Research Paper :

Analysis of Genetic Similarity of Rhizobacterial Strains Using RAPD-PCR Technique

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SUMMARY

Random amplified polymorphic DNA (RAPD) profiles using PCR for seven characterized, efficient rhizobacteria were generated with 30 random decamer primers. The primers generated 227 RAPD loci, of which 198 were polymorphic and exhibited 87.22 per cent polymorphism. The primers OPA-01, OPA-10, OPB-02, OPB-03 OPB-05 and OPB-06 showed 100 per cent polymorphism. Maximum genetic similarity was found between RB-31 and RB-50 (84.00%), while the lowest genetic distance was between RB-13 and RB-31 (40.00%). Dendrogram constructed using UPGMA showed two major clusters A and B at similarity coefficient of 0.52. The isolates RB-01, RB-10, RB-13, RB-22 and RB-43 in one cluster and RB-31 and RB-50 in other cluster.

Key words :
Genetic similarity, Rhizobacteria, Molecular marker, RAPD-PCR

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Seven rhizobacteria isolated from coleus rhizospheres were screened and tested *in vitro* and *in vivo* as biocontrol agents against different soil borne pathogens as well as for plant growth promotion by diverse mechanisms. It was difficult to distinguish these species using traditional morphological and biochemical differences. In recent years, limitations of morphological and biochemical markers have been overcome by molecular markers, some are relatively cheaper and simple to use in variety of applications in plant research. One of such markers is Random Amplified Polymorphic DNA (RAPD) and is one of the Polymerase Chain Reaction (PCR) based DNA markers, and defined as an assay based on the amplification of genomic DNA with single primer of arbitrary nucleotide sequence (Weising *et al*., 1995). RAPD can be used in studying genetic diversity, varietal identification *etc*. It is simple to operate and does not involve radio active labeling. With this in view, seven native isolates of rhizobacteria were isolated and screened against different soil borne pathogens causing root-knot and wilt complex in coleus were selected to understand more variation among the isolates of efficient rhizobacteria by subjecting them to RAPD analysis.

MATERIALS AND METHODS

Seven rhizobacteria isolates showing high efficacy against *Fusarium*, *Ralstonia* and *Meliodogyne in vitro* were studied during 2007. DNA was extracted by using rapid method from the rhizobacteria. Bacterial cultures were grown in 5ml NB with 10% glycerol (v/v) for 72 h at 28±2ºC. Eppendorf tube of 1.5 ml was used to centrifuge the cells at 13,000 rpm for 5 minutes. The pellet was suspended in 200 ml Tris 0.1 mol L^{-1} and added with 200 ml of lysis solution (NaOH 0.2 N and 1% SDS). Above solution was mixed and deproteinized with 700 ìl of phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v) and centrifuged at 13000 rpm for 10 min. Top layer containing bacterial DNA was taken without disturbing the bottom layer and it was transferred to fresh 1.5 ml microcentrifuge tube. To this, 700 ml of ice cold 95% ethanol/isoproponol was added to precipitate the DNA and spinned. Final washing was given with 70% ethanol and centrifuged at 8000 rpm for 5 min. Precipitated DNA was dried at room temperature and resuspended in 100 ml of water. The DNA obtained was further quantified and electrophoresed on 0.8% agarose gel stained with ethidium bromide and photographed under UV light. The PCR amplification for RAPD analysis was performed according to Williams