Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

MOLECULAR CHARACTERIZATION OF PLANT GROWTH PROMOTING BACTERIA FROM SOIL OF CENTRAL AND UPPER HIMALAYAN REGION

AGRAWAL PAVAN KUMAR*, AGRAWAL SHRUTI[2], VERMA SATISH KUMAR, [3] SINGH SANTOSH KUMAR[4] AND SHUKLA KESHAV PRASAD[5]

[3]Department of Biotechnology, [2]Department of Microbiology, Sai institute of paramedical and allied Sciences, Dehradun-248001, [4]Department of Biotechnology, SBS PG Inst. of Biomedical Science & Research,Balawala, Dehradun-248001, [5]Department of Biotechnology, MNNIT, Allahabad, India. Email: p_k_agarwal@rediffmail.com

ABSTRACT

Natural ecosystems are directly dependent on beneficial microorganisms present in the rhizosphere for soil health and plant productivity. Plant growth promoting bacteria were isolated from soil of central and upper Himalayan region with a view to screen/evaluate their phylogenetic relationship among the isolates by using amplified Restriction DNA Analysis (ARDRA).

Keywords: Plant growth promotory rhizobacteria, Soil, ARDRA.

INTRODUCTION

Bacterial diversity is of particular importance in human sustenance since these small creatures comprise the majority of earth's species diversity. Bacterial diversity is considered as one of the most useful resource with considerable significance in the global form of bioremediation and bio-prospecting (Homer-Devin et al 2004).

To understand rhizobacterial diversity, different approaches are now followed by taxonomists, in order to characterize and identify isolates up to species level.

The genomic era has resulted in development of new molecular tools and techniques for study of culturable microbial diversity including the DNA base ratio (mole % G+C) , DNA-DNA hybridization, DNA microarray, reverse sample genome probing, 16S rDNA sequencing and amplified rDNA restriction analysis. In addition, non-culturable diversity which makes a larger proportion of the existing population can be characterized employing tools such as denaturing gradient gel electrophoresis (DGGE) Muyzer *et al.* (1993), temperature gradient gel electrophoresis (TGGE) and single stranded conformational polymorphisms (SSCP) (Peters *et al.,* 2000).

Liu *et al.* (1997) have reported that PCR amplified 16S rDNA is digested with a 4-base pair cutting restriction enzyme. Pace (1996) has reported that banding patterns can be used to screen clones and used to measure bacterial community structure (Mossol-Deya *et al.,* 1995). Liu *et al.* (1997) have reported that this is useful for detecting structural changes in microbial communities but not as a measure of diversity or detection of specific phylogenetic groups.

Taxonomy based on comparative analysis of 16S rRNA genes, first introduced by Carlwoose, presents a radical departure from classical taxonomic. Life could be divided into three primary phylogenetic 'Domains, Archara, Bacteria and Eucarya (Woese, 1990). Olsen and Woese (1987) have reported use of rDNA and rRNA in phylogenetic analysis.

For microorganisms, molecular data often provides greatest information because microorganisms such as bacteria simply do not have the diversity of form to make morphological characteristics useful in establishing phylogeny.

Aside from derivation of taxonomies, phylogenetic analyses are important in identifying similarity between organisms, leading to the ability to understand physiology and ecology of non culturable species.

The Himalayan region represents a unique combination of plant and soil type that changes drastically with altitude however only limited efforts have so far been made to explore the available bacterial diversity. In the present study soil samples were collected from Chaubatia (Ranikhet) Uttaranchal Himalayas and Leh region, for assessment of molecular characterization of plant growth promoting rhizobacteria.

MATERIAL AND METHODS

Molecular characterization of bacteria isolates

Seven cultures (FQPR-2, MFA-1R-3, FQPR-3, MFB-1R-1, MFA-1SD2, FMPPB-3, FPASD-1) isolated from different sites of central Himalayan region and two isolates FA_2K100^3 and $FA_2K_{10}{}^5$ from cold deserts of Leh showing best PGPR properties were characterized employing amplified r DNA restriction analysis (ARDRA).

DNA extraction

Genomic DNA was extracted by NaCl-CTAB method. 1.5 ml culture was centrifuged at 12,000 rpm for 10 min at 4^0C. Cells were washed twice in TRIS-Cl (0.1 M, pH 8.0) and lysed with 0.5% SDS and 0.001% proteinase K followed by treatment with 1% CTAB. Protein was precipated by phenol: chloroform and DNA extracted using absolute alcohol. DNA was checked on 0.8% agarose gel in TBE buffer at 70 volts for 45 min, after staining with EtBr visualized under UV (Gel Doc system, Biosystematica) (Bazzicalupo and Fani 1994).

Amplified r DNA restriction analysis (ARDRA)

ARDRA was performed using four tetracutter restriction endonucleases, *viz., Rsa*I, and *Taq*I (Bangalore Genei, India). 15 µl of (120 µg) of 16S rDNA amplification product was digested with restriction endonuclease in the reaction mixture. For 30 µl reaction mixture, added the following: Restriction enzyme (10 U), 1.0 U (MBI Fermentas); Restriction buffer (10 X), 1.0 X (MBI Fermentas); 16S rDNA amplicon 120 µg, and Milli Q water.

The reaction mixture was incubated at 37 °C for *Rsa*I, and at 65 °C for *Taq*I enzyme for 4 h. Restriction product was resolved on 2.5% agarose gel in 1X TBE at 60 V for 5 h and visualized on a UV transilluminator (Gel DocMega, Biosystematica). The restriction profile was analyzed using NTSYS pc version 2.02i. The clustering was done using Jaccard's similarity coefficient based on presence and absence of band ignoring their intensities.

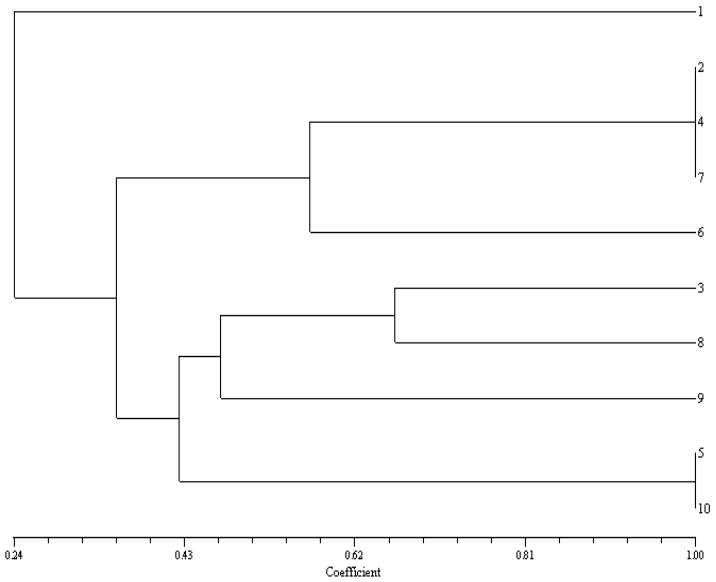

Fig. 1: Phylogenetic tree of ARDRA profile using Jaccard's coefficient

RESULTS AND DISCUSSIONS

Molecular characterization

Genetic relatedness amongst strains was assessed employing ARDRA. 16S region of the nine isolates and reference strain *(P. fluorescens,* ATCC 13525) was PCR amplified. Restriction analysis of 16S r DNA was performed using *Taq*1, and *Rsa*1 and revealed discriminatory 5-6 band pattern. Each pattern corresponded to a particular genotype. ARDRA generates species specific band pattern by Heyndricks1996 and six genotypes were detected within the nine bacterial isolates. On the basis of ARDRA with *Taq*1 and *Rsa*1, isolates were places in six groups. Restriction profiles of isolates MFA2 SD2 and FMP PB3 were identical. Three isolates FQP R3, FQPR2 and MFA-1R3 were identical and closely related with FA2 K1003, FA2K1005, and exhibited different profile. Isolate MFB1 R3 was closely related with FPASD1 however, only MFB-1R3 showed close similarity with reference strain when digested with *Taq*1. Reference strain (*P. fluorescens*) exhibited different profile that did not match with that of other bacterial isolates(Fig. 1a and 1b). Further analysis of the phylogenetic relatedness among the isolates was assessed based on UPGMA dendrogram (Fig. 1) A total of six genotypic clusters were formed. Two major clusters, A and B, were formed at 37% similarity level. Cluster A showed two genotypes at 58%-100% similarity level. One cluster was represented by three bacterial isolates i.e., FQPR-3, FQPR-2, and MFAR-3 at 100% similarity level whereas the second cluster was formed at 58% similarity level. Cluster B, represented three genotypes at 43-100% similarity level; GroupB1 was represented by two bacterial isolates i.e., MFB1 R3 and FPA SD1 at 67% similarity level. Group B2 had a distinct genotype (FA2K105) at 48% similarity level. Group B3 was also represented by two bacterial isolates at 100% similarity level. Group B1 and B2 was 48% similar and these were similar to B3. Reference strain (*P. fluorescence*) was quite distinct from this group since it showed only 24% similarity (Fig. 1).

Molecular tools for the identification of soil bacteria were used and 16S rDNA gene analysis was intensively used to understand the phylogenetic relationships. Among the 16SrDNA gene analysis, amplified ribosomal DNA restriction analysis (ARDRA) was performed. This molecular technique has been successfully used for bacterial community analysis in a great variety of environments (Lagace2004). ARDRA analysis revealed that although bacterial isolates were recovered from nearly similar locations, they showed wide genetic diversity on account of niche diversification (Mittal 2004).

Phylogenetic analysis on the basis of 16s rDNA sequences provided better understanding in evaluation of genetic diversity of rhizobacteria isolated from same and different ecological niche; phylogenetic analysis of 500 bp of terminal region of 16S rDNA from cultivated strain has been found to show existence of large bacterial diversity by Hunter-Cerva J C, 1998 .

ACKNOWLEDGMENT

The author wish to thank to chairmain Mr Harish Arora and Vicechairperson Mrs Rani arora of SIPAS, Dehradun. We acknowledge the sincere thanks to Dean life science and Principal SIPAS for providing us research facilities.

REFERENCES

1. Homer-Devine M C, Carney K M & Bohannan B J M, (2004)An ecological perspective on bacterial biodiversity, *Proc. R. Soc. Lond. B*, 271: 113-122.
2. Heyndricks M, Vauterin, Vandamme P, Kersters K, Devers P. (1996) Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) pattern of bacterial phylogeny and taxonomy. *J Microbiol Meth*. 26 : 257-259.
3. Mittal S. 2004Diversity and taxonomy of pseudomonads from wheat rhizosphere. PhD thesis, G.B. Pant University of Agriculture and Technology, Pantnagar.
4. Hunter-Cerva J C, (1998) the value of microbial diversity. *Curr. Opin. Microbiol,* 1 : 278-285.
5. Muyzer, G.; Dewall, E.C. and Uitterlinden, A.G. 1993. Profiling of complex bacterial opulation by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59: 695-700.
6. Massol-Deya, A.A., Odelson, D.A., Hickey, R.F. and Tiedje, J.M. 1995. Bacterial community fingerprinting of amplified 16S and 16S-23S ribosomal DNA gene sequences and restriction endonuclease analysis (ARDRA). *In*: Akkermans, A.D.L., Van-Elsas, J.D., de-Bruijin, F.J. (Eds.). Molecular Microbial Ecology Manual. Kluwer Academic Publishing. Boston. Pp. 3.3.2.1-3.3.28.
7. Liu, W.T.; Marsh, T.L.; Cheng, H.; Forney, L.J. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl. Environ Microbiol.* 63: 4516-4522.
8. Pace, N.R. 1996. New perspective on the natural microbial world: molecular microbial ecology. ASM News. 62: 463-470
9. Woese, C.R. 1987. Bacterial Evolution. *Microbiol. Rev.* 51: 221-271.