonnes (http://www.agricoop.nic.in, Annual report 2009–2010), which corresponds to about 12% of total world production (http://dacnet.nic.in). More than 143.88 million

tonnes of chemical fertilizers are used worldwide to increase the yield of crop plants. Despite their efficiency in promoting crop yields, such fertilizers have proved to be hazardous for soil health as well as for the wellbeing of human and animal populations. Applying microbial inoculants helps to reduce the use of chemical pesticides, and the use of inorganic fertilizers and PGPR inoculation hence represent promising agricultural approaches that can play a vital role in crop protection, growth promotion or biological disease control, and sustained soil fertility (Dilantha et al. 2006).

PGPR are known to employ one or more direct and indirect mechanisms of action to improve plant growth and health, although the major mode of action of many PGPRs is through increasing the availability of nutrients for the plant in the rhizosphere region (Glick 1995). Several studies have reported that the influence of PGPR is sometimes crop- or niche-specific, or their benefits are limited due to the climatic variability and inconsistency of soil (Khalid et al. 2004; Ryu et al. 2003; Wu et al. 2005). Therefore, in the present investigation, our attempts were directed towards the selection of strains exhibiting the highest number of traits associated with the PGP ability of wheat under in vitro conditions and pot experiments, as a prelude to their use at field level.

Materials and methods

Isolation and preliminary screening of the bacterial isolates

A set of ten wheat plant rhizospheric soil samples was collected from a field at the Indian Agricultural Research Institute, New Delhi, and stored at 4°C. These soil samples were used to isolate the most predominant bacteria using the serial dilution method on nutrient agar. The bacterial strains were bioassayed for their ability to promote seed germination and growth of wheat seedlings (Shende et al. 1977). Seeds were dipped for 10 min in log phase bacterial culture containing at least 10^6 cells ml^{-1}. Dipped seeds were placed on soft agar plates (0.8% sterile agar) with three replicates for each treatment at 28°C for 4–5 days. Seed vigor index was calculated by multiplying germination (%) by seedling length (mm) (Abdul Baki and Anderson 1973).

Detection of plant growth promoting traits of the selected isolates

Indole acetic acid and phosphate solubilization

Production of indole acetic acid (IAA) in the bacterial isolates was estimated by inoculation of bacterial suspension (10% v/v) into Luria Bertani (LB) broth containing 50 μg ml^{-1} L-tryptophan. The cultures were kept at 28±2°C for 48 h and then centrifuged at 10,000 *g* for 10 min. IAA concentration in the culture supernatant was estimated using Salkowski reagent (Gordon and Weber 1951).

A qualitative assay for P solubilization was performed in Pikovskaya medium containing tricalcium phosphate (Pikovskaya 1948). Bacterial culture was spotted on the medium and incubated at 30°C for 48 h. The presence of a clear halo zone around the culture spot indicates the P solubilization capacity of the isolate.

HCN and ammonia production

Hydrogen cyanide (HCN) production was evaluated by the qualitative method of Kremer and Souissi (2001) by streaking the bacterial isolates on King's B agar medium amended with 4.4 g glycine l^{-1}. Circular Whatman no.1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. The plates were then sealed air-tight with Parafilm and incubated at 30°C for 48 h. A color change of the disc from yellow to reddish-brown was considered as an indication of HCN production. Detection of ammonia production was done by adding 1 ml Nessler's reagent to a 72-h-old culture grown in peptone broth and recording the presence of the yellowish brown color (Dye 1962).

Siderophore production

A qualitative assay of siderophore production was conducted in Chrome Azurole's (CAS) agar medium (Schwyn and Neilands 1987). CAS agar plates were prepared and spot inoculated with test organism and incubated at 30°C for 3–5 days. Development of a yellow–orange halo around the colony was considered as a positive result.

1-Aminocyclopropane-1-carboxylate deaminase assay

The activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase in the induced bacteria was quantified by measuring the amount of α-ketobutyrate produced as a result of the enzymatic cleavage of ACC (Penrose and Glick 2003).

Acetylene reduction assay

An acetylene reduction assay (ARA) was used as an index of nitrogen fixation. Pure cultures of the isolates were inoculated in semi-solid nitrogen-free medium (Rennie 1981) in a wide test tube and incubated at 30°C for 48 h under stationary conditions. N-free semi-solid medium was

prepared by mixing solution A (0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.1 g NaCl, 0.028 g Na_2Fe EDTA, 0.025 g $Na_2MoO_4 \cdot 2H_2O$, 0.10 g yeast extract, 5.0 g mannitol, 5.0 g sucrose, 0.5 ml of 60% v/v sodium lactate for 900 ml distilled water, pH 7) and solution B (0.20 g $MgSO_4 \cdot 7H_2O$, 0.06 g $CaCl_2$ for 100 ml distilled water) with 1% agar. Solutions A and B were autoclaved separately then mixed together. Filter sterilized biotin (5 μg l^{-1}) and PABA (10 μg l^{-1}) was also added to the above mixture. After incubation, the air was replaced with acetylene (10% v/v) and incubated at 30°C for 6 h. Ethylene production was measured using a Gas chromatograph (Model Hewlett Packard Series II-5890). The total cell pellet was digested by adding 500 μl of 2 N NaOH and kept at 100°C for 30 min. After cooling, equal amounts of 2 N HCl were added to neutralize the aliquot. The protein concentration of the cell suspension was estimated by Lowry et al. (1951) and the values of ARA expressed in terms of nmol ethylene mg^{-1} proteins h^{-1}.

Antifungal activity

The antifungal activity of the bacteria was assessed against the test pathogen (*Macrophomina phaseolina*) by dual culture technique on potato dextrose agar (PDA) medium (Dennis and Webster 1971). The diameter of the zone of inhibition was measured after 48 h.

Evaluation of plant growth promoting ability of bacterial isolates

The selected set of ten bacterial isolates was tested under controlled conditions at the National Phytotron Facility, IARI, New Delhi. The isolates were applied along with one-third the recommended dose of N + full dose P and K fertilizers as a basal dose in the soil. Wheat (var. HD. 2687) seeds were soaked in culture suspensions of the individual cultures (10^6 cells ml^{-1}) for 15 min. Control seeds were soaked in sterile medium. Seeds were sown in plastic pots (15-cm diameter) containing sterile soil, and placed in a temperature-controlled growth chamber for 120 days. Five plants per pot were maintained throughout the experimental period. The soil was maintained at 60% water-holding capacity (WHC). The treatments were arranged in a completely randomized design with three replications. At the end of the experimental period, the plants were uprooted and plant height was measured. Biomass was dried to constant weight in oven at 80°C. The dry weight of plants was recorded. Subsequently, root, shoot, panicle weight was estimated. Statistical analysis was performed with the SPSS (SPSS, Chicago, IL) package, version 10.0, and treatment means were compared at 5% level of significance.

Morphological and biochemical characterization of isolates

The selected bacterial isolates were examined for their morphological features. Gram staining and biochemical tests, including oxidase test, MRVP test (Methyl Red, Voges-Proskauer), starch hydrolysis, casein hydrolysis, urease test, catalase test, gelatin liquefaction, and nitrate reduction test, were performed (Cappuccino and Sherman 1992).

Identification of bacterial isolates by 16S rDNA sequence analysis

Genomic DNA was extracted by the phenol/chloroform extraction method (Sambrook et al. 1989). The 16S rRNA gene was amplified using specific primers (Maatallah et al. 2002): forward 41f (5′-GCTCAAGATTGAACGCTGGCG-3′) and reverse 1488r (5′-GTTACCTTGTTACGACTT CACC-3′). The reaction was carried out in a 100 μl reaction mixture containing 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 25 pmoles of forward and reverse primers, 50 ng DNA template and 5 U *Taq* DNA polymerase with its reaction buffer. A 30-cycle reaction was performed at 94°C for 1 min, 62°C for 30 s and 72°C for 90 s followed by a final extension of 10 min at 72°C. The reaction was carried out in a thermocycler. The resulting 1.5 kb DNA fragment was extracted and purified using a gel extraction purification kit. The purified product was sequenced (Ocimum Link Biotech, Hyderabad). The isolates were identified by aligning the nucleotide sequence with the other nucleotide sequences submitted to NCBI. The gene sequences were also submitted to GenBank and accession numbers were assigned.

Results

Selection of bacterial isolates

A total of 100 bacterial isolates were picked up on the basis of their colony characteristics. On the basis of the seed germination assay, ten bacterial isolates that performed better and enhanced the root and plumule length of wheat significantly compared to untreated controls were selected (Fig. 1). Maximum percentage germination (100%) and vigor index (2,300) was recorded in isolate AW5, followed by AW7.

PGP traits of the selected isolates

The PGP traits of the selected bacterial isolates are shown in Table 1. Among the ten bacterial isolates, five (AW1, AW3, AW5, AW7 and AW10) were able to solubilize P in the plate-based assay, as evidenced by the formation of a clear halo around the colony. All the isolates produced a

Table 1 Characterization of selected bacterial isolates for specific plant growth promoting rhizobacteria (PGPR) traits. Values indicate the mean ± SE for three replications. Upper case letter indicate ranking order obtained by Duncan's multiple range test

PGPR trait	Production of ammonia	Phosphate solubilization	Siderophore production	Hydrogen cyanide	Antifungal activity	Indole acetic acid (μg ml^{-1})
Isolate no.						
AW1	+[a]	+	-	-	-	22.9±0.3 B
AW2	-	-	-	-	-	16.97±0.3 DE
AW3	+	+	-	-	-	12.68±0.9 F
AW4	+	-	-	-	-	46.88±4.1 A
AW5	+	+	+	+	+	17.37±3.0 C
AW6	+	-	-	-	-	15.02±2.0 DE
AW7	+	+	-	-	-	13.96±2.0 DE
AW8	+	-	-	+	+	21.96±1.3 B
AW9	-	-	-	-	-	12.96±1.8 DE
AW10	+	+	-	-	-	15.85±2.1 DE

[a] + /– indicates presence/absence of trait

significant amount of IAA and tested positive for ammonia production except AW2 and AW9. IAA production ranging from 12.68 to 46.88 μg ml^{-1} was observed, with isolate AW4 showing maximum IAA production. Only AW5 was positive for siderophore production, showing a yellow zone on the CAS agar medium plate. Isolates AW5 and AW10 exhibited ACC deaminase activity (3.13 and 9.5 μm α-ketobutyrate mg^{-1} h^{-1} respectively). Among the ten isolates, AW4 displayed highest nitrogenase activity in terms of ARA (29.31 nmol C_2H_4 mg^{-1} protein h^{-1}) while the activity ranged from (3.62 to 18.28 nmol C_2H_4 mg^{-1} protein h^{-1}) in the other isolates (Fig. 2). Antagonistic activity of the bacterial isolates was evaluated in terms of inhibition zone diameter as an indicator of the reduction in growth of pathogenic fungi *Macrophomina phaseolina*. AW5 and AW8 were recorded positive for HCN production and antifungal activity. The maximum zone of inhibition (3.6 cm) was observed in isolate AW5.

Evaluation of PGP abilities under controlled conditions

Under pot culture conditions, all the isolates promoted root and shoot growth of wheat seedlings, with a concomitant increase in shoot and root dry biomass over untreated controls (Table 2). The maximum enhancement was observed in treatments involving the three isolates: AW1, AW5 and AW7. In these treatments, a significant enhancement in root weight (85.71%, 71.4% and 64.2%, respectively), panicle weight (37%, 48.1% and 38.9%) and plant height (40.91%, 41.31% and 29.14%) was recorded over untreated controls.

Morphological, biochemical and molecular characterization of the selected isolates

All the isolates were motile rods, testing positive for catalase and oxidase except AW4 and AW5. Seven isolates were Gram-negative, while AW1, AW3 and AW6 were Gram-positive, exhibiting endospore formation. All the isolates tested positive for nitrate reduction, casein hydrolysis and citrate utilization, except AW7 and AW9. None of

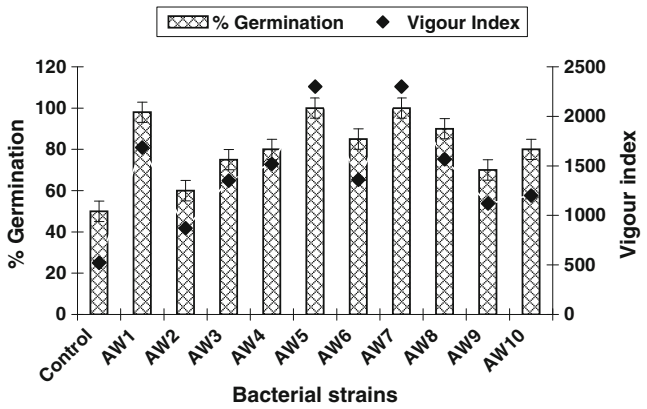

Fig. 1 Effect of bacterial cultures on seed germination and vigor index of wheat after 4 days germination. Error bars depict standard error of means of three independent replicates

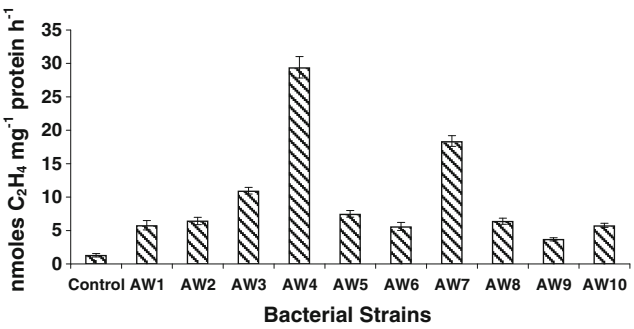

Fig. 2 Effect of bacterial inoculants on acetylene reduction assay (ARA) in terms of ethylene produced. Error bars depict standard error of means of three independent replicates

Table 2 Influence of selected bacterial PGPRs on biometric parameters of wheat crop under controlled conditions. Upper case letters denote ranking based on Duncan's multiple range test. Values followed by different letters in a column were significantly different ($P<0.05$)

Wheat rhizosphere PGPR or treatment	Shoot weight (g pot^{-1})	Root wt (g pot^{-1})	Panicle wt (g pot^{-1})	Biomass (g pot^{-1})	Plant height (cm)
AW1	1.06 F	0.56 AB	0.74 AB	2.36 D	24.66 BC
AW2	1.00 FG	0.36 BC	0.17 E	1.53 HI	21.16 D
AW3	0.55 H	0.39 BC	0.51 ABCD	1.45 I	19.70 EF
AW4	0.85 G	0.37 BC	0.66 ABC	1.88 GH	21.16 D
AW5	1.55 E	0.48 ABC	0.80 A	2.83 CD	24.73 AB
AW6	1.57 E	0.41 ABC	0.25 DE	2.23 CDE	17.33 G
AW7	2.12 C	0.41 ABC	0.75 AB	3.28 AB	22.60 BC
AW8	2.54 A	0.36 BC	0.72 ABC	3.62 A	20.00 E
AW9	1.41 E	0.26 C	0.43 CDE	2.10 DE	18.33 FG
AW10	2.31 B	0.34 C	0.54 ABCD	3.19 BC	18.40 F
Un-inoculated	1.12 F	0.28 C	0.54 ABCD	1.94 G	17.50 G
1/3 N dose	1.77 D	0.29 C	0.46 BCD	2.52 DE	20.73 C
2/3 N dose	2.47 A	0.41 ABC	0.67 ABC	3.55 AB	21.50 BCD
SEM	0.054	0.012	0.025	0.176	0.085
CD@ 5%	0.149	0.033	0.069	0.487	0.235

the isolates were positive for VP reaction. Three isolates (AW4, AW5, AW8) exhibited golden ring formation during indole test, and only two (AW4 and AW5) were positive for methyl red test. Five of the isolates (AW6–AW10) were positive for urease test. Six isolates (AW1, AW2, AW3, AW4, AW7 and AW8) found positive for fat hydrolysis, two isolates (AW8, AW10) were positive for H_2S production, and two isolates (AW5, AW8) were positive for gelatin liquefaction (Table 3). AW4 and AW5 were found positive for indole test, methyl red test and negative for urease test, which aided in designating them as belonging to *Providencia* genus. AW7 and AW9 were identified as

Table 3 Biochemical characterization of selected PGPR isolates from wheat rhizosphere. + Positive, − negative test

	Isolate									
Test	AW1	AW2	AW3	AW4	AW5	AW6	AW7	AW8	AW9	AW10
Gram's staining	+	-	+	-	-	+	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	-	-	+	+	+	+	+
Catalase	+	+	+	+	+	–	+	+	+	+
Endospore formation	+	-	+	-	-	+	-	-	-	-
Indole production	-	-	-	+	+	-	-	+	-	-
Methyl red	-	-	-	+	+	-	-	-	-	-
Voges proskauer's	-	-	-	-	-	-	-	-	-	-
Citrate utilisation	+	+	+	+	+	+	-	+	+	+
Casein hydrolysis	+	+	+	+	+	+	-	+	-	+
Starch hydrolysis	+	+	-	+	+	+	+	+	+	+
Fluorescent pigment production	-	-	-	-	+	-	-	-	-	+
H_2S production	-	-	-	-	-	-	-	+	-	+
Gelatin hydrolysis	-	-	-	-	+	-	-	+	-	-
Urease	-	-	-	-	-	+	+	+	+	+
Fat hydrolysis	+	+	+	+	-	-	+	+	-	-
Lysine decarboxylase	+	-	+	+	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	-	+	+	+
Oxidative/fermentative	+	+	+	F[a]	F	-	-	+	-	-

[a] Fermentative

belonging to *Brevundimonas* genus. As per *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994), AW2 and AW10 matched well with genus *Alcaligenes* and AW1, AW3 and AW6 with *Bacillus* genera (Gram-positive, oxidase, catalase positive, endospore forming rods).

The sequenced PCR products of bacterial isolates were matched with the available sequences in the GenBank database. On the basis of 16S rDNA sequencing data, bacterial isolates AW1, AW6 and AW3 showed 99% similarity with the *Bacillus* genus. Further, they were distinguished on the basis of biochemical characteristics, according to *Bergey's Manual of Determinative Bacteriology*, as *B. pumilis*, *B. cereus* and *Paenibacillus* sp. AW2 and AW10 showed 99% similarity with *Alcaligenes* sp., AW4 and AW5 exhibited 99% and 96% similarity with *Providencia* sp. respectively, AW8 showed 98% similarity with *Pseudomonas aeruginosa*. AW7 and AW9 revealed 99% similarity with *Brevundimonas* sp. The GenBank accession numbers of the isolates AW1 to AW10 are HM452309, GQ368701, HM452311, GQ368694, FJ866760, HM452310, FJ843099, GQ368695, HM452312 and GQ368696, respectively.

Discussion

The beneficial plant–microbe interactions in the rhizosphere are known to be important determinants of plant health and soil fertility (Jeffries et al. 2003). Plant growth promoting rhizobacteria (PGPR) represent a diverse range of soil bacteria that stimulate the growth of their host when grown in association. Such rhizosphere microbes benefit by utilization of metabolites secreted by plant roots as a nutrient for their growth and promote plant growth through more than one mechanism, including production of growth stimulating hormones and suppression of plant pathogens.

It has often been reported that an effective biological control/biofertilizer strain isolated from one region may not perform in the same way in other soil and climatic conditions (Duffy et al. 1997). Therefore, in our study, selection of an effective PGPR was based on a wide range of attributes, making them adaptable to diverse environments and soil types.

In our investigation, ten PGPR isolates were selected on the basis of their performance in a seed germination assay. All the strains enhanced the seedling length significantly but *Providencia* (AW5) and *Brevundimonas diminuta* (AW7) also showed a two-fold increase in percentage germination as compared to untreated controls. A similar enhancement of seed germination in wheat crop was also reported by Zarrin et al. (2009); however, the vigor index observed in other cereals crop such as maize (Gholami et al. 2009) was much lower as compared to our study. The selected set of isolates was further tested for specific PGP traits under in vitro conditions. IAA production was quantified as ranging from 12.96 to 46.88 μg ml^{-1}. Similar observations for IAA production have been reported by other researchers (Selvakumar et al. 2008; Zarrin et al. 2009).

Some rhizobacteria promote plant growth by production of siderophores. The role of siderophore production in plant growth promotion is described by two mechanisms: one is direct supply of iron to plants (Glick et al. 1999), the other is indirect, in depriving fungal pathogens of iron (Ahmad et al. 2008). In the present study, only *Providencia* sp. (AW5) was positive for siderophore production. Plants take iron up directly in the form of an iron–siderophore complex. Siderophore production by *Pseudomonas* has shown to promote rootlet elongation on cucumbers grown under gnotobiotic conditions (Bellis and Ercolani 2001).

Providencia sp. (AW5) and *Pseudomonas aeruginosa* (AW8) also exhibited HCN production. Both siderophores and HCN are known to be intricately related to antifungal activity. HCN production has been shown to be both a beneficial and a harmful property for plants (Cattelan et al. 1999). The production of HCN in excess may play a critical role in the control of fungal diseases in wheat seedlings (Flaishman et al. 1996). Blumer and Hass (2000) reported that IAA production promotes plant growth and HCN production has been proposed as a defense regulator against phytopathogens.

Nitrogen-fixing potential is an important attribute for PGPR and, among the ten isolates tested, *Providencia* sp. (AW4) and *Brevundimonas diminuta* (AW7) exhibited highest activity (29.31 and 18.29 nmol ethylene mg^{-1}protein h^{-1}, respectively). Interestingly, a positive correlation of nitrogen-fixing potential (measured as ARA) was recorded with panicle weight and plant height. This, in turn, is indicative of the significant role of nitrogen fixation in enhancing plant growth. Production of ACC deaminase by PGPR has reported as a direct PGP trait of benevolent isolates (Penrose and Glick 2003). In our study, *Providencia* sp. (AW5) and *Alcaligenes* sp. (AW10) possess ACC deaminase activity (3.13 and 9.5 μm α-ketobutyrate mg^{-1} h^{-1}, respectively). Similar findings have been reported by Fischer et al. (2007) and in *Pseudomonas fluorescens* by Javier et al. (2004). In our study, all isolates exhibited motility, which is another interesting feature, since it promotes colonization of roots.

Soil is a storehouse of several forms of phosphate, including inorganic and organic phosphate, mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. In our study *Bacillus* sp., *Providencia* sp., *Brevundimonas* and *Alcaligenes* were recorded positive for P solubilization. *Alcaligenes* and *Bacillus* sp. have been reported previously for P solubilization, among several other genera (Forchetti et al. 2007). Diverse groups of microorganisms including *Pseudomonas*,

Serratia, *Acinetobacter* sp. employ a variety of solubilization reactions, such as acidification, chelation, exchange reactions, and production of gluconic acid, to release soluble from insoluble P (Pandey et al. 2006). *Pseudomonas*, *Burkholderia*, *Bacillus* and *Alcaligenes* sp. were reported as PGPRs and were found positive for specific PGPR traits (Cattelan et al. 1999).

Bacterial plant growth promotion is a well-established and complex phenomenon that is often achieved by the activities of more than one PGP trait exhibited by plant-associated bacteria. In our study, 50% of the isolates exhibited more than four PGP traits, and *Providencia* (AW5) is reported as a novel PGPR that was positive for all the PGP attributes tested. Isolates AW4 and AW5 tested positive for indole test, methyl red test and negative for urease test, which was essential for their identification (Holt et al. 1994) as *Providencia* genera. Rauss (1962) proposed the nomenclature and identification of this genus in the *International Bulletin of Bacteriological Nomenclature and Taxonomy* on the basis of their biochemical tests.

An important factor to consider during screening of new isolates is their activity in the range of environments/soil types wherein they would be expected to be used (Ross et al. 2000). In our investigation, the bacterial isolates increased plant growth parameters as compared with uninoculated plants. The growth parameters, namely plant height (40.91%), panicle weight (37%), root weight (85.71%), increased significantly due to PGPR inoculants. Maximum enhancement in panicle weight and plant height was recorded by only three isolates (AW1, AW5 and AW7) among the ten bacterial isolates. The higher panicle and shoot weight response to all inoculants compared to controls clearly showed the beneficial role of these rhizobacteria, which might be attributed to IAA production, phosphorus solubilization, nitrogen-fixing capacity of bacteria and any other PGPR activity in favour of plant growth response. All the selected PGPRs had promising positive effects on the plant growth parameters of wheat under controlled conditions. A similar trend in observations was recorded by Burd et al. (2000). Salantur et al. (2006) also showed the effect of PGPR in terms of enhancement of plant height and productivity by synthesizing phytohormones, increasing the availability of nutrients, facilitating the uptake of nutrients by the plants, and antagonizing plant pathogens. Inoculation of apple trees with PGPR isolates significantly increased cumulative yield (from 26.0 to 88.0%) as compared to controls (Karlidag et al. 2007). In our earlier investigation, wheat crop yield was also enhanced significantly (48.76%) in treatments comprising cyanobacteria-bacteria PGPR consortia, as compared to uninoculated control/fertilizer controls (Nain et al. 2010).

Bacteria with a number of PGP characteristics can constitute a significant part of the protective flora that benefits plants by enhancing root function, suppressing disease and accelerating growth and development. In our study, the enhancement in plant parameters by the bacterial inoculants, especially AW1, 5 and 7, was supported by the greater number of PGPR traits possessed by these isolates. This can lead to better survival of the bacterial inoculants as a result of their ability to utilize a large number of carbon/nitrogen substrates, along with the additional feature of motility in these isolates.

Conclusions

This study illustrates the significance of screening of rhizobacteria under in vitro conditions for multiple PGPR traits and their evaluation under controlled conditions in a pot experiment. This led to the selection of effective PGPR isolates—AW1, AW5 and AW7—which, as a result of their multiple PGPR traits, could prove effective in improving the productivity of wheat crop and maintenance of soil fertility.

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