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Screening and identification of soil bacteria for growth promotion of wheat (*Triticum aestivum* L.)

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Abstract

Plant growth promoting rhizobacteria (PGPR) are well known to impact plant growth by various direct or indirect mechanisms. To investigate the effect of PGPR, a total of nineteen bacterial strains were isolated from wheat rhizospheric soil. These bacterial strains were characterized for their plant growth promoting (PGP) traits like indole-3-acetic acid (IAA) production, phosphate solubilzation, *nif*H gene amplification, catalase, oxidase, siderophore production, and gram staining. Identification of bacterial strains was done by using 16S rRNA gene sequencing and all bacterial strains were able to produced IAA and solubilize inorganic phosphate. The range for IAA and inorganic phosphate was between 2.81-107.39 µg mL^{-1} and 14.55 to 556.63 µg mL^{-1}, respectively. Only two bacterial strains were successfully amplified for 400bp *nif*H gene and most of the isolated bacterial strains were positive for siderophore production test. On the basis of PGP activities nine strains were selected as most promising and tested for wheat growth under controlled chamber experiment. All tested PGPR significantly increased root length, shoot length, dry root weight, and dry shoot weight over control. Three strains i.e. (i) A18, (ii) A28 and (iii) A29 were selected on the basis of their performance under controlled chamber and further testing under pot and field conditions along with application of different doses of NP fertilizer. In pot and field experiments, significant increase was recorded in T6. These results support our hypothesis that use of PGPR or combinations of PGPR and chemical fertilizer can enhance the nutrient use efficiency of fertilizers and crop production.

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Introduction

Plant growth promoting rhizobacteria (PGPR) are advantageous for many plant species by being colonized in their rhizosphere. The plant species are benefited by those PGPRs in the form of improvement in their growth rate and susceptibility against different diseases (Kloepper and Schroth, 1978). Surface and subsurface soils are normally nutrient-poor environments for microbes but rhizosphere is enriched in nutrients due to the release of root exudates, monosaccharides, organic acids and amino acids, which enhance the active growth and different microbial activities within the surrounding of the roots. Therefore isolation, screening, characterization and selection of proficient PGPRs is important. These PGPRs are further utilized in integrated practices to significantly stimulate the yield and growth of agricultural crops and to achieve sustainable stabilized agro-ecosystems. The use of PGPRs is now well recognized and large number of evidences have supported the fact that PGPRs play a vital role in enhancing plant's tolerance against different environmental stresses, which include drought stress, salt stress, weed infestation, heavy metal contaminations and nutrient deficiency (Sheng, 2005; Egamberdieva, 2008; Zahir *et al.,* 2008; Babalola, 2010).

To fulfill the needs of growing population, the cultivation of wheat (*Triticum aestivum* L.) in rain-fed areas is enhanced, which is a major cereal crop in Pakistan. Improved varieties with high yield production and different chemicals are used for this purpose. The chemicals are used as fertilizers (providing nourishment) and as protecting agents (against phytopathogens). Extreme use of these chemicals and change in traditional agricultural practices has ruined the physical, chemical and biological health of soil. Sustainable agriculture production is attained if the soil is enriched with microbial variety. The use of pesticides can be minimized by application of microbial inoculants. The addition of inorganic fertilizers and PGPR play a vital role in growth promotion, crop protection against diseases and maintaining soil fertility. Therefore, in the present study, main focus was to isolate, identify and screen the potential bacterial strains from wheat rhizosphere soil using 16SrRNA gene tagging and evaluate their effectiveness for improving growth and yield of wheat.

Materials and methods

Isolation and Screening of Soil Bacteria

Nineteen bacterial strains were isolated from wheat rhizospheric soil collected from farmer's fields of Northern and Pothwar regions of Pakistan and stored at 4°C. These bacterial strains were isolated by using the serial dilution plate technique on Tryptic Soy Agar (TSA; Difco) and incubated at 28°C for 48-72 hours depending on rate of bacterial growth. For purification, single colonies were picked and streaked on plates containing media and purified cultures were stored in 75% (w/v) at -80°C for further characterization (Hayat *et al.,* 2012).

Plant Growth Promoting Activities

Isolated bacterial strains were assayed for their capability to synthesize indole-3-acetic acid (IAA), siderophore, phosphate solubilzation, *nif*H gene amplification and resistance against antibiotics. For determination of IAA, bacterial cultures were grown at 28±2 °C by using their respective media. After 48-72 hrs fully grown culture were centrifuged at 3000 rpm for 30 min. Salkowski reagent (1ml 0.5M $FeCl_3$ solution, 50ml 35% of perchloric acid) 4 mL and two drops of orthophosphoric acid were mixed with 2 mL of supernatant (Brick *et al.,* 1991) and quantified colorimetrically at 530 nm using Indole-3-acetic acid pure solution as standards. For quantitative estimation of phosphate, all the bacterial strains were grown in Nautiyal broth containing tricalcium phophate $Ca_3(PO_4)_2$ as the source of inorganic phosphates followed by seven day shaking at 28 °C and centrifuge at 15,000 rpm for 30 min. The amount of soluble phosphate was estimated in supernatant and the absorbance of the developing color was recorded at 700 nm and concentration of soluble phosphorus was measured with the help of standard

curve of KH_2PO_4 (King, 1932). Siderophores production was detected by the formation of orange halos around bacterial colonies on Chrome Azural S (CAS) agar plates after incubation for 24 hrs at room temperature. The presence of catechol-type and hydroxamatetype siderophores was determined by Arnow's assay (Schwyn and Neilands, 1987). Antibiotic resistant was determined by using the disc-diffusion following the protocol of Bauer *et al.,* (1966). The nitrogen fixing potential of bacterial strain was determined through PCR using reverse and forward *nif*H gene primers $PolF^b$ (5'TGCGAYCCSAARGCBGACTC3') and $PolR^b$ (5'ATSGCCATCATYTCRCCGGA3') (Poly *et al.*, 2000). Enzyme activity tests for selected strains were performed using the API20E and API ZYM system (biomerieux) according to the manufacturer's instructions. The procedures were carried out in the Laminar flow Hood to ensued sterilized environment. Gram staining, catalase and oxidase were also determined by using (Bio Mérieux) kit and according to the procedure described by Cowan and Steel (2004).

Identification of Bacterial Strains by 16S rRNA Sequence Analysis

The bacterial strains were identified using standard method of PCR amplification of 16S rRNA genes sequencing with forward and reverse primers 27F (5'AGAGTTTGATCATGGCTCAG3') and 1541R (5'AAGGAGGTGATCCAGCCGCA3') (Macrae *et al.*, 2000). The reaction mixture (50 µL) contained 5 µL of 10x PCR buffer (Takara), 4 µL of each deoxyribonucleotide triphosphate (dNTP, Takara), 0.3 µL of Taq DNA polymerase (Takara), 1 µL of each oligonucleotide primer, and 1µL of template DNA. PCR was performed by 30 cycle reactions at 95°C for 4 min, 95°C for 30s and 56°C for 30s followed by 10 min extension at 72°C. Amplified PCR products were separated on 1% agarose gels in 1x TE buffer and purified with a QIA quick Gel Extraction Kit (Qiagen). The purified samples were sequenced and matched to those data available in Gen Bank DDBJ using BLAST search on http://www.ezbiocloud.net. Phylogenetic analyses were performed using bioinformatics software MEGA 6 (Tamura *et al.,* 2011). BioEdit and CLUSTAL X were used for comparison and sequence alignment, respectively. Sequences of all bacterial strain were submitted to DNA Data Bank of Japan (DDBJ) and accession numbers of each bacterial strain were obtained.

Wheat Inoculation

On the basis of PGP activities, most promising nine identified PGPR strains were tested to see their effect on wheat crop under growth chamber. Sterilized wheat seed were soaked in inoculums of individual bacterial strain for 10-15 min except control. Treated seeds were sown in small plastic container containing sterile soil and placed in growth chamber (25±1 °C). Three plants per container were maintained for period of four week and treatments were arranged in a completely randomized design (CRD) with three replications. Crop parameters like root length, root dry weight, shoot length and shoot dry weight were recorded at the end of experiment.

Pot and Field Experiment

Three strains were selected on the basis of their performance from growth chamber experiment and tested in greenhouse to evaluate the effect of most potential bacterial strains on wheat growth. These three bacterial strains enhanced growth parameter of wheat under growth chamber. A pots having capacity of 10 kg sterilized soil (sandy clay loam) were used in green house experiment. Sterilized seeds were treated with inoculums of three selected bacterial strains in consortium with half and full rate of recommended NP fertilizer on wheat. Uninoculated pot was considered as control. The pot experiment was carried out in completely randomized design (CRD) with three replications. To estimate the response of treatments on different growth parameters, data regarding plant height, root length, dry root weight, and dry shoot weight were recorded at the time of maturity.

Field experiment was also conducted at research farm

of the Department of Soil Science and Soil & Water Conservation, PMAS-Arid Agriculture University Rawalpindi, Pakistan. The soil of the experimental field (33° 38′ 48″ N, 73° 04′ 59″ E) was sandy clay loam and belonged to Rawalpindi soil series (week medium and coarse sun angular blocky with nearly continuous thin cutans, Typic Ustocrepts, Eutric Cambisols) with a pH 7.5. The field experiment was performed in randomized complete block design (RCBD) with three replications. ANOVA was applied on each set of data, using statistix 8.1 and least significant difference (LSD) were used to compare the means at α 00.05 level (Steel *et al*., 1997).

Result

PGP Activities and Biochemical Characterization

The results of PGP activities of all bacterial strains are presented in Table 1**.**

Table 1. Characterization of isolated bacterial isolates for plant growth promoting rhizobacteria (PGPR) traits.

I.D.	Location of Sampling	P-solubilization	IAA with Tryptophane	IAA Without Trytophane	siderophore assay	*Nif*H gene	Catalase	Oxidase	Gram Staining
		----------μg mL^{-1}----------							
A2	Gilgit	104.5±0.1	43.3±0.2	26.2±0.0	+	–	+	–	–
A4	Gilgit	110±5.8	16.6±0.2	9.5±0.1	+	–	+	–	–
A5	Gilgit	106.7±1.3	16.8±0.1	12.4±0.0	+	–	+	–	–
A6	Gilgit	14.5±0.2	16.8±0.5	15.5±0.3	+	–	+	–	+
A9	Abbotabad	136.5±15.4	15.4±0.2	14.4±0.2	+	–	+	–	–
A18	Attock	192.9±1.2	107.3±0.0	14.5±0.0	+	–	+	–	–
A25	Chakwal	345.7±2.4	6.1±0.0	5.3±0.0	+	–	–	+	+
A26	Chakwal	556.6±0.3	8.2±0.0	6.7±0.2	+	–	+	–	–
A27	Chakwal	332.8±2.6	12.3±0.0	9.1±1.4	+	–	+	–	+
A28	Chakwal	428.2±1.3	58.6±1.1	16.0±0.8	+	–	–	–	–
A29	Chakwal	204.5±24.1	2.8±0.1	3.0±0.5	+	–	–	–	+
A33	Chakwal	209.0±0.5	16.7±0.2	13.9±0.1	+	–	+	–	+
A35	Chakwal	153.4±1.0	19.1±0.0	14.7±0.0	+	–	+	–	+
A42	Chakwal	72.9±1.2	11.1±0.3	7.1±0.0	+	+	+	–	–
A45	Chakwal	190.278	5.0±0.0	2.8±0.2	–	–	+	–	–
A44	Attock	459.3±3.5	7.9±0.6	6.9±1.6	–	–	+	+	–
A46	Attock	164.5±0.7	23.1±0.2	18.7±4.0	+	–	+	–	+
A51	Swat	373.7±0.3	10.1±0.0	9.6±0.8	+	–	+	–	+
A84	Attock	173.8±1.2	3.14±1.01	1.3±0.0	–	+	+	–	–

Values indicate the mean ± SE for three replications.

+ Positive, – negative.

All the nineteen bacterial strains were tested for their capability to produce IAA in pure bacterial culture in the absence and presence (500 μg mL^{-1}) of L-tryptophan. The bacterial strains produced significant amount of IAA ranging from 2.81 to 58.68 μg mL^{-1}. The isolates A28 (*Serratia proteamaculans*) and A2 (*Acinetobacter junii*) exibited maximum IAA production i.e., 58.68 μg mL^{-1} and 43.31 μg mL^{-1}, respectively with tryptophan while A29 (*Bacillus anthracis)* and A84 (*Enterobacter arachidis*) produced lowest amount of IAA (2.81-3.14 μgmL^{-1}). All the isolated strains showed the ability to solubilize inorganic phosphate (tri-calcium phosphate). P-solubilizing activity exhibited in the range of 14.55 to 556.63μg mL^{-1}. Isolate A26 (*Pseudomonas koreensis*) was found with the maximum solubilization for inorganic phosphate (556.63μgmL^{-1}) followed by A44 (*Serratia nematodiphil*) which solubilized inorganic phosphate up to 459.38 μg mL^{-1}. Results indicated that only fifteen strains showed positive result for siderophore production and clear orange zone was formed around the bacterial strains on the plates containing CAS agar medium. All bacterial strains were positive for catalase production except for A25, A28 and A29. Only two bacterial strains A25 and A44 were positive for oxidase production (Table 1). Only

two bacterial strains A42 (*Pseudomonas azotoformans*) and A84 (*Enterobacter arachidis*) were able to amplify for nearly 400bp *nif*H gene while *nif*H gene was absent in all others isolated strains. Out of nineteen, only nine strains were selected on the basis their PGP traits and tested on wheat growth under growth chamber experiment. Three strains performed best at growth chamber experiment were selected for further studies and also characterized for biochemical and antibiotic resistant activities (Table 3 and 4).

Molecular Identification of Bacterial Strain

Bacterial strains were identified based on the method of 16S rRNA gene sequencing (Table. 2). Nucleotides sequence were BLAST through the EzTaxon server and revealed that the isolated strains belonged to genera: *Acinetobacter, Arthrobacter, Bacillus, Enterobacter, Staphylococcus, Pseudomonas, Psychrobacter* and *Serratia* (Table 2). All bacterial strains shared more than 97% identity with their closest phylogenetic relatives. Sequences of all identified bacterial strains were submitted to the DDBJ Gene Bank to attain accession numbers (Table 2).

Table 2. Identification of bacteria based on 16S rRNA gene sequencing.

Strain I.D.	Location of Sampling	Closely related Taxa identified by BLAST search using ezbiocloud.net		
		Species strain	Highest similarity of 16SrRNA gene sequence (%)	DDBJ Accession Number
A2	Gilgit	*Acinetobacter junii*	99.2%	AM410704
A4	Gilgit	*Acinetobacter lwoffii*	99.8%	AIEL0100
A5	Gilgit	*Acinetobacter lwoffii*	99.7%	X81665
A6	Gilgit	*Arthrobacter phenanthrenivorans*	99.7%	CP002379
A9	Abbotabad	*Acinetobacter calcoaceticus*	100%	X81661
A18	Attock	*Psychrobactermaritimus*	100.0%	AB975345
A25	Chakwal	*Bacillus cereus*	99.8%	AE016877
A26	Chakwal	*Pseudomonas koreensis*	99.8%	AF468452
A27	Chakwal	*Bacillus cereus*	99.8%	AE016877
A28	Chakwal	*Serratia proteamaculans*	99.8%	AJ233430
A29	Chakwal	*Bacillus anthracis*	99.0%	AB190217
A33	Chakwal	*Bacillus safensis*	100.0%	AF234854
A35	Chakwal	*Staphylococcus equorum subsp. linens*	99.8%	AF527483
A42	Chakwal	*Pseudomonas azotoformans*	99.7%	D84009
A45	Chakwal	*Enterobacter arachidis*	100.0%	EU672801
A44	Attock	*Serratia nematodiphil*	99.8%	EU036987
A46	Attock	*Staphylococcus equorum subsp. equorum*	99.5%	AB009939
A51	Swat	*Bacillus aryabhattai*	99.8%	AB975358
A84	Attock	*Enterobacter arachidis*	99.8%.	EU036987

On the basis of IAA production, phosphate solubilizing and other PGP activities most promising nine bacterial strains were selected and further tested under temperature controlled growth chamber experiment.

Wheat Inoculation with Selected Potential PGPRs All the nine selected PGPR significantly increase the

root length (cm), dry root weight (g plant^{-1}), shoot length (cm) and dry shoot weight (g plant^{-1}) as compared to media (uninoculated) and control (uninoculated). Three PGPRs identified as *Bacillus anthracis* (A29), *Serratia proteamaculans* (A28) and *Psychrobacter maritimus* (A18) were performed best in growth chamber and selected as best potential strains the others.

Table 3. Biochemical characterization of selected PGPR from rhizospheric soil of wheat.

Biochemical test	(A18) *Psychrobacter maritimus*	(A28) *Serratia proteamaculans*	(A29) *Bacillus anthracis*
Alkaline Phospaha Estarase(C4)	+	+	+
Esterase (C4)	+	+	+
Estarase Lipase	+	+	+
Lipase(C14)	-	-	-
Leucine arylamidase	+	+	+
Vanile arylamidase	-	+	-
Cystine arylamidase	+	+	-
Trypsin	+	-	-
α-Chymotrypsin	+	+	+
Acid phosphatase	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	+
α-galactosidase	-	+	-
N-acetyl-β-glucosidase	-	-	-
α-mannosidase	-	-	-
α-fucosidase	-	-	-

+ Positive, – negative.

The strain A29 significantly enhanced root and shoot length by 128% and 10% respectively over control (uninoculated). However, A28 and A18 positively increase the dry shoot weight up to 166 and 299% and dry root weight up to 101 and 84% respectively over control (Table 5). Consequently, on the basis of growth chamber results three bacterial strains (*Psychrobacter maritimus, Bacillus anthracis* and *Serratia proteamaculans*) were selected as most potential PGPRs for further characterization and crop tests.

Pot and Field Experiment

In pot experiment, significant result was recorded in treatment T6 (*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp. + NP @ 25-20 mg kg^{-1}) which increases root length (cm), plant height (cm), dry root weight (g plant^{-1}), dry shoot weight (g plant^{-1}) up to 470%, 65%, 125% and 77% over T1 (control) followed by T5 (*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp. + NP @ 25-20 mg kg^{-1}) which increased above mentioned crop parameters by 430, 66, 125 and 78% over T1 (control) respectively (Table 6).

In field conditions, the bacterial inoculation along with fertilizer significantly showed better results as compare to control (T1). Biomass yield was increased by 34% and 85% by T6 (*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp. + NP @ 100-80 kg ha^{-1}) and T3 (NP @ 50-40 mg kg^{-1}) over T1 (control) respectively. Same trend was obtained in case of grain yield which increased by 27% and 81% by T6 (*Psychrobacter*sp. +

*serratia*sp. + *Bacillus* sp. + NP @ 100-80 kg ha^{-1}) and T3 (NP @ 50-40 mg kg^{-1}) over control respectively.

Discussion

In the present study all nineteen isolated bacterial strains were characterized for specific PGP traits and exhibited their potential for IAA production, P-solubilization, siderophore production, nitrogenase (*nif*H) gene amplification and identified by 16S rRNA gene sequence analysis. Among the phytohormones, IAA is characterized as most common hormone. It has been concluded that more than 80% bacterial strains isolated from rhizospheric soil are able to synthesize a hormone (Patten and Glick 1996). Isolated bacterial strain produced IAA ranging from 2.81 to 58.68µg mL^{-1} (Table.1). Other researchers also reported the same results (Selvakumar *et al.*, 2008; Zarrin *et al.*, 2009).

Table 4. Antibiotic resistance characterization of selected PGPR from rhizospheric soil of wheat.

Antibiotic test	(A18) *Psychrobacter maritimus*	(A28) *Serratia proteamaculans*	(A29) *Bacillus anthracis*
Erythromycin (15ug)	S	R	R
Chloronphenicol (30ug)	R	R	S
Tobramycin (10ug)	R	S	S
Clindamycin (2ug)	R	R	S
Rifampin (5ug)	R	R	S
Tetracycline	S	R	S
Ampicillin	R	R	S
Novobiocin	R	R	S
Penicillin (10ug)	S	R	R
Ciprofloxacin (5ug)	S	R	S
Vanomycin(30ug)	S	R	S
Gentamycin(10ug)	R	S	S
Amikacin(30ug)	R	S	S
Norfloxacin(10ug)	R	S	S

S-sensitive, R-resistant.

Usually, very low concentration of available P is present in soil, normally at levels of 1 ppm or less. To enhance crop yield, conversion from insoluble form of phosphates to soluble phosphates compound is an important trait of PGPRs (Rodríguez *et al.*, 2006). In present study, the selected bacterial strains solubilized insoluble phosphate in the range of 14.55 to 556.63 µg mL^{-1} (Table 1). The strain *Pseudomonas koreensis* (A26) solubilized maximum amount of phosphate. Microbes play a significant role in improving the nutrient availability and their supply to the plants and solubilize unavailable phosphorous compounds (Banik and Dey, 1983; Kang *et al.*, 2002). Many PGPRs are identified as very well solubilizers of insoluble rock phosphate P, soil accumulated-P or bounded and organic-P (Richardson, 1994). These microbes have been normally isolated from rhizospheric soil of various higher plants e.g. aubergine (Ponmurugan and Gopi, 2006), chilli (Ponmurugan and Gopi, 2006), soybean (Son *et al.*, 2006), mustard (Chandra *et al.*, 2007), wheat (Ahmad *et al.*, 2008) and rice (Chaiharn and Lumyong, 2009). Gluconic acid, 2-ketogluconic acid, acetate, citerate, glycolate, lactate, oxalate, succinate and tartarate etc. are the different organic acids released by microbes (Puente *et al.*, 2004; Hayat *et al.*, 2012) and inorganic phosphate solubilization is possible due to the release of these organic acids (Yadav and Dadarwal, 1997). Phosphate solubilizing bacteria can serve as an active biofertilizer supplier to

enhance the availability of P-nutrition to crop plants. The inoculation of isolated strains into the plant rhizosphere becomes easier and feasible when isolation is done from natural soils rather than from genetically modified environment.

Most of the rhizobacteria stimulate plant growth by release of siderophores. There are two mechanisms to describe the role of siderophore production in plant growth promotion: first is release of iron to plants and second is depriving fungal pathogens of iron (Ahmad *et al.*, 2008). In our present study, sixteen bacterial strains were positive for siderophore production and form a clear orange hallo zone around the bacterial strain on the CAS medium. Siderophore production (by rhizospheric bacteria) is a significant plant growth promoting trait to plants in even iron deficient soils (Glick *et al.,* 1999).

Table 5. PGPRs possessing high plant growth promoting activity significantly enhanced root, shoot length and weight of wheat under controlled conditions.

Treatments	Root length	root weight	Shoot length	shoot weight
	(cm)	(g plant^{-1})	(cm)	(g plant^{-1})
Control	4.7 g	0.02 c	23.7 f	0.92 c
Media	6.1 f	0.02 d	24.6 def	1.13 bc
Psychrobacter maritimus	7.2 cd	0.03 c	25.6 bc	1.31 abc
Staphylococcus equorum	7.0 cde	0.03 bc	25.2 cd	1.26 abc
Bacillus anthracis	10.7 a	0.02 c	26.6 a	1.66 a
Pseudomonas libanensis	6.7 def	0.03 c	25.3 cd	1.20 bc
Bacillus safensis	8.9 b	0.02 c	25.6 bc	1.51ab
Bacillus aryabhattai	6.5 ef	0.02 d	24.2 ef	1.05 c
Serratia proteamaculans	9.1 b	0.04 a	26.3 ab	1.69 a
Acinetobacter calcoaceticus	7.5 c	0.04 a	24.8 cde	1.21 bc
Pseudomonas koreensis	6.7 def	0.03 ab	22.6 g	0.99 c
LSD	0.6348	6.972	0.9908	0.4449

The mean (n=4) with different small letters indicate significant difference at probability level < 0.05.

Nine multi traits PGPRs were selected on the basis of their performance in improving shoot length, root length, shoot dry weight, root dry weight under controlled conditions. In our study selected PGPRs *Bacillus, Enterobacter, Serratia* and *Kosakonia spp.* were found most prominent among isolated genera which were significantly effective on growth of wheat. Hayat *et al.,* (2012, 2013) also reported that *Bacillus, Enterobacter, Pseudomonas* and *Serratia* sp. are very good PGPRs with PGP traits like IAA production, phosphate solubilization and N_2-fixation and are also being used for crop production as bioinoculants. The *Bacillus* species is also reported to increase the yield in wheat (Çakmakçi *et al.*, 2007), maize (Pal, 1998) and beans. Kishore *et al.,* (2005) also stated that many *Serratia* species have antifungal characters along with PGP traits and enhanced the growth and yield of legumes, maize and sorghum. All the PGPRs significantly enhance the growth parameters but A18 (*Psychrobacter maritimus*), A28 (*Serratia proteamaculans)* and A29 (*Bacillus anthracis*) significantly perform better as compare to other and these three PGPRs were also characterized for antibiotic and biochemical. Results of antibiotic sensitivities and API biochemical tests of selected PGPRs are illustrated in Table 2 and 3. The results recommended that in vitro experiments of auxin production and growth promotion by PGPR can be a good parameter to screen the potent PGPR to be used as biofertilizer. Effects of selected strains A18 (*Psychrobacter maritimus*), A28 (*Serratia proteamaculans*) and A29 (*Bacillus anthracis*) were

further verified under green house and field conditions. It is considered that combined application of bacterial inoculations and chemical fertilizer markedly improved wheat total biomass and grain yield as compared to control (uninoculated). According to Zahra *et al.,* (2012), use of rhizobacterial inoculants as biofertilizer significantly improved the growth parameters of cereals. Various researchers reported that under controlled conditions, root and seed inoculation with PGPRs enhance root growth through PGP activity. The better root growth generally resulted in good shoot and grain yield. Similar results are presented by Shaharoona *et al.,* (2008), who reported improved efficiency of nutrients uptake by inoculation of PGPRs which resulted in increased root growth and hence efficient uptake of nutrients by plants. Likewise, the use of PGPRs for improvement of plant nutrition under sustainable agriculture has been reported by Karlidag *et al.,* (2007).

Table 6. Effect on root and shoot growth of wheat by inoculation of PGPRs with and without NP fertilizer. Growth significantly increases by combination of PGPRs and NP fertilizer (Full recommended rate), under pot experiment.

Treatments		Root length	Root weight	Shoot length	Shoot weight
		(cm)	(g plant^{-1})	(cm)	(g plant^{1})
T1	Control	4.71 f	1.58 d	2.19 f	1.58 d
T2	NP @ 25-20 mg kg^{-1}	7.86 e	1.77 c	2.66 e	1.77 c
T3	NP @ 50-40 mg kg^{-1}	18.20 d	1.74 c	3.59 d	1.74 c
T4	*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp.	21.51 c	1.80 c	4.12 c	1.80 c
T5	*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp. +NP @ 25-20 mg kg^{-1}	25.02 b	2.57 b	4.66 b	2.57 b
T6	*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp. + NP @ 50-40 mg kg^{-1}	26.90 a	2.81 a	4.96 a	2.81 a
	LSD	0.4933	0.2390	2.0110	0.1204

The mean (n=3) with different small letters indicate significant difference at probability level < 0.05
PGPRs: (P*sychrobacter maritimus* + S*erratia proteamaculans* + *Bacillus anthracis*).

Table 7. Effect on yield of wheat as affected by inoculation of PGPRs with and without NP fertilizer. Yield significantly increases by combination of PGPRs and NP fertilizer (Full recommended rate), under field experiment.

Treatments		Biomass yield	Grain yield	Total shoot N
		t ha^{-1}	t ha^{-1}	%
T1	Control	3.84 c	1.66 d	1.56 b
T2	NP @ 50-40 kg ha^{-1}	4.44 b	1.90 bc	1.52 b
T3	NP @ 100-80 kg ha^{-1}	4.88 a	2.19 bc	1.85 a
T4	*Bcillus* sp. + *Enterobacter* sp.+ *Serratia* sp.	4.45 b	1.72 d	1.37 c
T5	*Bcillus* sp. + *Enterobacter* sp.+ *Serratia* sp. +NP @ 50-40 kg ha^{-1}	4.27 b	1.95 cd	1.55 b
T6	*Bcillus* sp. + *Enterobacter* sp.+ *Serratia* sp. +NP @ 100-80 kg ha^{-1}	5.18 a	2.65 a	1.88 a
	LSD	0.3910	0.3654	0.1045

The mean (n=3) with different small letters indicate significant difference at probability level < 0.05
PGPRs: (*Psychrobactermaritimus*+ *Serratia proteamaculans* + *Bacillus anthracis*).

In field conditions, best results were recorded by treatment T6 (*Psychrobacter* sp. + *serratia* sp. + *Bacillus* sp. + NP @ 100-80 kg ha^{-1}) which significantly increased biomass yield and grain yield up to 34% and 84% over control respectively. These results are in agreement with the findings of many other workers (Xia *et al.*, 1990; Chen *et al.*, 1994; Zahir *et al.*, 1996; Naveed *et al.*, 2008). The results obtained support the hypothesis that inoculation of PGPRs, especially mixture of PGPRs strains along with fertilizers promote the plant's ability in the uptake of N from fertilizer. The same results were concluded by Adesemoye *et al.,* (2010) who reported that PGPRs applied along with fertilizers promote plant growth and yield. On the basis of present results, we proposed that these selected PGPRs could serve as very good bio-fertilizer and have capability to be utilized as an alternatives to inorganic fertilizers.

Conclusion

The present study demonstrates the importance of screening of rhizobacteria and evaluates their potential under controlled conditions, pot and field experiment. This led to the selection of effective PGPRs isolates A18 (*Psychrobacter maritimus*), A28 (*Serratia proteamaculans*) and A29 (*Bacillus anthracis*) which, as a result of their multiple PGPRs traits proved more effective in improving the productivity of wheat crop and maintenance of soil fertility. Additional inoculation experiments under different field conditions and on different crops are needed to confirm these isolates as potential PGPRs and to verify their survival in natural conditions.

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