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Genetic Diversity of Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric Soil of Wheat Under Saline Condition

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Abstract In this study, a total of 130 rhizobacteria was isolated from a saline infested zone of wheat rhizosphere, and screened for plant growth promoting (PGP) traits at higher salt (NaCl) concentrations (2, 4, 6, and 8%). The results revealed that 24 rhizobacterial isolates were tolerant at 8% NaCl. Although all the 24 salt tolerable isolates produced indole-3-acetic acid (IAA), while 10 isolates solubilized phosphorus, eight produced siderophore, and six produced gibberellin. However, only three isolates showed the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Diversity was analyzed through 16S rDNA-RFLP, and of these isolates with three tetra cutter restriction enzymes (*Hae*III, *Alu*I, and *Msp*I), the representative cluster groups were identified by 16S rDNA sequencing. *Bacillus* and *Bacillus*-derived genera were dominant which showed PGP attributes at 8% NaCl concentration. Out of 24 isolates, nitrogen fixing ability (*nif* H gene) was detected in the two isolates, SU18 (*Arthrobacter* sp.) and SU48.

Introduction

Plant growth-promoting rhizobacteria bacteria (PGPR) are free-living soil bacteria that can either directly or indirectly facilitate rooting [20] and growth of plants [13]. In the last 10 years, a number of PGPR that have been identified has seen a great increase, mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere and also because mechanisms of action of PGPR have been deeply studied. Bacteria from the genera *Bacillus* have evolved highly sophisticated regulatory networks for protection against sudden unfavorable environmental changes, including nutrient starvation, changes in temperature and humidity, oxidative stress, sudden elevation in medium salinity. Plant productivity in saline soils is considerably reduced due to improper nutrition of plant along with osmotic and drought stress [3, 22].

The soil gains importance, especially in saline agricultural soils, where high salinity results from irrigation practices and application of chemical fertilizer. This effect is always more pronounced in the rhizosphere as a result of increased water uptake by the plants due to transpiration. Hence, the rhizobacteria form a group of the best adapted microorganisms [33, 35]. The rhizobacteria often plays crucial roles in increasing crop productivity in the plants they colonize due to their close proximity with the roots they inhabit [27]. They are known to take on many functions ranging from plant growth promotion to soil nutrient recycling. For more than a decade, rhizobacteria have been investigated as possible replacements for chemical fertilizers due to the severe deterioration of the chemical, physical, and biological health of the cultivated land [2].

Spore-forming bacteria, typically *Bacillus* species, are one of the major types of soil bacteria. Common physiological traits important to their survival include production of a multilayered cell wall structure, formation of stress-resistant endospores and secretion of peptide antibiotics, peptide signal molecules and extracellular enzymes [12]. Quantitative and qualitative variations in these traits allow

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for these bacteria to inhabit diverse niches in agro-ecosystems.

Wheat (*Triticum aestivum* L.) is one of the major cereal crops in India. Wheat is grown both as spring and winter crop, however, winter crop is more extensively grown than spring. The wheat crop is mainly cultivated under rain fed conditions where precipitation is less than 900 mm annually. Salinity is one of the major constraints which hamper wheat production in India. About 65% yield loss in moderately saline area. The use of plant growth promoting rhizobacteria (PGPR) may prove useful in developing strategies to facilitate wheat growth in saline area. PGPR inoculants are inexpensive, simple to use, and have no adverse effects to land. The bacterial adaptation to the highly saline cultivated soil depends on their genetic diversity. Very few attempts have been made to study the genotypic diversity and PGP attributes viz., production of indole-3-acetic acid (IAA), gibberellins, siderophore, and phosphate solubilization, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and nitrogen fixing ability (*nif* H gene) of the rhizospheric bacteria of wheat in saline area [25, 33]. Therefore, the present study aims to (i) isolation of bacteria from rhizosphere of wheat grown in saline—sodic soils, (ii) identification of plant growth promoting (PGP) attributes, (iii) diversity analysis through 16S rDNA restriction analysis and (iv) analyze the nitrogen fixing ability through *nif* H region.

Materials and Methods

Sample Collection

The root adhering soil (RAS) samples were collected from wheat crop grown field at 60 days of growth. The plants were sampled from different locations in the four districts viz., Varanasi, Mau, Ballia, and Ghazipur of Uttar Pradesh (UP) (23°52′ to 30°25′ N latitude and 77°3′ to 84°39′ E longitude), India. Physicochemical parameters of soil were analyzed and most of the soils have electrical conductivity (ECe) ≥8.5, pH ranging from 8.0 to 9.5, and organic carbon from 0.27 to 2.29%. The plants were carefully removed from the soil and the roots with adherent soil were put in plastic bags for isolation of rhizospheric bacteria.

Isolation of Rhizobacteria

For the isolation of rhizospheric bacteria, the roots were shaken to remove excess soil and 10 g of closely associated rhizospheric soil from each sample was added to 90 ml of sterile water and shaken for 30 min on a mechanical rotary shaker. Ten fold dilutions were made and plated on to five media, Jensen's N free medium, King's B (KB) medium, nutrient agar (NA), trypticase soy agar (TSA), and soil extract agar (SEA) [28]. Bacterial cultures were maintained on the respective slants and stored at 4°C till further use.

Screening for Salt Resistance in Rhizobacteria

The intrinsic resistance of the rhizobacteria isolates against salinity was evaluated by observing the growth on NA medium amended with various concentrations of NaCl (2, 4, 6, and 8% (w/v)). Control plate was also maintained with 0.05% NaCl (w/v). The plates were incubated for 48 h at 28 ± 2°C and the growth on NaCl amended broth were compared with control plates [35].

Screening for Plant Growth Promoting Attributes

All the isolates that showed salt tolerance up to 8% NaCl were screened for the expression of plant growth promoting attributes at different NaCl concentrations (2, 4, 6, and 8%). The growth promoting attributes were estimated as total cellular protein by the Bradford method [5]. IAA production and gibberellins were estimated by colorimetric methods as described earlier [34], phosphate solubilization was observed on Pikovskaya's agar plates [21], ACC deaminase activity was analyzed by using DF Salt [8] minimal medium with ACC as sole source of nitrogen [8, 26] and siderophore production was also examined [30].

Genetic Diversity of Salt Tolerant PGPR

Extraction of Total Genomic DNA

Genomic DNA of the selected 24 rhizobacterial isolates was isolated by GenElute Bacterial Genomic DNA Kit (Sigma, USA). Amplification of 16S rDNA region was done by using universal primers pA (5′-AGAGTTTGATCCTGGCTAG-3′) and pH (5′-AGGAGGTGATCCAGCCGCA-3′) as described earlier [9].

PCR–RFLP Analysis of 16S rDNA

16S rDNA-PCR products were purified by QIA quick PCR purification kit (Qiagen), 100 ng purified PCR products were digested with 3 units of restriction endonucleases *Hae*III, *Alu*1 and *Msp*1 (Fermentas) as per the manufacturer instructions. The digested products together with marker (100 bp, Bangalore GeNei) were resolved by gel electrophoresis (60 V cm^{-1}) on 2.5% agarose gels in 1× TAE buffer containing 10 μg ml^{-1} ethidium bromide (EB). Gels were photographed by gel documentation system (Alphaimager, USA).

Sequencing of 16S rDNA for Identification of Salt Tolerant PGPR

Ten highly efficient bacterial strains were identified by partial sequence of 16S rDNA**.** The PCR amplified 16S rDNA were purified with a QIA quick PCR purification kit (Qiagen). The DNA sequence was double checked by sequencing both strands using primers pA and pH for forward and reverse reactions, respectively. The nucleotide sequences were di-deoxy cycle sequenced with fluorescent terminators (Big Dye, Applied Biosystems) and run in an Applied Biosystems ABI prism automated DNA sequencer (3130xl). Partial 16S rDNA sequence was analyzed by alignment with the GenBank database using BLAST (NCBI BLAST^{R} home page). The nucleotide sequences of 10 partial 16S rDNA segment determined in this study have been deposited in NCBI GenBank data base (Table 1).

Amplification of *nif* H Region in Salt Tolerant PGPR

For amplification of *nif* H region (781 bp), two primers, viz. *nif* H1 5′-CGT TTT ACG GCA AGG GCG GTA TCG GCA-3′ and *nif*H2 5′-TCC TCC AGC TCC TCC ATG GTG ATC GG-3′ were used [25].

Data Analysis

For the analysis of 16S rDNA-RFLP data, the character state '1' was given for a band, which could be clearly and reproducibly detected in the gel and '0' was assigned if it was absent or it was not possible to determine. The data matrix thus generated was calculated by Jaccard's similarity coefficient for each pair wise comparison. Dendrogram was constructed from the similarity matrix by the unweighted pair group method with arithmetic mean (UPGMA) [32].

Phylogenetic and molecular evolutionary analyses with the sequences of 10 strains were conducted by using a software included in MEGA4. All 10 sequences of 16S rDNA of bacteria were aligned using the multiple sequence alignment program CLUSTAL-X. The pair wise evolutionary distances matrix was generated as described by [15] and phylogenetic tree was inferred by the Neighbor joining method [29]. Tree topologies were evaluated by boot strap analysis [10] based on resamplings of 1000 times of the neighbor joining data set.

Results

Screening for Salt Resistance and Plant Growth Promoting (PGP) Traits

A total of 130 bacteria were isolated from the rhizosphere soil of wheat growing in the salt affected soils of Varanasi, Mau, Ballia, and Gorakhpur districts of UP. The number of isolates obtained on different media viz., nutrient agar, King's B, Jensen's N-free, soil extract agar, and trypticase soy agar were 55, 16, 26, 17, and 16, respectively. All the isolates were screened for salt tolerance at graded concentrations (2, 4, 6, and 8%) of NaCl. Out of the 130 isolates, 42 isolates were able to tolerate NaCl stress up to 8% (data not shown). Further screening of these isolates for the production of PGP traits at high salt concentrations revealed that 24 isolates produced IAA, 10 isolates solubilized phosphorus, eight produced siderophore, six produced gibberellins, while only three showed the production of ACC deaminase (Table 1).

Amplification of 16S rDNA and PCR–RFLP Analysis

The 16S rDNA of all the 24 isolates were amplified with the primers pA and pH. Gel electrophoresis of undigested PCR products revealed that all isolates produced a single band of about 1540 bp (Fig. 1). The RFLP analysis revealed large variations among the isolates. The sum of the estimated sizes of the digested fragments of the amplified product was close to that of the full size, i.e., 1540 bp. The restriction patterns obtained after digestion of the amplified 16S rDNA fragment with *Hae*III revealed 14 restriction patterns (Fig. 2a). The fragments with *Hae*III, 650, 450, 250, and 150 bp were common in SU17, SU18, and SU10 strains. Digestion of 16S rDNA product with *Alu*1 gave 17 restriction patterns (Fig. 2b). *Msp*1 was the least discriminatory among the three endonucleases and gave a total of nine restriction patterns (Fig. 2c). Again a DNA band of 600 and 350 bp were common in SU24 and SU30 strains in *Msp*1 digestion. Isolates SU17, SU24, and SU28 were clustered together with restriction endonucleases *Alu*I and *Msp*I, however, SU17 was segregated from the cluster when clustered with *Hae*III.

Dendrogram was derived from the distance matrix by the UPGMA, a total of 22 different combinations of restriction patterns were recorded among 24 isolates (Fig. 3). A critical analysis of the results shows a major division into two clusters at 27% similarity coefficient value. The cluster I comprised of 19 isolates whereas cluster II has only five isolates (Fig. 3).

On the basis of phylogenetic analysis of 16S rDNA (1.5 Kb) of 10 highly salt tolerant bacterial strains were identified as SU3 and SU10 were *Bacillus pumilus*, SU8 and SU44 were *B. aquimaris*, SU13 was *B. arsenicus*, SU16 was *B. sporothermodurances*, SU18 was *Arthrobacter* sp., SU24 was *B. cereus*, SU40 was *Pseudomonas medicona* and SU47 was *B. subtilis* (Table 1). Phylogenetic analyses of the strains based on the NJ method with 1000 bootstrap sampling were resulted into three major clusters

Table 1 Plant growth promoting attributes of salt tolerant rhizobacteria at 8% NaCl concentration

Isolates	IAA[a]		Gibberellin[b]		Phosphate solublization[c]		Siderophore production[d]		ACC deminase[e]		Identified isolates	
	0%	8%	0%	8%	0%	8%	0%	8%	0%	8%	Strain	
SU 3	77.11 ± 5.3	41.54 ± 3.7	ND	ND	5.5 ± 0.72	4.5 ± 0.62	–	–	–	–	EU927407	*Bacillus pumilus*
SU 8	119.63 ± 7.7	99.02 ± 6.3	109.94 ± 7.0	55.75 ± 3.7	–	–	–	–	–	–	EU927408	*Bacillus aquimaris*
SU10	35.08 ± 3.1	11.93 ± 2.1	54.73 ± 5.0	ND	–	–	3.5 ± 0.55	–	–	–	EU430990	*Bacillus pumilus*
SU13	55.62 ± 4.2	31.72 ± 2.8	ND	ND	4.5 ± 0.33	–	4.2 ± 0.35	3.1 ± 0.34	–	–	EU927409	*Bacillus arsenicus*
SU16	65.76 ± 5.0	42.65 ± 3.0	ND	ND	–	–	5.2 ± 0.45	3.5 ± 0.41	–	–	EU430991	*Bacillus sporothermodurances*
SU18	3.79 ± 0.55	80.49 ± 7.2	130.74 ± 9.5	ND	7.5 ± 0.35	–	4.2 ± 0.32	3.1 ± 0.25	–	–	EU927410	*Arthrobacter* sp .
SU24	77.90 ± 6.2	27.43 ± 5.1	73.32 ± 5.0	ND	5.8 ± 0.35	–	–	–	–	–	EU927411	*Bacillus cereus*
SU40	87.10 ± 5.7	56.84 ± 3.3	ND	ND	–	–	–	–	–	–	EU927412	*Pseudomonas medicona*
SU44	61.29 ± 5.3	51.15 ± 2.8	ND	ND	6.7 ± 0.23	5.2 ± 0.32	4.0 ± 0.23	1.9 ± 0.23	–	–	EU927415	*Bacillus aquimaris*
SU47	88.73 ± 4.2	48.85 ± 3.3	7.67 ± 1.2	3.75 ± 0.55	8.0 ± 1.2	3.0 ± 0.33	–	–	–	–	EU927413	*Bacillus subtilis*

Data showing only of ten identified isolates, all observation are in triplicate forms

[a] IAA production (μg mg^{-1} protein)

[b] Gibberellin production (μg mg^{-1} protein)

[c,d,e] Radius of halo zone in mm

(+) Zone observed, (–) Not zone observed

ND Not detected

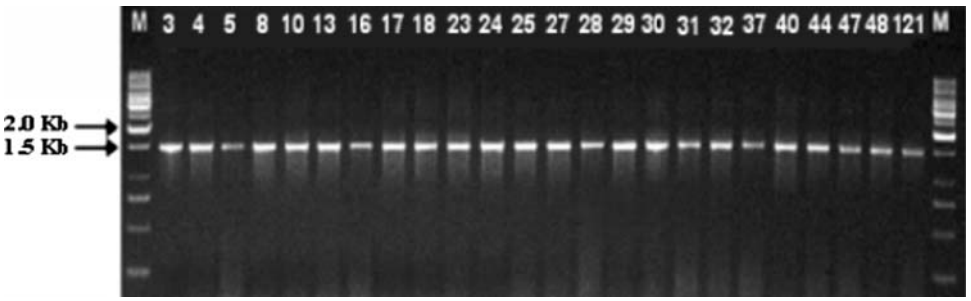

Fig. 1 Agarose gel electrophoresis of PCR amplified 16S rDNA; Lane name with respective isolates; M (1 Kb ladder), 3–(SU3), 4–(SU 4), 5–(SU 5), 8–(SU 8), 10–(SU 10), 13–(SU 13), 16–(SU 16), 17–(SU 17), 18–(SU 18), 23–(SU 23), 24–(SU 24), 25–(SU 25), 27–(SU 27), 28–(SU 28), 29–(SU 29), 30–(SU 30), 31–(SU 31), 32–(SU 32), 37–(SU37), 40–(SU 40), 44–(SU 44), 47–(SU 47), 48–(SU 48), 121–(SU 121), M (1 Kb ladder)

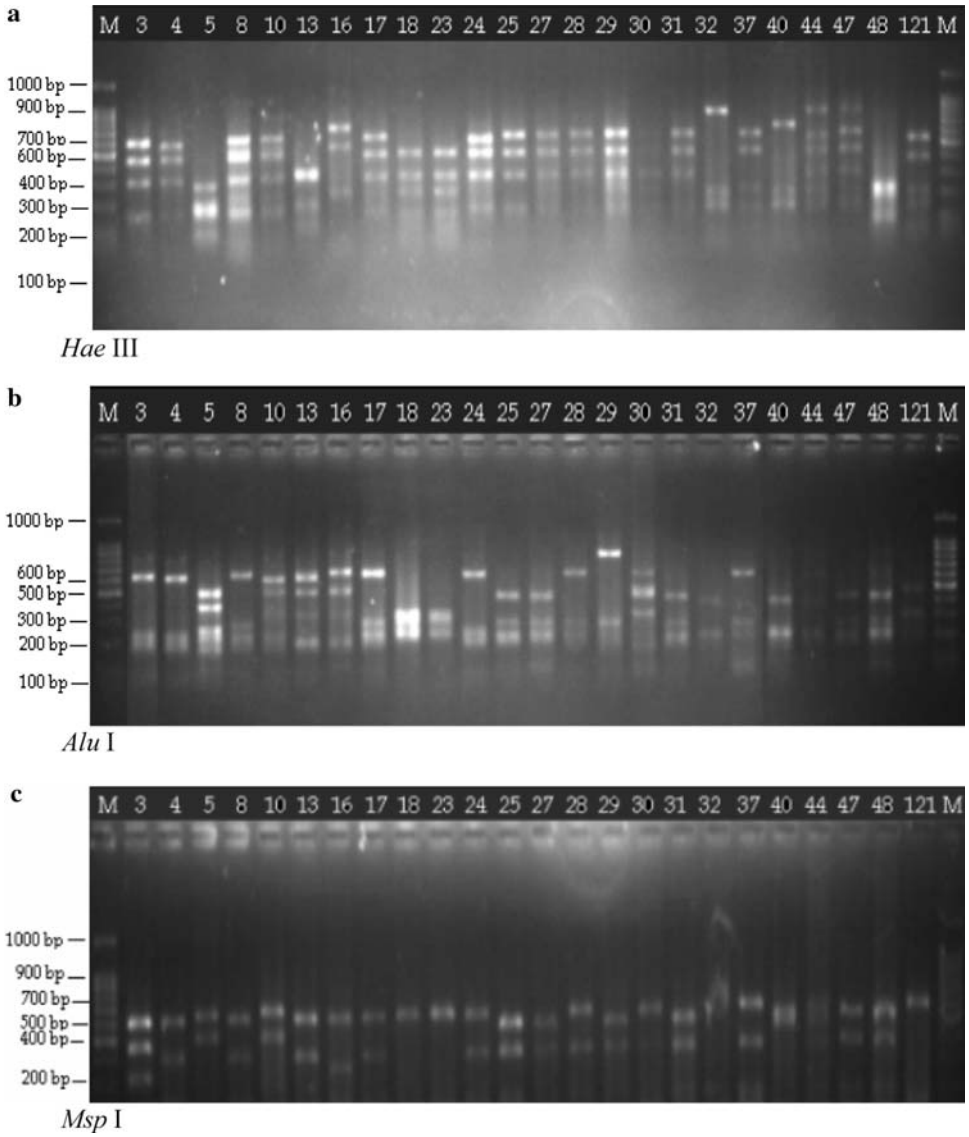

Fig. 2 **a**, **b**, **c** Restriction patterns of PCR amplified fragment of 16S rDNA digested with *Alu*I, *Msp*I and *Hae*III. Lane name with respective isolates; M (100 bp ladder), 3–(SU3), 4–(SU 4), 5–(SU 5), 8–(SU 8), 10–(SU 10), 13–(SU 13), 16–(SU 16), 17–(SU 17), 18–(SU 18), 23–(SU 23), 24–(SU 24), 25–(SU 25), 27–(SU 27), 28–(SU 28), 29–(SU 29), 30–(SU 30), 31–(SU 31), 32–(SU 32), 37–(SU37), 40–(SU 40), 44–(SU 44), 47–(SU 47), 48–(SU 48), 121–(SU 121), M (100 bp ladder)

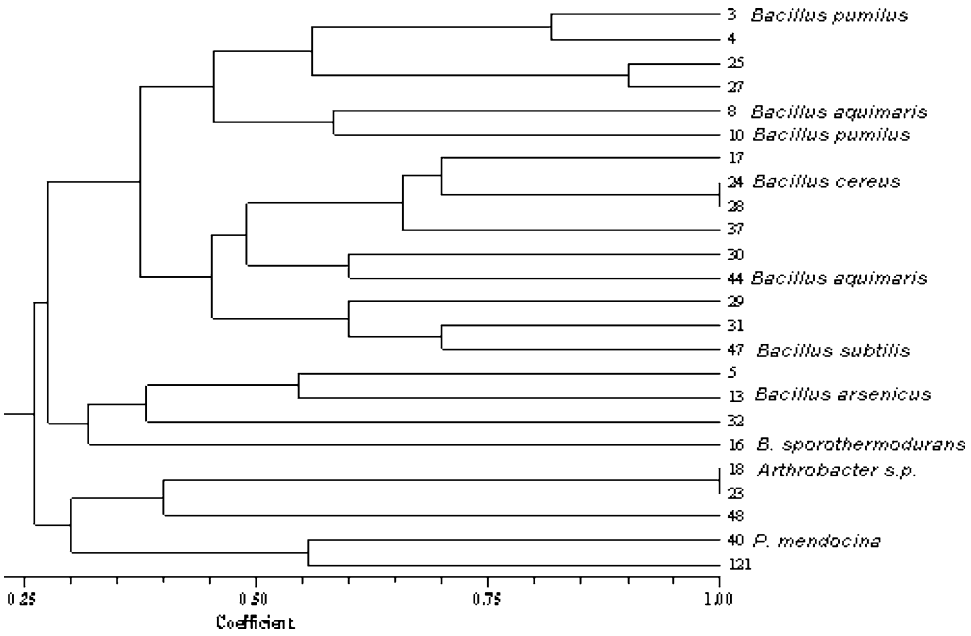

Fig. 3 Combined dendrogram of salt tolerant isolates based on cluster analysis of ARDRA products with restriction endonuclease *Hae*III, *Alu*I and *Msp*I using the UPGMA algorithm and the Jaccard coefficient

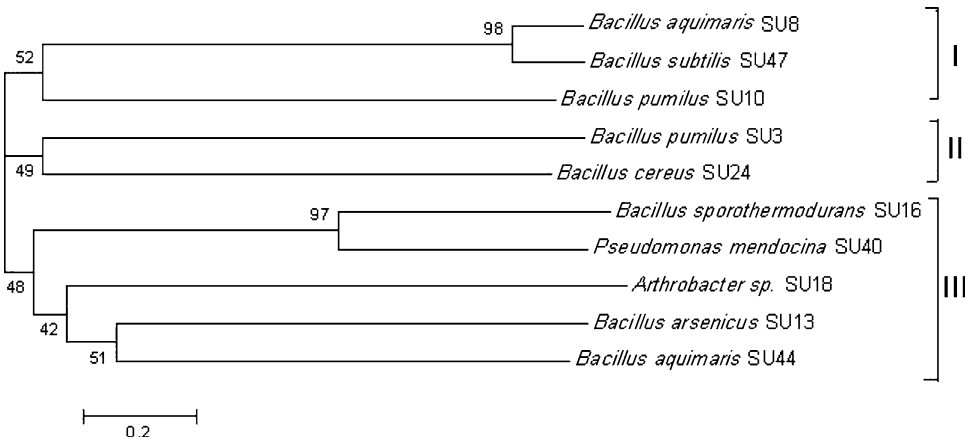

Fig. 4 Phylogenetic tree based on the 16S rDNA sequence of the 10 highly salt tolerant bacterial strains

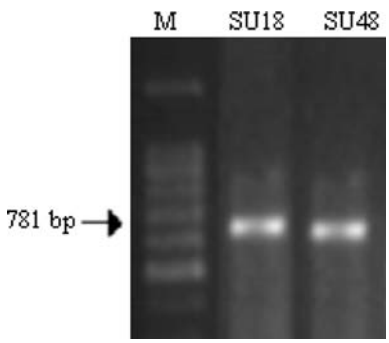

Fig. 5 PCR-amplification of *nif* H region, Lane-M, 100 bp ladder; isolate SU 18-*Arthrobacter* sp. and isolate SU 48 (unidentified)

(Fig. 4). Of the 10 strains, cluster I formed with three strains, cluster II formed with two strains and cluster III formed with five strains.

Amplification of *nif* H Region in Salt Tolerant PGPR

Out of 24 salt tolerant rhizobacterial isolates only two isolates (SU18—*Arthrobacter* sp. and SU48) showed significant amplification of 781 bp and confirmed the presence of nitrogen fixing ability (Fig. 5).

Discussion

The salinity of the soil plays a prominent role in the microbial selection process as environmental stress has been shown to reduce bacterial diversity [4]. Earlier reports also indicate that bacteria isolated from a saline environment are more likely to survive inhibitory salt concentrations than their counterparts from non-saline habitats [7, 14]. Wheat is considered to be moderately tolerant to salinity [19] and species variations to salinity tolerance have

been reported [1]. The population and activity of these PGPRs is greatly influenced by the soil conditions. The rigorous screening of the 130 isolates for salt tolerance and PGP attributes finally led to the selection of only 24 isolates that were evaluated for generic diversity. Similar approach was applied by Principe et al. [27] where they screened approximately 1,000 native strains isolated from saline soils of Cordoba Province (Argentina) to identify the strains which had plant growth promoting and biocontrol activities, as well as salt tolerance. Production of IAA by PGPR isolates is an important attribute for improvement of plant growth. IAA was detected in 80% of bacteria isolated from the rhizosphere [18]. However, in the present study, out of 130 isolates only 24 isolates (18%) showed the production of IAA at 8% NaCl (Table 1) and its level decreased in all isolates with the increase in NaCl concentration (data not shown). The establishment and performance of phosphate-solubilizing microorganisms are severely affected by environmental factors, especially under stressful conditions [21] making it essential to isolate microorganisms from these conditions (such as saline–alkali soils) with high efficiency. In the present study 10 isolates were found to solubilize phosphorus at a concentration of 8% NaCl. These isolates appeared to have potential to be used in saline soils as earlier reports could only identify bacteria capable of solubilizing P up to 5% NaCl [31]. Siderophore production is another important PGP attributes, 48 isolates produced siderophore, out of 311 isolates isolated from wheat field in Southern Brazil [2]. In present study, eight isolates produced siderophore, while only three showed the production of ACC deaminase at high concentration of salt. Cheng et al. [6] reported that ACC deaminase bacteria conferred salt tolerance onto plants by lowering the synthesis of salt-induced stress ethylene and promoted the growth of canola in saline environment. Plant growth promoting substances—gibberellic acid was produced by bacterial isolates from vertisols was reported [34]. In present study, six isolates were positive for gibberellic acid production at 8% NaCl concentration.

In the present study, a combination of three tetra cutter restriction endonuclease *Hae*III, *Alu*I, and *Msp*I permitted a resolution level as comparable to the earlier report [17]. In 16S rDNA-RFLP with *Hae*III and *Alu*I, there was a significant difference observed in all 24 rhizobacterial isolates belonged to the wheat rhizosphere (Fig. 2a, b). However, with *Msp*I showed an almost common restriction pattern in all salt tolerant PGPR isolates (Fig. 2c). Out of 24 salt tolerant rhizobacterial isolates only two isolates (isolate SU18—*Arthrobacter* sp. and isolate SU48) were shown amplification of 781 bp and confirmed the presence of *nif*H for nitrogen fixation in these two isolates. Amplification of *nif* gene in bacteria were isolated from wheat field was reported earlier [2]. The results from PCR–RFLP cluster and potent PGP attributes showing rhizobacterial isolates were sequenced through 16S rDNA and identified with online BLAST. *Bacillus* and *Bacillus*-derived genera were dominant in root adhering soil of wheat under saline soil. Out of 10 identified isolates, eight belongs to the group of *Bacillus*, and these 10 strains formed three major clusters. These strains will ultimately help in development of bio-fertilizers which can be used in saline soil and increasing the availability of nutrients for growth and yield of wheat crop.

It is also observed that the two strains *B. aquimaris* SU8 and *B. aquimaris* SU44 have set of two clusters. The genetic variation of these strains may be due to mutation [24] and other genetic changes like recombination [16, 23]. Mutation rates in bacteria are known to generally increase under stress because of the SOS response and reduced ability to deal with DNA-damaging free radicals generated by metabolism [11].

From this study, we can conclude that—(i) rhizobacteria strains isolated from the rhizospheric soil of wheat in the districts of UP could tolerate NaCl concentration up to 8% and having plant growth promoting traits; (ii) *Bacillus* and *Bacillus*-derived genera were dominant in root adhering soil of wheat and showed genetic diversity.

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References

1. Ali Z, Salam A, Azhar FM, Khan IA (2007) Genotypic variation in salinity tolerance among spring and winter wheat (*Triticum aestivum* L.) accessions. S Afr J Bot 73:70–75
2. Beneduzi A, Peres D, da Costa PB, Zanettini MHB, Passaglia LMP (2008) Genetic and phenotypic diversity of plant-growth-promoting bacilli isolated from wheat fields in southern Brazil. Res Microbiol 159:244–250
3. Benlloch-Gonzalez M, Fournier J, Ramos J, Benlloch M (2005) Strategies underlying salt tolerance in halophytes are present in *Cynara carbunculus*. Plant Sci 168:653–659
4. Borneman J, Skroch PW, O'Sullivan KM (1996) Molecular microbial diversity of an agricultural soil in Wisconsin. Appl Environ Microbiol 62:1935–1943
5. Bradford MM (1976) A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254
6. Cheng Z, Park E, Glick BR (2007) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918
7. Douka CE, Apostalakis CG, Skarloy VD (1978) Studies of *Rhizobium meliloti* isolated from salt-affected soil. Ann Appl Biol 88:457–460
8. Dworkin M, Foster J (1958) Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 75: 592–601

9. Edwards U, Rogall TH, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853
10. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
11. Friedberg E, Walker G, Siede W (1995) DNA repair and mutagenesis. American Society for Microbiology, Washington, DC
12. Gardener BBM (2004) Ecology of *Bacillus* and *Paenibacillus* spp. in agricultural systems. Symposium: the nature and application of biocontrol microbes: *Bacillus* spp. Phytopathology 94: 1252–1258
13. Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 4:109–117
14. Hua SST, Tsai V, Lichens GM, Noma AT (1982) Accumulation of aminoacids in *Rhizobium* spp. WR 10001 in response to sodium chloride salinity. Appl Environ Microbiol 44:135–140
15. Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism, vol 3. Academic Press, New York
16. Kiewitz C, Tümmler B (2000) Sequence diversity of *Pseudomonas aeruginosa*: impact on population structure and genome evolution. J Bacteriol 182:3125–3135
17. Laguerre G, Allard MR, Revoy F, Amarger N (1994) Rapid identification of rhizobia by restriction fragment length polymorphism analysis of PCR amplifies 16S rRNA genes. Appl Environ Microbiol 60:56–63
18. Loper JE, Schroth MN (1986) Influence of bacterial sources of indole-3-acetic acid biosynthetic on root elongation of sugar beet. Phytopathology 76:386–389
19. Maas EV (1986) Salt tolerance of plants. Appl Agric Res 1:12–26
20. Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18:49–53
21. Mehta S, Nautiyal CS (2001) An efficient method for qualitative screening of phosphate solubilizing bacteria. Curr Microbiol 43:51–56
22. Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 28:239–250
23. Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405: 299–304
24. Oliver A, Canton R, Campo P et al (2000) High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science 288:1251–1254
25. Pandey P, Sahgel M, Maheshwari DK, Johri BN (2004) Genetic diversity of rhizobia isolated from medicinal legumes growing in the sub-Himalayan region of Uttaranchal. Curr Sci 86:202–207
26. Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant 118:10–15
27. Principe A, Alvarez FA, Castro MG, Zachi L, Fischer SE, Mori GB, Jofre E (2007) Biocontrol and PGPR features in native strain isolated from saline soils of Argentina. Curr Microbiol 55: 314–322
28. Rouatt JW, Katznelson H (1961) A study of bacteria on the root surface and in the rhizosphere soil of crop plants. J Appl Bacteriol 24:164–171
29. Saitou N, Nie M (1987) The Neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
30. Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56
31. Sharan A, Shikha, Darmwal NS, Gaur R (2008) *Xanthomonas campestris*, a novel stress tolerant, phosphate-solubilizing bacterial strain from saline–alkali soils. World J Microbiol Biotechnol 24:753–759
32. Sneath PHA, Sokal RR (1973) Numerical taxonomy—the principles and practice of numerical classification. W. H. Freeman, San Francisco
33. Tripathi AK, Mishra BM, Tripathi P (1998) Salinity stress responses in the plant growth promoting rhizobacteria, *Azospirillum* spp. J Biosci 23:463–471
34. Vikram A, Hamzehzarghani H, Alagawadi AR, Krishnaraj PU, Chandrashekar BS (2007) Production of plant growth promoting substances by phosphate solubilizing bacteria isolated from vertisols. J Plant Sci 2:326–333
35. Yildirim E, Turan M, Donmez MF (2008) Mitigation of salt stress in radish (*Raphanus sativus* l.) by plant growth promoting rhizobacteria. Roumanian Biotechnol Lett 13:3933–3943