International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 6(2): 187-196 June 2013 .

Biotechnology

Profiling of Plant Growth Promoting Bacteria Associated with Jaunpuri Giant Raddish Rhizosphere

Rishi Srivastava[1], Binu Mani Tripathi[2,3], Rahul Kunwar Singh[4], Pratima Srivastva[1], Priyanka Kumari [2], Madhusudan Srivastav[1], Alok K Srivastava[2], Sudheer Kumar[2], Prem Lal Kashyap[2], Rajesh Sharma[5] and Shree Prakash Tiwari[1*]

[1]Department of Microbiology, VBS Purvanchal University, Jaunpur, India
[2]National Beareu of Agriculturally Important Microorganisms, Mau Nath Bhanjan, India
[3]School of Biological Sciences, Seoul National Uniersity, Seoul, Republic of Korea
[4]Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, India
[5]Department of Biotechnology, VBS Purvanchal University, Jaunpur, India

Email: sptiwarimicro@gmail.com

Paper No. 102 Received: September 13, 2012 Accepted: May 13, 2013 Published: June 1 , 2013

Abstract

Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. Jaunpuri Giant Newar variety of radish was selected to study the PGPR profiling due to its peculiar growth features.The analysis of PGPR was based on their plant growth promotion (PGP) attributes, requirement of carbon substrates, amplified ribosomal DNA restriction analysis (ARDRA) and 16S rDNA homology. In search of efficient PGPR strains associated with radish cv. Jaunpuri Giant Newar rhizosphere, a total 115 isolates were randomly selected and screened for PGP attributes, out of which twenty isolates showing multifarious PGP traits were selected for detailed study. Metabolic characterization of representative strains revealed a large versatility with respect to carbon utilization. ARDRA revealed ten clusters composed of 1–5 strains. Based on 16S rRNA gene sequencing, the isolates were identiûed as *Micrococcus luteus*, *Enterobacter cloacae*, *Bacillus subtilis*, *Pseudomonas* sp., *Agrobacterium tumefaciens*, *Leifsonia* sp., *P. aeruginosa*, *B. cereus*, *B. licheniformis*, *Nesterenkonia terrae*. To the best of our knowledge, this is the first report from India as far as systematic, genetic and functional diversity of PGPR in radish rhizosphere region is concerned. Additionally, the generated information may serve as a baseline data for developing effective bio-inoculants to improve plant growth and biotic stress management of radish in an eco-compatible manner.

Highlights

- A total of 115 isolates were obtained from rhizospheric region of Radish cv. Jaunpuri Giant Newar.
- Among115, 20 isolates showing multiple PGP traits were characterized by BIOLOG, RFLP and ARDRA.
- Representative isolates from each group resulted from ARDRA were identified by 16S rDNA sequence homology.
- Most of the identified isolates were related to genera Bacillus followed by *Pseudomonas, Enterobacter, Agrobacterium, Micrococcus, Leifsonia* and *Nesterenkonia.*

Keywords: ARDRA, biolog, PGPR, raddish, rhizobacteria

The study of rhizobacteria, which establish positive interactions with plant roots, has increased because of their potential use in sustainable agriculture. Plant growth promoting rhizobacteria (PGPR) are a class of beneûcial free- living bacteria inhabiting the soil and are capable of stimulating plant growth either indirectly or directly. The indirect promotion of plant growth occurs when PGPR

lessen or prevent the deleterious effects of one or more phytopathogenic organisms. The direct promotion of plant growth, for the most part, of PGPR, entails either providing

the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients like nitrogen (N) or phosphorus (P) from the environment (Glick, 1995).

In recent years, a number of PGPR have been characterized from rhizosphere of various crops (Solanki *et al*., 2012; Naik *et al*., 2008; Upadhyay *et al*., 2009; Monteiro *et al.,* 2009; Saikia *et al*., 2011) Radish (*Raphanus sativus*) is an important commercial root vegetable and occupies an important position among vegetable crops in India. One of the cultivar of tropical radish is Jaunpuri Giant Newar cultivated around Jaunpur, Uttar Pradesh, India. Its growth feature is very peculiar because it grows up to 75 -90cm in length and 50 – 60 cm in girth (Figure 1). Although there is report about the isolation of plant growth promoting rhizobacteria from radish rhizosphere (Sharma *et al*., 2009), no systematic genetic and functional diversity of PGPR in radish rhizosphere has been explored. Moreover, the characterization of PGPR prevalent in the Jaunpuri Giant cultivar of radish is essential due to the novelty in its growth feature. Therefore, the present investigation was aimed to observe the genetic and functional diversity of bacteria associated with radish rhizosphere through the examination of PGP attributes, utilization of various carbon substrates by Biolog ECO plates, amplified ribosomal DNA restriction (ARDRA) and sequencing of 16S rRNA gene.

Figure 1: Photograph of Raddish (*Raphanus sativus*) cv. Jaunpuri Gi- ant Newar

Materials and methods

Sample Collection

The root adhering samples were collected from radish cv. Jaunpuri Giant grown field at 45 days of growth. The plants were sampled from radish grown area of district Juanpur ($82^{o}44$'E longitude and $25^{o}46$'N latitude), Uttar Pradesh (UP), India. Physicochemical parameters of soil were analyzed and most of the soils have electrical conductivity (ECe) 8.5 dSm^{-1}, pH ranging from 8.0 to 9.5, and organic carbon >1%. The plants were carefully removed from the soil and the roots with adherent soil were put in plastic bags for isolation of rhizobacteria.

Isolation of Rhizobacteria

For the isolation of rhizospheric bacteria, the roots were shaken to remove excess soil. Ten gram 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 four different media *viz.* king's B (KB), nutrient agar (NA) medium, trypticase soy agar (TSA) and soil extract agar (SEA). Bacterial cultures were maintained on the respective slants and stored at 4^{o}C till further use.

Screening for Plant Growth Promoting Attributes

All the isolates were screened for the expression of plant growth promoting attributes. *In vitro* IAA production was estimated colorimetrically (with and without tryptophan in the medium) (Glickmann *et al*., 1995). Tricalcium phosphate solubilization was estimated at 30^{o}C using pikovskaya broth (100 ml) inoculated with 1 ml of bacterial suspension (3 x 10^{5} cells/ml) following the procedure of Pandey *et al*. (2006). Siderophore production was examined on chrome-azurol-S agar medium (Schwyn and Neilands, 1987). Isolates were qualitatively screened for

protease activity by spot inoculating them on nutrient agar plates (pH 6.5) containing 10% skimmed milk (w/v) and incubating for 48 h at 37°C. After the incubation period, the formation of the clear zone around the colony was taken as an indication of protease activity (Kanekar *et al*., 2002). Production of ammonia was tested in peptone water. Freshly grown cultures were inoculated into 10 ml peptone water per tube and incubated for 48 h at 30°C. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to yellow color was a positive test for ammonia production (Cappucino and Sherman, 1992). The method of Bakker and Schippers (Bakker and Schippers, 1987) was used to estimate HCN production.

Microbial Diversity of Radish Rhizosphere

In Vitro Screening for Antimicrobial Activity

Antagonistic activities of the isolates were screened towards the fungal pathogens, viz., *Alternaria brassicae*, *Rhizoctonia soloni* and *Fusarium oxysporum*um, using secondary metabolite of the culture broth. Secondary metabolites were extracted as described by Singh *et al.* (2012). The crude extract was dissolved in 10% dimethyl sulfoxide (DMSO) and used for screening of antimicrobial activity by agar well diffusion method (Schillinger and Lucke 1989). Zone of inhibition was recorded after 5 days of incubation at 28 ± 1°C.

Seed germination assay

Surface sterilized radish (cv. Jaunpuri Giant) seeds were immersed in 0.5% NaOCl solution for 15 min followed by subsequent washing with sterilized distilled water. Dried seeds (100) were soaked in exponentially growing bacterial culture broth for 30 min and then dried in air. Seeds soaked in respective sterile culture medium were served as control. Seeds were placed in Petri plates containing 0.8% water agar and incubated at 25°C in the dark for three days. All the experiments were done in triplicates. Seed germination percentage, shoot and root length were recorded in each treatment with their respective control (Naik and Srinivasa, 2009).

Genomic DNA extraction and Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Total genomic DNA extraction from selected isolates was performed as per the method of Pospiech and Neumann (1995). The gene encoding 16S rDNA from selected isolates was amplified using universal primers pA (5'-AGAGTTTGATCCT GGCTAG-3') and pH (5'-AGGAGG TGATCCAGCCGCA-3') as described earlier (Edwards *et al*., 1999). The PCR products were monitored through gel electrophoresis (1% agarose, w/ v), followed by ethidium bromide staining and UV transillumination. Approximately 1 µg of PCR-amplified 16S rRNA gene fragments were restricted with endonucleases *Hha* I, *Rsa* I and *Bst*U I (Fermentas, USA) separately at 37°C for overnight and resolved by electrophoresis in 2.5% agarose. Banding pattern was visualized by ethidium bromide staining and documented in gel documentation and analysis system (Alphaimager, USA). Strong and clear bands were scored for similarity and clustering analysis using the software, NTSYS- PC2 package (Numerical taxonomy analysis program package, Exeter software, USA). Similarity among the strains was calculated by Jaccard's coefficient and dendrogram constructed using UPGMA method (Nei and Li, 1979).

16S rDNA gene sequencing and phylogenetic analysis

Purified 16S rRNA gene of representative isolates from each cluster was used as a template in cycle sequencing reactions with fluorescent dye-labeled terminators (Big Dye, Applied Biosystems). Both primers pA and pH were used for sequencing and run in ABI prism automated DNA sequencer (3130xl). Partial 16S rDNA sequence was analyzed by alignment with the GenBank database using BLAST (NCBI BLASTR home page). Identification to the species level was determined based on 16S rRNA gene sequence similarity (>97%) with that of a prototype strain sequence. Multiple sequence alignment of approx 1500-bp sequences was performed using CLUSTAL W, version 1.8 (Thompson *et al*., 1994). A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987). Tree topologies were evaluated through bootstrap analysis of 1,000 data sets by MEGA 4.0 package (Tamura *et al.*, 2007). The nucleotide sequences of partial 16S rDNA segment determined in this study have been deposited in NCBI GenBank data base under accession numbers HM367733 – HM367742.

Carbon substrate usage

Carbon substrate usage by isolates was measured using the BIOLOG^{TM} ECO automated identification system (Hayward, California). The BIOLOG^{TM} ECO microplates were inoculated with 125 µl of bacterial cell suspension per well and were incubated at 37°C in sealed plastic bags for 24 h. Substrate oxidation was measured with a

microplate reader at 590 nm. Clustering was based on binary data (usage/nonusage) for each of the 31 substrates. Data were submitted to cluster analysis using a simple matching coefficient (S) (Sokal and Michener, 1958) and clustering was achieved by the unweighted-pair-group method of association (UPGMA) (Sneath and Sokal, 1973).

Results

In vitro screening of isolates for PGP traits

A total of 115 isolates were randomly selected and screened for their PGP attributes and 20 isolates were further selected for the physiological and phylogenetic analysis based on their positivity for multiple PGP traits (Table 1). All the 20 isolates produced IAA in the medium with and without the addition of tryptophan. The concentration was ranged between 0.12 to 4.84 mg/ml and 0.84 - 7.38 µg/ml respectively. Four isolates (RR18, RR15, RR8 and RR13) were able to solubilize mineral phosphate with the concentration ranging from 76.7 to 258.3 µg /mg protein. Eight isolates (RR1, RR2, RR3, RR4, RR5, RR7, RR8 and RR9) were found positive for siderophore production. On the basis of halo zone formation around the bacterial colonies, all isolates except RR19, RR17, RR10, RR9 and RR15 were reported as protease producer. Ammonia production was Microbial Diversity of Radish Rhizosphere reported in fourteen isolates whereas, four isolates (RR18, RR6, RR5 and RR15) were found to be positive for HCN production.

In Vitro Screening for Antimicrobial Activity

Among 20 isolates, three isolates (RR5, RR13, and RR18) showed strong antifungal activity against all the fungal pathogens tested (Table 1). However, other isolates showed mixed reactions in antagonistic activity. For instance, RR12 exhibited strong antifungal activity against *R. soloni*, and *F. oxysporum*um except *A. brassicae*. Similarly, RR19 strongly inhibited the growth of *A. brassicae*, but could not inhibit *R. soloni*, and *F. oxysporum*.

Seed germination assay

In King's B medium, treated seeds with isolate RR17 and RR19 showed highest (90±5%) and lowest (70±10%) percentage of germination, respectively (Table 1). In nutrient agar medium, highest (83.3+10.82%) and lowest (56.7±5.77%) percentage of seed germination was recorded with seeds treated with isolate RR6 and RR3 respectively. The seeds treated with isolate RR11 and RR5 showed highest (96.7 ±2.77) and lowest (43.3 ±05.77) percentage of seed germination, respectively in TSA medium. Similarly, seeds treated with isolate RR9 and RR7 showed highest (90±07.32%) and lowest (43.33±5.77%) percentage of seed germination respectively in SEA medium. Treatment with the extract of isolate RR17 for five days brought the highest enhancement in both root (2.3 fold) and shoot (2.5 fold) length in radish seedling (Table 1).

Amplified ribosomal DNA restriction and 16S rDNA homology

Restriction digestion of 16S rRNA gene using three endonucleases (*Hha* I, *Rsa* I and *Bst*U I) yielded 7 to 9 distinct restriction patterns for each enzyme. About 2 to 5 restricted fragments of varying sizes were rcommon to each of the restriction patterns (Figure 2a) Cluster analysis of combined

16S rRNA gene restriction pattern based on Jaccard's similarity index, grouped all the 20 isolates under ten distinct groups with similarity percentage ranging from 22 -100% (Figure 2b). For sequence analysis, a total 10 representative isolates were chosen from each of group generated by ARDRA. The representative isolates from all four clusters were identified as *Micrococcus luteus*, *Enterobacter cloacae*, *Bacillus subtilis*, *Pseudomonas* sp., *Agrobacterium tumefaciens*, *Leifsonia* sp., *P. aeruginosa*, *B. cereus*, *B. licheniformis*, *Nesterenkonia terrae* (Figure 3).

Carbon substrate usage

The result of clustering analysis based on use/non-use of substrates studied by BIOLOG^{TM} system is shown in Figure 4. The substrate usage pattern for the isolates tested revealed a broad variability. Isolate RR3 identified as *Bacillus subtilis* used about 61% (19/31) of the total number of substrates tested, whereas isolate RR2 (*Enterobacter cloacae*) and RR4 (*Pseudomonas* sp.) were able to utilize only 32% (10/31) of the substrates tested.

Discussion

The study of rhizospheric bacterial population structure gained attention because of the positive response of many plants inoculated with suitable plant growth promoting bacterial strains. In the present study, using a culture-dependent approach, 115 strains were isolated from the rhizosphere of radish cv. Jaunpuri Giant on various media.

Production of IAA by PGPR isolates is an important attribute for improvement of plant growth. In the present

Table 1: Multiple plant growth promoting attributes of the bacterial isolates isolated from radish cv. Jaunpuri Giant Newar rhizosphere

Isolate No	IAA Prod. without Trp µg/mg protein	IAA Prod. with Trp µg/mg protein	Phosphate Solubilization µg/mg protein	Siderophore Production[a]	Protease production[b]	NH_3 Production	HCN production	Seed Germination Assay (%)	Root Length (in cm)	Shoot Length (in cm)	Antifungal activity *Alternaria brassicae*	Antifungal activity *Rhizoctonia solani*	Antifungal activity *Fusaium oxysporium*
KB control								83.3 ±11.50	0.96 ±0.35	0.56 ±0.37			
RR18	0.79	0.84	225.0	ND	0.8	+	+	76.7 ±5.77	1.77 ±0.76	1.11 ±0.48	+	+	+
RR19	2.94	5.20	ND	ND	ND	+	ND	70.0 ±10.00	1.93 ±0.74	1.26 ±0.59	+	ND	ND
RR17	2.75	3.16	ND	ND	ND	+	ND	90.0 ±05.00	2.22 ±1.03	1.43 ±0.55	ND	ND	ND
NA control			-					63.3 ±5.77	2.84 ±0.72	1.66 ±0.37			
RR1	0.12	1.46	ND	1.3	1.7	+	ND	66.7 ±5.77	3.28 ±0.97	1.67 ±0.67	ND	ND	ND
RR2	0.91	1.17	ND	0.9	0.7	ND	ND	73.3 ±5.77	3.13 ±0.86	1.73 ±0.76	ND	ND	ND
RR3	0.16	1.07	ND	1.9	1.8	ND	ND	56.7 ±5.77	2.55 ±0.82	1.37 ±0.47	ND	ND	ND
RR4	1.00	3.48	ND	2.1	0.6	ND	ND	80.0 ±10.00	2.30 ±0.97	1.44 ±0.76	ND	ND	ND
RR6	0.72	0.93	ND	ND	1.3	+	+	83.3 ±10.82	2.66 ±0.90	1.40 ±0.62	ND	ND	ND
TSA control								70.0 ±17.30	2.02 ±1.43	1.03 ±0.67			
RR14	2.56	3.02	ND	ND	1.0	+	ND	90.0 ±07.32	1.72 ±0.72	0.93 ±0.38	ND	ND	ND
RR5	0.64	1.12	210.0	1.7	1.0	+	+	43.3 ±05.77	1.70 ±0.91	0.92 ±0.43	+	+	+
RR8	0.19	4.31	258.3	1.2	0.9	ND	ND	90.0 ±04.00	2.46 ±0.69	1.12 ±0.43	ND	ND	ND
RR20	1.56	1.59	76.7	ND	1.3	+	ND	70.0 ±17.32	2.15 ±0.74	1.40 ±0.71	ND	ND	ND
RR13	0.64	2.96	ND	ND	1.2	ND	ND	63.3 ±5.77	2.76 ±0.81	0.97 ±0.38	+	+	+
RR12	0.79	2.26	ND	ND	1.6	ND	ND	83.3 ±10.82	2.24 ±0.80	1.2 ±0.45	ND	+	+
RR11	2.00	5.24	ND	ND	1.0	+	ND	96.7 ±2.77	1.06 ±0.57	0.87 ±0.62	ND	ND	ND
RR10	0.54	1.88	ND	ND	ND	+	ND	90.0 ±4.00	1.05 ±0.52	0.89 ±0.52	ND	ND	ND
SEA control								43.3 ±5.77	0.79 ±0.46	0.38 ±0.29			
RR16	1.76	3.04	ND	ND	0.4	+	ND	56.6 ±11.54	1.12 ±0.68	0.52 ±0.40	ND	ND	ND
RR7	4.84	4.29	ND	1.8	0.6	+	ND	43.3 ±5.77	0.92 ±0.59	0.42 ±0.36	ND	ND	ND
RR9	2.26	7.38	ND	1.5	ND	+	ND	83.3 ±5.77	1.10 ±0.29	0.47 ±0.38	ND	ND	ND
RR15	0.33	4.25	ND	ND	ND	+	+	45.3 ±5.77	0.90 ±0.50	0.38 ±0.33	ND	ND	ND

a, b Radius of halo zone in mm, (ND) not detected, (+) positive for the attributes

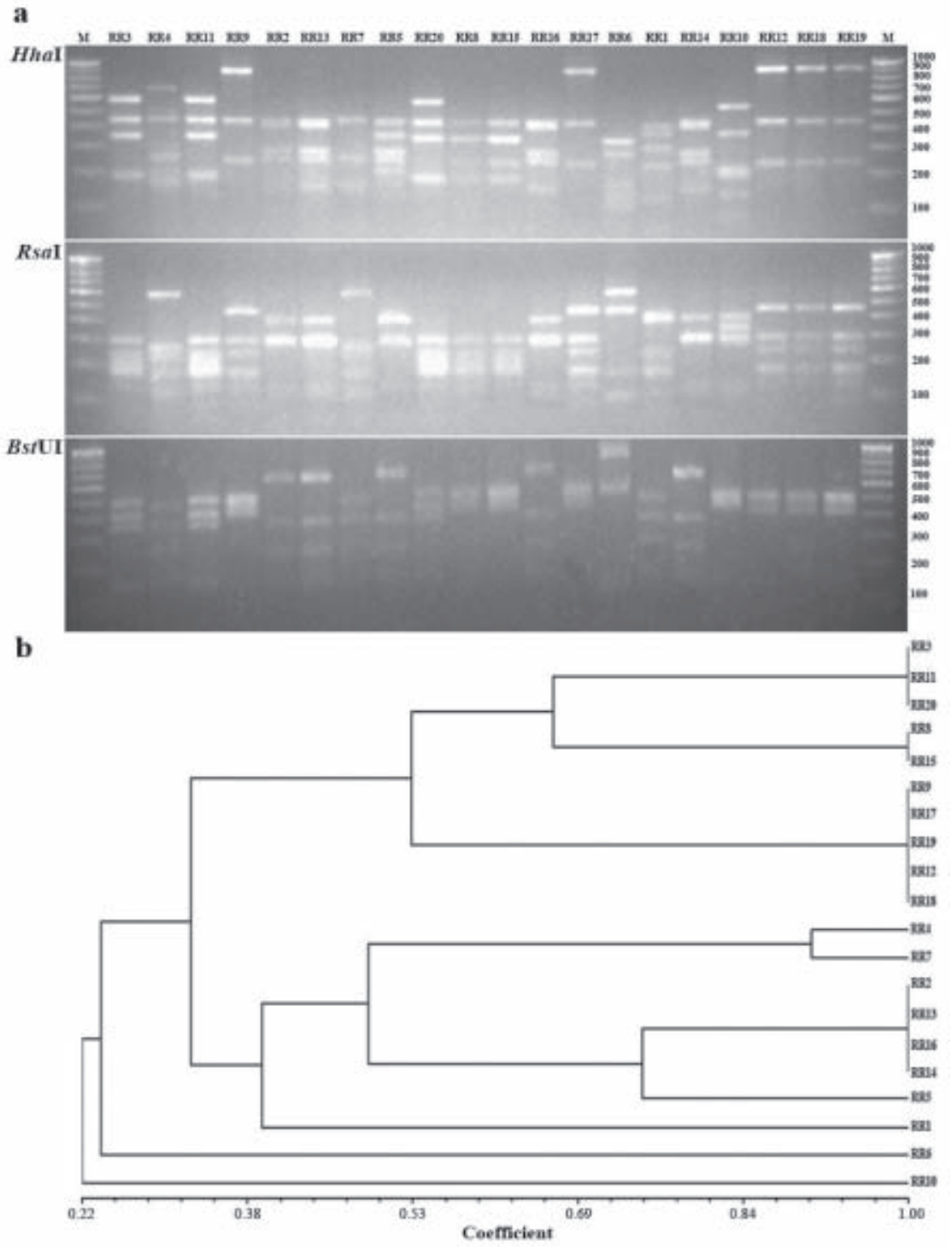

Figure 2: Clustering based on ARDRA. **a)** Restriction patterns of PCR-amplified fragments of 16S rDNA digested with (i) *Hha*I, (ii) *Rsa*I and (iii) *Bst*UI. Lane name with respective isolates; M (100 bp ladder) **b)** Dendrogram showing the clustering of 20 bacterial isolates from radish rhizosphere generated from restriction analysis of 16S rDNA amplicon, using the UPGMA algorithm and the Jaccard's coefficient.

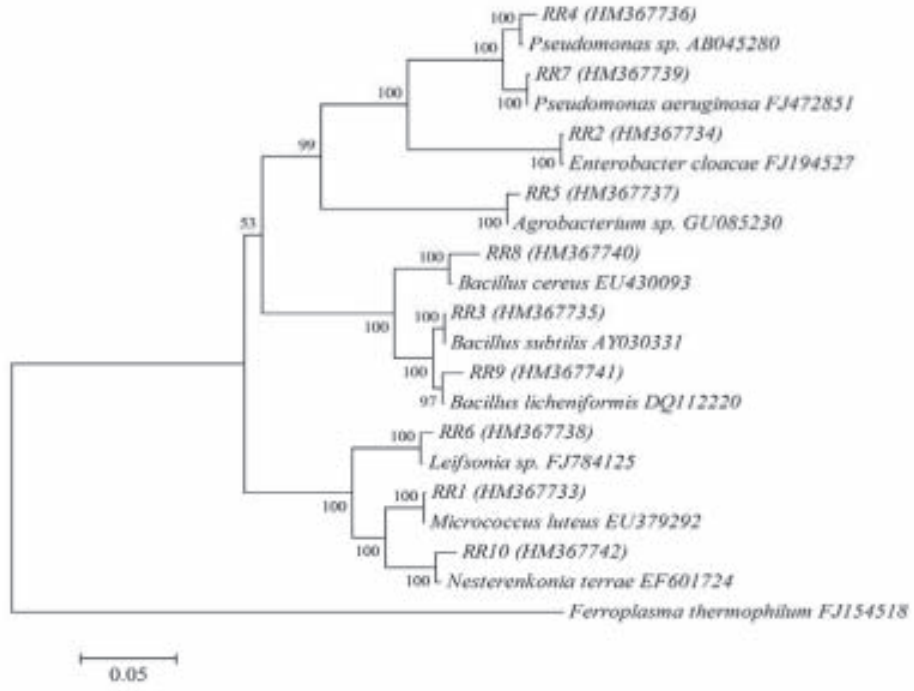

Figure 3: Phylogenetic tree based on the 16S rRNA gene sequences of bacterial isolates from radish cv. Jaunpuri Giant rhizosphere and their closest phylogenetic relatives. The tree was created by the neighbour-joining method. The numbers on the tree indicate the percentage of bootstrap sampling derived from 1000 replicates. 16S rRNA gene sequences of *Ferroplasma thermophilum* was used as out-group.

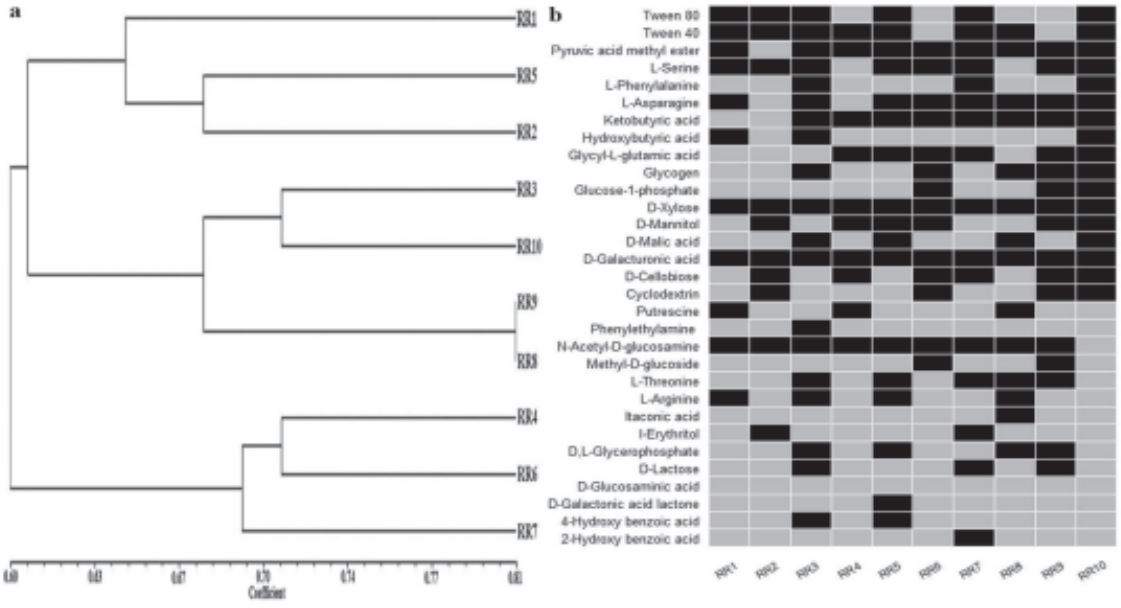

Figure 4. Cluster analyses of BIOLOG substrate usage data by bacterial isolates from radish. **a)** Dendrogram showing clustering of 10 represen- tative bacterial isolates based on simple matching (S_{SM}) coefficient and unweighted-pairgroup method with average (UPGMA) clustering. **b)** Carbon source usage of representative isolates via BIOLOG ECO assay plates. Black boxes represent statistically significant substrate usage at any point during experiments. Experiments were conducted in triplicate.

study, it was found that all 20 bacterial isolates were able to produce IAA in the medium without the addition of tryptophan and tryptophan supplementation showed increased production of IAA **(**Table 1**)**. These isolates appeared to have potential to be used to promote the growth of radish, because radish produces nine times more tryptophan in its exudate per seedling than other plants (Lugtenberg and Kamilova, 2009).

Phosphate solubilizing bacteria are common in rhizospheres and they convert insoluble phosphate into a soluble form, mobilizing the fertilizers added to soil (Rodriguez and Fraga, 1999). In the present investigation, four isolates were found to solubilize phosphorus and among these three belonged to the genus *Bacillus*, which is a well-known phosphate solubilizer (Chen *et al*., 2006).

Siderophore production is one of the most important attributes of the microorganisms. Siderophores chelate Fe and deprive the phytopathogens from iron nutrition. Production of siderophores in rhizospheric bacteria has been already reported by many researchers (Rajkumar *et al*., 2010). In present investigation, eight out of twenty isolates exhibited the production of siderophores. HCN and ammonia production also constitute a very important part of plant growth promotion. It is reported that HCN and ammonia production has beneficial effects on plants (Kloepper *et al.,* 1988; Weller *et al.,* 1988). In this study 14 isolates produced ammonia, whereas only 4 isolates showed the production of HCN.

In the present study, a combination of three tetra cutter restriction endonuclease (*Hha* I, *Rsa* I, and *Bst*U I) were used and found superior for the detection and differentiation of bacterial taxa based on criteria of RFLP size-frequency distribution patterns as reported by earlier workers (Moyer *et al*., 1996). Representatives of the different ARDRA groups were used for partial 16S rRNA gene sequencing. After identification by Blast search tool of the NCBI GenBank database, the majority of the sequences were related to the genus *Bacillus*. Enhancement of plant growth by root- colonizing *Bacillus* is well known (Thompson *et al*., 1994; Timmusk and Wagner, 1999). Although several PGPR have been identified within several distinct bacterial taxa, most commercially developed PGPR formulations belong to *Bacillus* as it forms heat- and desiccation resistant endospores, which confer population stability during formulation and storage of products (Yadav *et al.,* 2011). The other identified bacteria were related to genus *Pseudomonas*, *Enterobacter*, *Agrobacterium*, *Micrococcus*, *Leifsonia* and *Nesterenkonia*, strains of many of these genera have been already described as plant growth-promoting rhizobacteria (Naik *et al*., 2008; Shoebitz *et al*., 2009; Boddey *et al*., 1995; Antoun *et al*., 2004).

Relating the Biolog carbon utilization profiling to the *in situ* ecology of PGPR growth is a big challenge and one could hypothesize that pysiological activity is a reflection not just of the species but is also of the specific environmental conditions. Biolog ECO plate based physiological profiling generated a large number of characters and produced robust data and more realistic representation of relationships among the strains. The Biolog metabolic profiling analysis has been demonstrated to be a highly effective method for differentiating among the species groups by cluster analysis as reported by Dawson *et al.* (2002).

Conclusion

The present study was a preliminary attempt to identify and characterize PGPR associated with the rhziosphere of radish cv. Jaunpuri Giant. To the best of our knowledge this is the first report from India as far as systematic, genetic and functional diversity of PGPR in radish rhizospheric region is concerned. Additionally, the generated information may serve as a baseline data for developing effective bio-inoculants to improve plant growth and biotic stress management of radish in an eco-compatible manner.

References

Antoun, H., C.J. Beauchamp, N. Goussard, R. Chabot and R. Llande. 2004. Potential of Rhizobium and Bradyrhizobioum species as plant growth promoting rhizobacteria on non-legumes: effect on radishes. *Plant & Soil* **204**: 57-67.

Bakker, A.W., B. Schippers. 1987. Microbial Cyanide Production in the Rhizosphere in Relation to Potato Yield Reduction and Pseudomonas Spp-Mediated Plant Growth-Stimulation. *Soil Biology & Biochemistry* **19** (4):451-457.

Boddey, R.M., O.C. Deoliveira, S. Urquiaga, V.M. Reis, F.L. Deolivares, V.L.D. Baldani, J. Dobereiner. 1995. Biological Nitrogen-Fixation Associated with Sugar-Cane and Rice - Contributions and Prospects for Improvement. Plant & Soil **174** (1-2): 195-209.

Cappucino, J.C., N. Sherman. 1992. In: Microbiology: A Laboratory Manual. 3rd edn. Benjamin/Cumming Pub. Co., New York

Chen, Y.P., P.D. Rekha, A.B. Arun, F.T. Shen, W.A. Lai and C.C. Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* **34** (1): 33-41.

Dawson, S.L., J.C. Fry and B.N. Dancer. 2002. A comparative evaluation of five typing techniques for determining the diversity of fluorescent pseudomonads. *Journal of Microbiological Methods* **50** (1): 9-22.

Edwards, U., T. Rogall, H. Blocker, M. Emde, E.C. Bottger. 1989. Isolation and Direct Complete Nucleotide Determination of Entire Genes - Characterization of a Gene Coding for 16s-Ribosomal Rna. *Nucleic Acids Research* **17** (19): 7843-7853.

Glick, B.R. 1995. The Enhancement of Plant-Growth by Free-Living Bacteria. *Canadian Journal of Microbiology* **41** (2):109-117. Glickmann, E., Y. Dessaux. 1995. A Critical-Examination of the Specificity of the Salkowski Reagent for Indolic Compounds Produced by Phytopathogenic Bacteria. *Applied Environmental Microbiology* **61** (2): 793-796.

Kanekar, P.P., S.S. Nilegaonkar, S.S. Sarnaik, A.S. Kelkar. 2002. Optimization of protease activity of alkaliphilic bacteria isolated from an alkaline lake in India. *Bioresource Technology* **85** (1): 87-93.

Kloepper, J.W., D.J. Hume, F.M. Scher, C. Singleton, B. Tipping, M. Laliberte, K. Frauley, T. Kutchaw, C. Simonson, R. Lifshitz, I. Zaleska and L. Lee. 1988. Plant Growth-Promoting Rhizobacteria on Canola (Rapeseed). *Plant Diseases* **72** (1): 42-46.

Lugtenberg, B., F. Kamilova. 2009. Plant-Growth-Promoting Rhizobacteria. *Annual Reviews of Microbiology* **63**: 541-556.

Monteiro, J.M., R.E. Vollu, M.R.R. Coelho, C.S. Alviano, A.F. Blank, L. Seldin. 2009. Comparison of the bacterial community and characterization of plant growth-promoting rhizobacteria from different genotypes of chrysopogon zizanioides (L.) Roberty (Vetiver) Rhizospheres. *Journal of Microbiology* **47** (4):363-370.

Moyer, C.L., J.M. Tiedje, F.C. Dobbs and D.M. Karl. 1996. A computer-simulated restriction fragment length polymorphism analysis of bacterial small-subunit rRNA genes: Efficacy of selected tetrameric restriction enzymes for studies of microbial diversity in nature? *Applied Environmental Microbiology* **62** (7): 2501-2507.

Naik, N., M.N. Sreenivasa. 2009. Influence of bacteria isolated from panchagavya on seed germination and seed vigour in wheat. *Karnataka Journal of Agricultural Science* **22**:231-232.

Naik, P.R., N. Sahoo, D. Goswami, N. Ayyadurai and N. Sakthivel. 2008. Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of banana. *Microbial Ecology* **56** (3):492-504.

Nei, M and W.H. Li. 1979. Mathematical-Model for Studying Genetic-Variation in Terms of Restriction Endonucleases. *Proceedings* Microbial Diversity of Radish Rhizosphere *of National Academy of Sciences, USA* **76** (10): 5269-5273.

Pandey, A., P. Trivedi, B. Kumar, L.M.S. Palni. 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine location in the Indian Central Himalaya. *Current Microbiology* **53** (2):102-107.

Pospiech, A., B. Neumann. 1995. A versatile quick-prep of genomic DNA from gram-positive bacteria. *Trends in Genetics* **11:**217

Rajkumar, M., N. Ae, M.N.V. Prasad and H. Freitas. 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology* **28** (3):142-149.

Rodriguez, H and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* **17** (4-5): 319-339.

Saikia, R., R.K. Sarma, A. Yadav, T.C. Bora. 2011. Genetic and functional diversity among the antagonistic potential fluorescent pseudomonads isolated from tea rhizosphere. *Current Microbiology* **62** (2):434-444.

Saitou, N., M. Nei. 1987. The Neighbor-Joining Method - a New Method for Reconstructing Phylogenetic Trees. *Molecular Biology & Evolution* **4**: 406-425.

Schillinger, U. and F.K. Lucke. 1989. Antibacterial Activity of Lactobacillus-Sake Isolated from Meat. *Applied Environmental Microbiology* **55** (8): 1901-1906.

Schwyn, B., J.B. Neilands. 1987. Universal Chemical-Assay for the Detection and Determination of Siderophores. *Annals of Biochem* **160** (1): 47-56.

Sharma, N., G. Kapoor, N. Gautam, B. Neopaney. 2009. Characterization of a partially purified bacteriocin of *Bacillus* sp MTCC 43 isolated from Rhizosphere of radish (*Raphanus sativus*) & its application as a potential food biopreservative. *Journal of Scientific & Industrial Research India* **68** (10): 881-886.

Shoebitz, M., C.M. Ribaudo, M.A. Pardo, M.L. Cantore, L. Ciampi and J.A. Cura. 2009. Plant growth promoting properties of a strain of Enterobacter ludwigii isolated from Lolium perenne rhizosphere. *Soil Biology & Biochemistry* **41** (9): 1768-1774.

Singh, R.K., D.P. Kumar, M.K. Solanki, P. Singh, A.K. Srivastva, S. Kumar, P.L. Kashyap, A.K. Saxena, P.K. Singhal and D.K. Arora. 2012. Optimization of media components for chitinase production by chickpea rhizosphere associated *Lysinibacillus fusiformis* B-CM18. *Journal of Basic Microbiology* **52**: 1-10.

Sneath, P.H.A and R.R. Sokal. 1973. Numerical taxonomy : the principles and practice of numerical classification. Series of biology books. W.H. Freeman, San Francisco.

Sokal, R.R., C.D. Michener. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* **38**:1409-1438.

Solanki, M.K., S. Kumar, A.K. Pandey, S. Srivastava, R.K. Singh, P.L. Kashyap, A.K. Srivastava, and D.K. Arora. 2012. Diversity and antagonistic potential of *Bacillus* spp. associated to the rhizosphere of tomato for the management of *Rhizoctonia solani*. *Biocontrol Science & Technology,* **22**(2):203-217.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology & Evolution* **24:**1596-1599.

Thompson, J.D., D.G. Higgins, T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**:4673-4680.

Timmusk, S., Wagner, E.G.H. 1999 The plant-growthpromoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: A possible connection between biotic and abiotic stress response. *Plant-Microbe Interaction,* **12**:951-959.

Upadhyay, S.K., D.P. Singh, R. Saikia. 2009. Genetic Diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. *Current*

Microbiology **59**(5): 489-496.

Weller, D.M. 1988. Biological-Control of Soilborne Plant-Pathogens in the Rhizosphere with Bacteria. *Annual Reviews of Phytopathology* **26**: 379-407.

Yadav, S., R. Kaushik, A.K. Saxena and D.K. Arora. 2011. Diversity and phylogeny of plant growth-promoting bacilli from moderately acidic soil. *Journal of Basic Microbiology,* **51**(1): 98-106.

