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DETERMINATION OF GENETIC DIVERSITY OF *Rhizobium* species ISOLATED FROM ROOT NODULES AND DNA FINGERPRINTING BY RAPD

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ABSTRACT:

Rhizobia are Gram-negative, motile, non-spore forming rods soil bacteria that fix nitrogen (diazotrophs) after becoming established inside root nodules of legumes. Nitrogen is the most commonly deficient nutrient in many soils and it is the most commonly supplied plant nutrient. Supply of nitrogen through fertilizers has severe environmental concerns. In this study four different plant species were collected and their root nodules were taken for the isolation of Rhizobia. The present study deals with, establishing a phylogenetic relationship between four Rhizobia species isolated for different plant nodules by RAPD marker. The genomic DNA of good quality without any degradation was successfully isolated from Rhizobia. In total four Rhizobia isolates were studied for their polymorphism at molecular level. Out of four random primers three primers namely OPZ 8, 9 and 10 produced clear banding patterns. From the electrophoretic banding pattern, cluster analysis was carried out using frequency similarity co-efficient. The results of this study indicated that RAPD provided a high degree of discrimination between the strains. The use of molecular markers to study genetic diversity will help in characterization and development of new species of Rhizobia.

KEYWORDS: *Rhizobium*, DNA fingerprinting, RAPD

INTRODUCTION

Rhizobia are usually defined as nitrogen fixing soil bacteria capable of forming root or stem nodules on leguminous plants fixing atmospheric nitrogen and reducing to ammonia for the benefit of the plant. Due to their considerable agricultural and environmental significance, these legumes have been extensively studied. Rhizobia require a plant host, they cannot independently fix nitrogen. Rhizobia are important members of plant growth promoting rhizobacteria (PGPR) showing several plant growth-promoting (PGP) activities [1]. Biological nitrogen (N) fixing microbes are free living in soil media also found in association with rhizosphere and the tissues of the healthy plant are beneficial for plants [2]. These bacteria can infect the roots of leguminous

plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place.

These N fixing organisms have ability to enhance the N fixation performance and increase nutrient level in soil. This is due to the production of substances like hormones, siderophores, phosphate solubilization, improvement of nutrients, water uptake and also these microbes helps to enrich soil fertility and counteract agro environmental problems [3]. An enzyme called nitrogenase catalyses the conversion of nitrogen gas to ammonia in nitrogen-fixing organism. One of the most important factors in the generation of high yields from modern rice cultivars is nitrogen fertilizer. That is why farmers are applying high amounts of the fertilizers which are very costly and make the environment hazardous. In addition, more than 50% of the applied N fertilizers are somehow lost through different processes which denote a cash loss to the farmers and pollute the environment [4]. Biofertilizer, an alternative source of N fertilizer, especially rhizobia in legume symbiosis is an established technology. Use of the biofertilizers can also prevent the depletion of the soil organic matter [5]. Inoculation with bacterial biofertilizer may reduce the application of fertilizer N by increasing N uptake by plants [6, 7]. Although most studies were conducted on crop plants, less explored legumes like different shrubs and herbaceous plants have important roles in certain ecosystems [8].

Nitrogen fixation by rhizobia is of great importance in agriculture in several ways. Legumes such as peas, beans, lentils, soybeans, alfalfa and clover help to feed the meatproducing animals of the world as well as humans. The availability of sensitive and accurate PCR-based genotyping among closely related bacterial strains and the detection of higher rhizobial diversity have been greatly considered [9]. The Rhizobia strains were studied by using randomly amplified polymorphic DNA (RAPD) fingerprinting, as the technique is frequently used recently for exploring genetic polymorphisms [10, 11]. The aim of the present study was to investigate the suitability of the RAPD methods to distinguish Rhizobium strains and to detect genetic diversity in field populations.

MATERIALS AND METHODS

Samples Collection

Four leguminous plant species were collected from rural farms in Tumkur & their root nodules were taken for the isolation of Rhizobium species by smashing it within a sterile test tube and then root nodules were serially diluted by distilled water after that spread plated on YEMA agar media. The inoculated media plates were incubated at room temperature for 24-48hrs. Gram's stain was done in order to see the morphology of bacteria under microscope.

DNA Isolation

Genomic DNA was extracted from fin tissues as described previously. In brief, approximately 50 mg of fin tissues was cut into small pieces and taken into 2 ml microfuge tube. The fin tissue was digested with proteinase-K in extraction buffer (100 mM Tris, 10 mM EDTA and 250 mM NaCl, pH= 8.0 and 1% Sodium Dodecyl Sulfate) overnight at 37ºC. DNA was purified once with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol (24:1) and precipitated using 0.6 volumes of chilled isopropanol.

PCR

The polymerase chain reaction was carried out in final volume of 25 µl containing 100 ng DNA, 2 U of Taq DNA polymerase (Chromous Biotech, Bangalore), 2.5 mM $MgCl_2$ (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 p mol of primers (Eurofins Genomics, Bangalore). The DNA amplification was performed in the Corbett RG

6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94 °C for 45 sec, 35 °C for 1 min and 72 °C for 1.5 min) followed by 30 cycles of amplification (94 °C for 45 sec, 38 °C for 1 min and 72 °C for 1 min) and the final elongation step (72 °C for 5 min).

Data Analysis

Total volume of the amplified product (25µl) of each sample was subjected to electrophoresis on 1.5 % agarose gel containing ethidium bromide in 1xTAE buffer at 100V for 1 hr. Finally, the DNA bands were observed on a Gel Doc system and the photographs were captured. The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of Frequency similarity. The matrix of genetic distance was used for grouping the fish samples based on the dendrogram constructed by UPGMA (Unweighed Pair Group Method with Arithmetic averages).

RESULTS AND DISCUSSION

The colonies on YEMA media were globose, jelly like with entire margine. Gram's staining revealed bacteriods in different gram negative forms.

Figure 1: Isolation of Rhizobium on YEMA Plates

The genomic DNA was isolated from four Rhizobia by Phenol-chloroform extraction method. The qualitative estimation of the DNA on 0.8% agarose gel gave single, sharp and distinct bands devoid of any smear for two samples. Hence the DNA isolation was repeated for those two samples which showed good sharp bands. Thus, genomic DNA of good quality without any degradation was successfully isolated from all the eleven samples.

All the isolates were further genotypically characterized by RAPD fingerprinting. The electrophoretic profile of Rhizobium isolates generated by RAPD-PCR by using the primer OPZ 8-11 are presented in (Figures 2-5) respectively. Out of four random primers three primers namely OPZ 8, 9 and 10 produced clear banding patterns. OPZ 11 was unable to amplify any bands for sample 3 hence not considered for dendrogram analysis. OPZ 8 produced a total 23 bands ranging from 3-7, OPZ 9 produced a total 30 bands ranging from 5-11, OPZ 10 produced a total 26 bands ranging from 6-7.

Figure 2: DNA fingerprint for OPZ 8

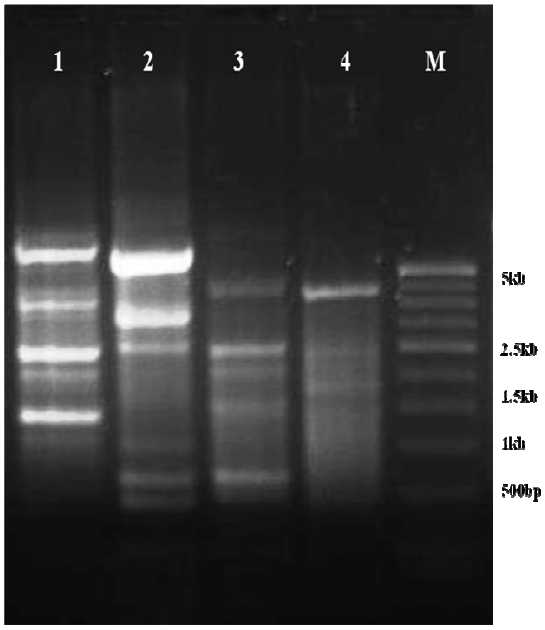


Figure 3: DNA fingerprint for OPZ 9

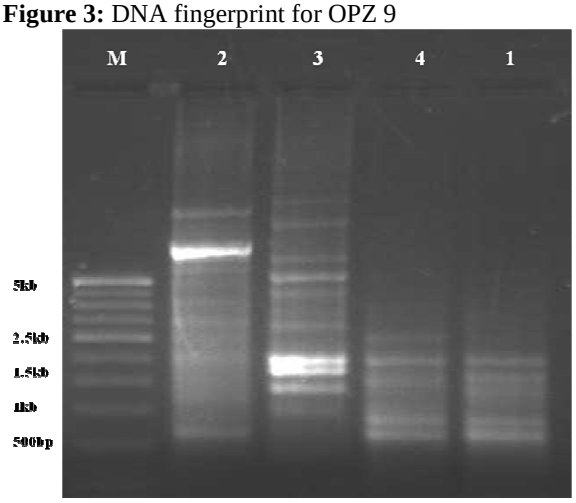


Figure 4: DNA fingerprint for OPZ 10

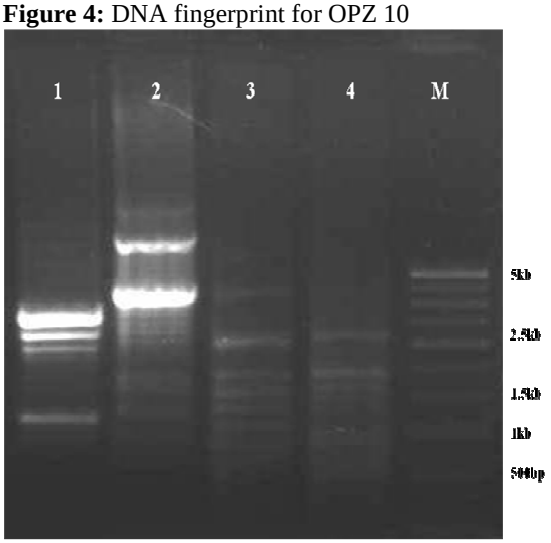
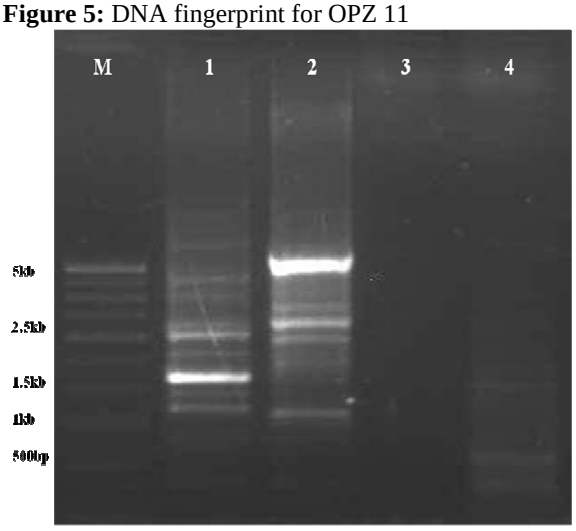
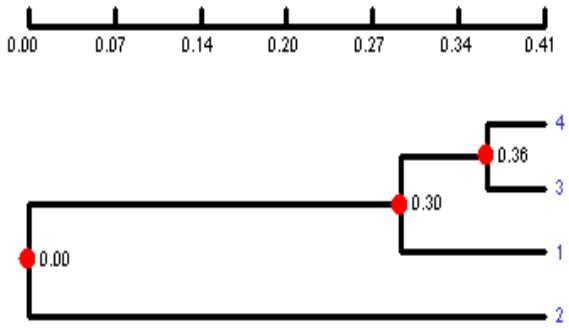


Figure 5: DNA fingerprint for OPZ 11



From the electrophoretic banding pattern, cluster analysis was carried out using frequency similarity co-efficient (Figure 6). The results of this study indicated that RAPD provided a high degree of discrimination between the strains. DNA fingerprinting, a tool widely used in forensic science is also useful in a variety of applications with plants. It is used to identify cultivars; to positively identify and differentiate accessions; genetic diversity within breeding populations, and species that might be difficult to characterize due to similar morphological characteristics or indistinct traits.

Figure 6: Dendrogram generated by frequency similarity using UPGMA



A number of molecular tools and procedures are being employed to establish DNA fingerprinting profiles and each of these procedures has its strengths and weaknesses. The use of molecular markers to study genetic diversity will help in characterization and development of new species of Rhizobium.

REFERENCES

1. Badawi, F.S., Biomy, A.M.M. & Desoky, A.H. 'Peanut plant growth and yield as influenced by co-inoculation with *Bradyrhizobium* and some rhizo-microorganisms under sandy loam soil conditions', Ann. Agric. Sci., vol. 56, 2011 pp. 17 - 25.

2. Bashan, Y. & de-Bashan, L.E. 'Freshweight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination', *Soil Biology and Biochemistry*, vol. 37, 2005 pp. 1795 - 1804.
3. Choudhury, A. & Kennedy, I.R . 'Prospects and potentials for system of biological nitrogen fixation in sustainable rice production', Biol. Fertil. Soils. vol. 39, 2004 pp. 219-227.
4. Glick, B.R. 'The enhancement of plant growth by free-living bacteria', Can. J. Microbiol., vol. 41, 1994 pp. 109–117.
5. Jarabo-Lorenzo, A.E., Velazquez, R., Perez-Galdona, M.C., VegaHernandez, E., Martinez-Molina, P.F., Meteos, P., Vinuesa, E., Martinez-Romero & Leon-Barrios M. 'Restriction Fragment Lenght Polymorphism Analysis of 16S rDNA and Low Molecular Weight RNA Profiling of Rhizobial Isolates from Shrubby Legumes Endemic to the Canary Islands', Syst. Appl. Microbiol., vol. 23, 2000 pp. 418–425.
6. Jeyabal, A. & Kupuswamy, G. 'Recycling of organic wastes for the production of vermicompost and its response in rice-legume cropping system and soil fertility', Eur. J. Agron., vol. 15, 2001 pp. 153-170.
7. Kennedy, I.R., Choudhury, A., & Kecskés, M.L. 'Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited?', Soil Biol. Biochem. vol. 36, 2004 pp. 1229-1244.
8. Ladha, J.K., Padre, A.T., Punzalan, G.C., Castillo, E., Singh, U. & Reddy, C.K. 'Nondestructive estimation of shoot nitrogen in different rice genotypes', Agron. J., vol. 90, 1998 pp. 33-40.
9. Tan, Z., Hurek, T., Vinuesa, P., Muller, P., Ladha, J.K. & Reinhold- Hurek, B. 'Specific detection of Bradyrhizobium and Rhizobium strains colonizing rice (Oryza sativa) roots by 16S-23S ribosomal DNA intergenic spacer-targeted PCR', Appl. Environ. Microbiol., vol. 67, 2001 pp. 3655- 3664
10. Teaumroong, N. & Boonkerd, N. 'Detection of Bradyrhizobium spp. and B. japonicum in Thailand by primer based technology and direct DNA extraction', Plant Soil, vol. 204, 1998 pp. 127-134.
11. Versalovic, J. Schneider, M. de Brujin, F.J. & Lupski, J.R. 'Genomic fingerprinting of bacteria using repetitive sequence based polymerase chain reaction', Meth. Mol. Cell. Biol., vol. 5, 1994 pp. 25-40.