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 promorty 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 cultivable 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 Carlwoese, presents a radical departure from classical taxonomic. Life could be divided into three primary phylogenetic ‘Domains, Archarya, 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 taxonomy, phylogenetic analyses are important in identifying similarity between organisms, leading to the ability to understand physiology and ecology of non cultivable 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) Uttarakhand 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 :K1002 and FA :Ku3 from cold deserts of Leh showing best PGPR properties were characterized employing amplified rDNA 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°C. Cells were washed twice in TRIS-CI (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 precipitated 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, Bio-systematica) (Bazicalupi and Fani 1994).

Amplified rDNA restriction analysis (ARDRA)

ARDRA was performed using four tetracutter restriction endonucleases, viz., RsaI, 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 16h, and at 65 °C for TaqI 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 2.02l. The clustering was done using Jaccard’s similarity coefficient based on presence and absence of band ignoring their intensities.
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 rDNA was performed using TaqI, and RsaI and revealed discriminatory 5-6 band pattern. Each pattern corresponded to a particular genotype. ARDRA generates species specific band pattern and was closely related with FPASD1 however, only MFB1R3 showed close similarity with reference strain when digested with TaqI. 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 showed three genotypes at 43-100% similarity level; Group B1 was represented by two bacterial isolates i.e., MFB1R3 and FPA-SD1 at 67% similarity level. Group B3 was also represented by two bacterial isolates at 100% similarity level. Group B1 and B2 were 48% similar and these were similar to B3. Reference strain (P. fluorescens) 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 ( Mittal2004).

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 JC, 1998.

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