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RESEARCH ARTICLE :

Molecular characterization of plant growth promoting Rhizobacteria associated with ground nut (*Arachis hypogea*) and sorghum (*Sorghum bicolor*)

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SUMMARY : In search of efficient PGPR strains with multiple activities, a total of 32 bacterial isolates belonging to *Bacillus* (20) *and Rhizobium* (12) were isolated from different rhizospheric soils of ground nut and sorghum in the Mahaboobnagar district. These isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like phosphate solubilization, production of indoleacetic acid (IAA), hydrogen cyanide (HCN) and siderophore. The molecular diversity of 10 selected plant growth promoting rhizobacterial isolates *viz*., *Bacillus cereus* and *Rhizobium* spp. was studied by PCR-RAPD technique. The 10 plant growth promoting rhizobacterial isolates were selected based on their multifunctional PGPR traits *i.e*. each isolate is having best or good PGPR property among the 32 isolates and some isolates that having multiple PGPR traits also taken in to consideration.

KEY WORDS :
Rhizobacteria, Groundnut, Sorghum

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BACKGROUND AND OBJECTIVES

The soil acts as a reservoir for millions of microorganisms, of which approximately more than 85% are beneficial for plant life. Thus, the soil is a resilient eco system and soil microorganisms provide precious life to soil systems catering to plant growth. Soil microorganisms play a vital role in the evolution of agriculturally use full soil conditions and in stimulating plant growth. Beneficial rhizosphere microorganisms that are closely associated with roots have been termed Plant Growth Promoting Rhizobacteria (PGPR).

Molecular analysis of genomic DNA of the organism is useful for distinguishing the bacterial strains better at intra-species level these techniques provide valuable information on the magnitude of genetic variability within and between organisms of different species.

With the advent of molecular techniques, several arbitrary primers based Randomly

Amplified Polymorphic DNA (RAPD) technique has been used for typing and identification of number of closely related species of bacteria and assessment of genetic relationships. Its results are usually consistent with those of DNA-DNA homology studies and can be used to estimate the genetic distances .

Random Amplified Polymorphic DNA (RAPD) markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessary in a reproducible way. Unlike, traditional PCR analysis RAPD does not require any specific knowledge of the DNA sequence of the target organism. The identical 10-mer primer will or will not amplify a segment of DNA, depending on positions that are complementary to the primer sequences. For example, no fragment is produced if primers annealed too far apart or 3' ends of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel. RAPD analysis is faster, technically less demanding and more economical than the other genomic typing methods like RFLP (Restriction Fraction Length Polymorphism) and AFLP.

RESOURCES AND METHODS

Isolation of plant growth promoting rhizobacteria :

The rhizospheric soil samples (twenty) were collected from fields growing ground nut and sorghum from different villages of Mahaboobnagar district, Andhra Pradesh, India. Different bacteria were isolated using their respective media; *Rhizobium* was isolated on yeast extract mannitol agar and *Bacillus* on nutrient agar. Bacterial cultures were maintained as slant cultures.

Isolates of *Bacillus* (20) and *Rhizobium* (12) were biochemically characterized by Gram's reaction, carbohydrate fermentation, oxidase test, H_2S production, IMViC tests, NO_2 reduction, starch and gelatin hydrolysis as per the standard methods (Cappuccino and Sherman, 1992).

Chemicals :

All the chemicals used in this project were of molecular biology grade and obtained from Sigma, Amersham Biosciences. USB, Bangalore Genei, Life Technologies, Invitrogen, etc. other consumables like plastic ware were obtained from Axygen and Tarson. Standard solutions and buffers were prepared according to the procedures given by Ausubel *et al*. (1999).

Bacterial genomic DNA isolation :

Genomic DNA was isolated by following the method of Ausubel *et al*. (1999). DNA samples were quantified by running on agarose gels along with standard DNA and staining with ethidium bromide. Samples were mixed with appropriate amount of 6X loading dye and electrophoresis on 0.8% agarose gel along with varying concentrations of ë DNA. The ethedium bromide gels were placed on a UV transilluminator and visual comparisons were made with the standards to estimate the DNA concentration in samples.

Polymerase chain reaction :

The RAPD analysis was carried out following the method recommended by Williams *et al*. (1990) with required modifications. A total number of 20 primers supplied by Operon technologies IDT, USA were used in RAPD (Table A) analysis. Genomic DNA (25-50ng μl^{-1}) of the Plant growth promoting rhizobacterial isolates was used as template and PCR amplification was performed in a 20µl reaction mixture as constituted.

PCR reaction was carried out in a DNA Thermocycler with heated lid. Each of 20 µl reaction volume contained 11.2 µl of sterile distilled water, 2 µl template, 2 µl of 10X, PCR buffer (Biogene). 1.6 µl of $MgCl_2$, 2 µl dNTPs (Biogene), 1 µl RAPD primer and 0.2 µl Taq polymerase. The amplification conditions were as following:

Cycle	Stage	Temperature (^oC)	Duration (min)	No. of cycles
	Lid temperature	105		
I	Initial denaturation	94	5	1
II	Denaturation	94	1	
	Annealing	36	2	35
	Extension	72	2	
III	Extension	72	10	1
	Dump	4	-	-

The reaction mixture was given a short spin (Quilitron, Korea.)for thorough mixing of the cocktail components. Then 0.2 ml of PCR tubes were loaded on to a thermal

cycler eppendorf. After PCR, the samples were loaded in 1.5 % agarose gel along with a standard ladder of 100 bp and 1 kbp for comparision of the PCR products.

Data analysis :

The gels were scored for the presence or absence of the corresponding band among the standards as well as the local isolates. A score of '1' was given for the presence and '0' for the absence of bands. The binary data generated was analyzed for genetic similarity using unweighted pair group arithmetic mean (UPGMA) program of NTSYSpc version 2.11 software. The dendrogram obtained served as the basis for assessing the genetic relatedness of the strains with respect to the standard subspecies.

$$\text{Percent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

OBSERVATIONS AND ANALYSIS

The molecular diversity of 10 selected plant growth promoting rhizobacterial isolates *viz*., *Bacillus cereus* and *Rhizobium* spp. was also studied by PCR-RAPD technique. The 10 plant growth promoting rhizobacterial isolates were selected based on their multifunctional PGPR traits *i.e* each isolate is having best or good PGPR property among the 32 isolates and some isolates that having multiple PGPR traits also taken in to consideration. The studies also indicated lot of variation amongst the isolates with respect to their morphological, biochemical and physiological characteristics as well as functional properties. Some selected isolates were further subjected to molecular diversity analysis. The diversity analysis as well as fingerprinting of an individual can be achieved by using PCR based RAPD markers.

The genomic finger printing method employed for plant growth promoting rhizobacteria is based on the use of DNA primers corresponding to naturally occurring repetitive elements in bacteria, which is a simple method to distinguish closely related genotypes. Keeping this in view the present investigation was carried out to analyze the genetic diversity of plant growth promoting rhizobacterial isolates to reveal their polymorphism using RAPD technique.

RAPD analysis of genetic diversity of *Bacillus cereus* isolates :

Twenty primers screened with OPL series for appropriate amplification and pattern formation, of which 6 primers were selected to test the repeatability of the method. Genomic DNA of 5 isolates of *Bacillus cereus* was analyzed which revealed cent per cent polymorphism with all the primers. Screening of the entire set of samples was done twice to assess the repeatability of the RAPD profiles and identical patterns were obtained. The percentage of polymorphism observed for each isolate with all the primers is calculated, which is cent per cent for all the isolates individually. The primers with maximum number of polymorphic bands are OPL 7 which is having 19 bands and the primer with minimum number of polymorphic bands is OPL 19 with only 8 bands. The number of bands generated with all the primers ranged from 8 to 19. The number of loci and polymorphic bands varies widely between primers and isolates. A high level of genetic diversity was revealed when cluster analysis was carried out with *Bacillus cereus* isolates based on similarity matrix (Fig. 1).

The dendrogram analysis revealed that the genotypes were divided in to 4 clusters. The isolate GKsB

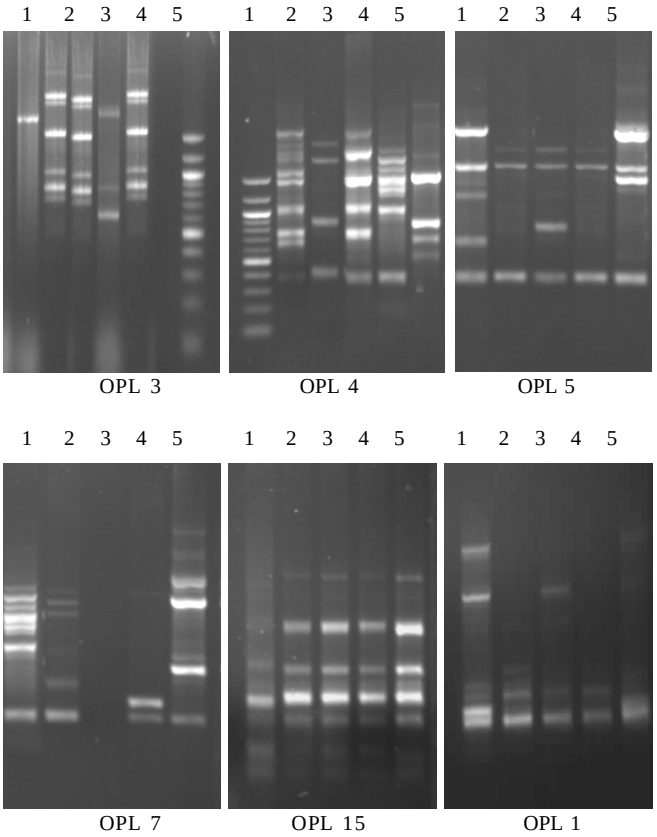

Fig. 1 : RAPD profiles of Bacillus cereus with respect to individual primers

is entirely falling in to a different single group with similarity co-efficient of 50 per cent in cluster I. remaining isolates fell in to different major clusters, in these SKcB formed a separate cluster II with 57 per cent similarity co-efficient. The other isolates divided in to 2 clusters which show their genetic relatedness according to Dendrogram. SIB formed in to cluster III with 67 per cent similarity co-efficient. GMhB and SLB are almost similar with 70 per cent similarity in cluster IV. The dendrogram constructed from the binary data which had maximum genetic similarity of 0.70 and minimum genetic similarity of 0.45. The highest genetic similarity of 0.70 could be noted between the isolates SLB and GMhB. The isolates that were taken for analyzing genetic diversity were also compared with their functional properties and the results revealed that the isolates which showed almost similar in their dendrogram analysis (GMhB and SLB) were also similar many of the functional properties.

Levy *et al.* (2005) used a PCR based method – Random Amplification of Polymorphic DNA (RAPD) – to identify genetic markers in *Bacillus* strains. They said that classification and differentiation of *Bacillus* isolates by genetic markers play an important role in future research. Joshi and Bhatt (2011) investigated the diversity of bacteria associated with the roots of wheat. They isolated 133 bacteria from four different locations of which two were rainfed and two were irrigated and said that 44 % were *Bacillus spp.* and 24% belong to *Pseudomonas spp.* on the basis of biochemical characterization. They revealed the diversity of bacterial isolates by using RAPD markers.

RAPD analysis of genetic diversity of Rhizobium isolates:

Five isolates were characterized by twenty primers of OPL series for appropriate amplification and pattern formation, of which 8 primers were selected to test the repeatability of the method. Genomic DNA of 5 isolates of *Rhizobium* was analyzed which revealed 88 per cent polymorphism with all the primers. Screening of the entire set of samples was done twice to assess the repeatability of the RAPD profiles and identical patterns were obtained. The per centage of polymorphism observed for each isolate with all the primers is calculated (Table 4), which is 88 per cent for all the isolates individually on an average. The primers with maximum number of polymorphic bands are OPL 1 which is showing 14 bands and the primer with minimum number of polymorphic bands is OPL 14 with only 4 bands. The number of bands generated with all the primers ranged from 4 to 14. The number of loci and polymorphic bands varies widely between primers and isolates. A high level of genetic diversity was revealed when cluster analysis was carried out with *Rhizobium* isolates based on similarity matrix (Fig. 2).

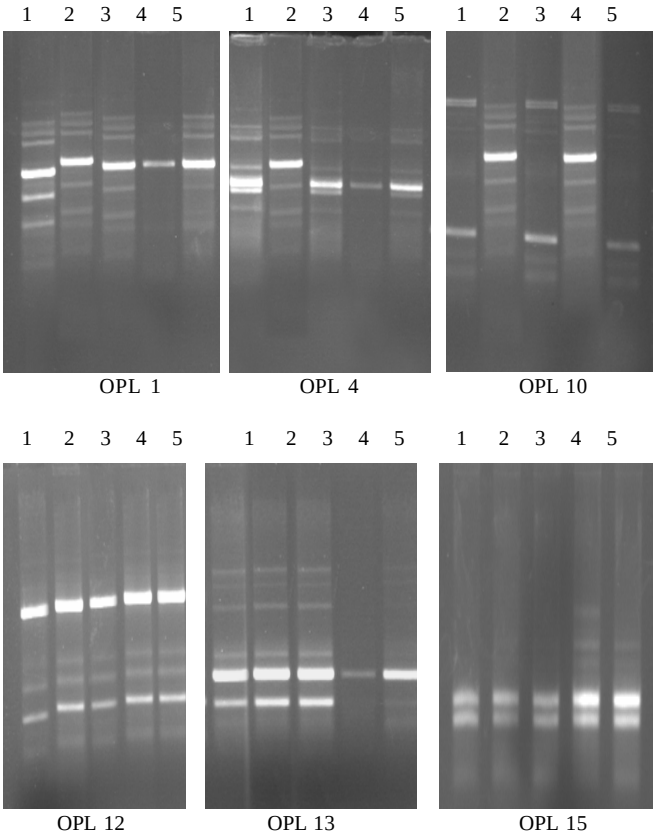

Fig. 2 : RAPD profiles of *Rhizobium* spp. with respect to individual primers

At 87 per cent similarity level 3 clusters were distinguished with 5 isolates of *Rhizobium* by RAPD using twenty primers (OPL series). Cluster III the largest one had 2 isolates closely related to SMdR and GMrR. Cluster I included 2 isolates shared 53 per cent similarity, which contained the GKsR and GMhR isolates. Only one isolate that showed a slight difference from rest of the isolates which was alone in Cluster II. The isolates that were taken for analyzing genetic diversity were also compared with their functional properties and the results revealed that the isolates which showed almost similar in their

dendrogram analysis (SMdR and GMrR) were also similar in their functional properties. Similarly the genetic diversity among five isolates of *Rhizobium* was evaluated by RAPD-PCR analysis (Naz *et al.*, 2009). Tamini (2002) investigated the genetic diversity and the N_2 fixation potential of 10 rhizobial isolates nodulating *Phaseolus vulgaris* in the soils of the Jordan valley. He used Random amplified polymorphic DNA (RAPD) analysis which showed that isolates consisted of two major genetic groups and revealed a low level of diversity among the isolates.

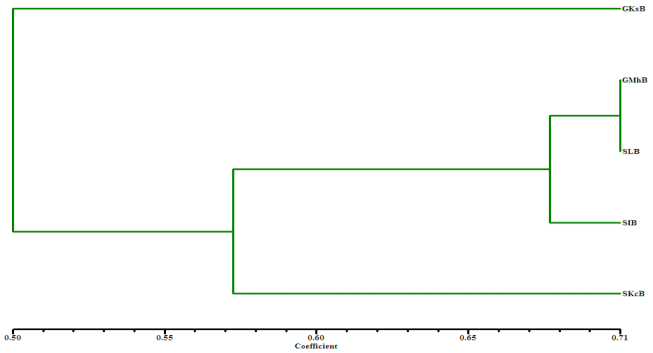

Fig. 3 : Dendrogram of the selected *Bacillus cereus* across six random primers

In the present investigation, ten plant growth promoting bacterial isolates (*Bacillus cereus* – 5 and *Rhizobium* sps. - 5 and) which showed multiple activities were initially screened with 20 RAPD primers (OPL series) to detect the primers showing polymorphism (or) genotypic variation between them. Of the twenty primers screened, amplification was obtained with most of the primers and they have recorded cent per cent polymorphism with all the isolates of *Bacillus cereus* (Dendrogram: 1). In case of *Rhizobium* spp. they have recorded 88 per cent polymorphism with all the isolates(Fig. 4).

The data on RAPD profiles were scored '1' presence of band and '0' absence of band (binary data) used to construct a Dendrogram to assess the genetic relatedness among the isolates. In the cluster analysis, based on polymorphic bands, the isolates are grouped in to different clusters based on their genetic similarity co-efficient. The purpose of our study was to identify and characterize the various plant growth promoting rhizobacterial isolates which were fulfilled by the PCR-based technique as the level of polymorphism detected was very high.

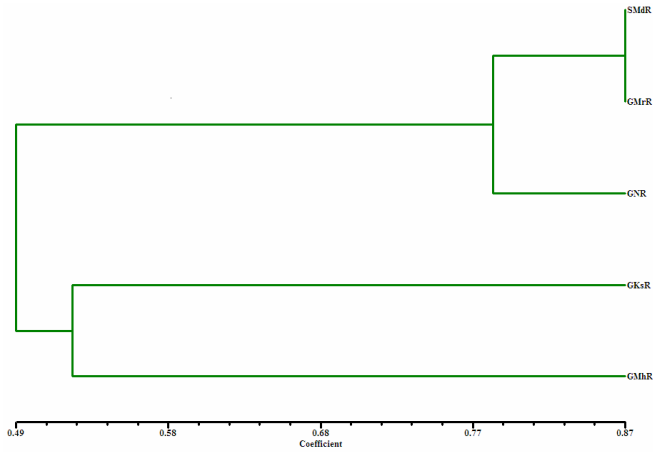

Fig. 4 : Dendrogram of the selected *Rhizobium* spp. across eight random primers

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REFERENCES

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Streehl, K. (1999). Short Protocols in Molecular biology, 4th edition. John willey and Sons, Inc., NEW YORK, U.S.A.

Cappuccino, J.C. and Sherman, N. (1992). In: Microbiology: A Laboratory Manual. New York. 125-179.

Joshi, P. and Bhatt, A.B. (2011). Diversity and function of plant growth promoting Rhizobacteria associated with wheat Rhizosphere in North Himalayan Region. *Internat. J. Environm. Sci.*, **1** : 1135-1143.

Levy, H., Fisher, M., Ariel, N., Altboum, Z. and Kobiler, D. (2005). Identification of strain specific markers in *Bacillus anthracis* by random amplification of polymorphic DNA. *FEMS Microbiology Letters,* **244** : 199-205.

Naz, I., Bano, A. and Hassan, T.U. (2009). Morphological, biochemical and molecular characterization of rhizobia from halophytes of khewra salt range and attock. *Pak. J. Bot*., **41**(6): 3159-3168.

Tamini, S.M. (2002). Genetic diversity and symbiotic effectiveness of rhizobia isolated from root nodules of common bean (*Phaseolus vulgaris* L.) grown in the soils of the Jordan valley. *Applied soil ecology*., **19**(2): 183-190.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids Res.,*. **18**: 6531-6535.*www.indiastat.com*