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RAPD CHARACTERIZATION OF RHIZOBIUM LEGUMINOSARUM

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ABSTRACT

Rhizobium is a gram negative aerobic, rod –shaped bacterium found in large numbers in rhizosphere of legume roots. Leguminous plants establish a symbiotic relationship with the soil bacterium Rhizobium. The Symbiosis is manifested in the formation of root nodules. Also, in the interaction of rhizobia and plants one observes a high degree of hostspecificity. Rhizobium invades roots of legumes and forms nodules on the roots. Inside the root nodules, the bacteria exist in various pleomorphic forms called bacteriods. The bacteriods fix the atmospheric nitrogen into ammonia. They provide the fixed nitrogen for plant use and draw nourishments from the root cells. Different species of Rhizobium can fix 50-200 Kg nitrogen/ha/year in leguminous crops. Hence, they have been recommendaed as nitrogen biofertilizers in agriculture. Rhizobium exhibits great degree of host. Although several species of Rhizobium live in the rhizosphere of a legume, a particular species alone can establish symbiotic association with its roots. The host specific infection of Rhizobium depends upon the specific flavonoid secreted by the roots of legumes. The root exudate induces certain genes of a particular species of Rhizobium to produce a host determinant compound on its cell wall. In most cases, the host determinant compound is a capsular polysaccharide. The development and increased availability of molecular biology techniques have made it possible to obtain information regarding the geneomic organization and diversity of rhizobia populations in different soils. Genomic DNA fingerprinting using random amplification of polymorphic DNA has been found to be useful in differentiating between very closely related bacteria, and recently this method has been used closely related bacteria, and recently this method has been

for Rhizobium identification and genetic analyses. The RAPD techinique is a polymerase chain reaction (PCR) based assay that was developed to detect polymorphisms in genenomic DNA. Besides being simpler and cheaper, this method is as effective as the more labor intensive RFLP for establishing genetic relationship and identifying Rhizobium strains. The present investigation was carried out to establish genetic pattern and genetic groups of different isolates of Rhizobium leguminosarum collected from the root nodules of Arachis hypogaea using techingue of RAPD –PCR.

KEYWORDS: Rhizobium, PCR, RAPD, RFLP, Leguminosarum, Arachis hypogaea.

INTRODUCTION

Rhizobium is a gram negative, aerobic, and rod-shapedBacterium found in large numbers in rhizosphere of the legume roots. Leguminous plants establish symbiotic relationship with the soil bacterium Rhizobium. The symbiosis is manifested in the formation of root nodules. Also, In the interaction of rhizobia and plants one observes a high degree of host specificity. Rhizobium invades roots of legumes and forms nodules on the roots. Inside the root noodles, the bacteria exist in various pleomorphic forms called bacteriods. The bacteriods fix the atmospheric nitrogen into ammonia. Theprovide the fixed nitrogen for plant's use and draw nourishments from the root cells. Different species of Rhizobium can fix 50-200kg nitrogen /ha/year in leguminous crops. Hence, they have been recommended as nitrogen biofertilizers in agriculture. Rhizobiumexhibits greater degree of host specificity. Although several species alone can establish symbiotic association with its roots. The host specific infection of Rhizobium depends upon the specific falconoid secreted by the roots of legumes (Redmond et al.., 1986). The root exudates induces certain genes of a particular species of Rhizobium to produce a host determinant compound on its cell wall. In most cases, the host determinant compound is a capsular polysaccharide.

The development and increased availability of molecular biology techniques have made it possible to obtain information regarding the genomic organization and and diversity of rhizobia.

Populations in different soils (Amarger et al., 1994; Nour et al., 1994 and Lauerre et al., 1996). Genomic DNA fingerprinting using random amplification of polymorphic DNA (RAPD) has been found to be useful in differentiating between very closely relaedbavcteria, and recently this method has been used for Rhhizobium identification and geneic analyses

(Kosier t al., 1993; Kay et al., 1994). The RAPD echnique is a polymerase chain reaction (PCR) based assay ha as developed o dec polymorphisms in genomic DNA (Welsh and McClelland, 1990 and Williamset al., 1990). Besides being simpler and cheaper, this method is as effective as the more labor intensive RFLP for establishing genetic relationshios and identifying Rhizobium strains (Lauguerre et al., 1996 and Selenska – Pobell et al., 1996). The present investigation was carried out establish genetic patern and genetic groups of different isolates of Rhizobium leguminosarum collected from the root nodules of Arachishypogaea using the technique of RAPD-PCR.

Table 1: Rhizobium leguminosarum isolated from the root nodules of Arachitshypogaea.

Isolate	Place	Crop
1	Achampathu, Madurai	Arachishypogaea
2	Alanganallur, Madurai	Arachishypogaea
3	Karumathur, Madurai	Arachishypogaea
4	Mellur, Madurai	Arachishypogaea
5	Vadipatti, Madurai	Arachishypogaea
6	Nathampatti, Viruthunagar	Arachishypogaea
7	Krishnankovil, Viruthunagar	Arachishypogaea
8	Vathrap, Viruthunagar	Arachishypogaea
9	Mamsapuram, Viruhunagar	Arachishypogaea
10	Koomapatti, Viruthunagar	Arachishypogaea

IV. RESULTS AND DISCUSSION

Rhizobia are exposed to highly external influences due to rapid changes in agronomical practices throughout world and ultimately they respond to the Impacts by changing their capacity mainly in terms of genetic modifications. Rhizobium leguminosarum isolates were collected from ten fields of Arachishypogaeacultivation. The genetic diversity of the biofertilizer is analysed using the RAPD PCR technology. Twenty RAPD [RAPD Kit A1 to Kit A20] decameric primers were tested for their ability to differentiate the ten different isolates of R.leguminosarum. of all the primers tested,RAPD Kita 9 [^{5}GGGTAACGCC 3] and KitA12 [^{5}TCGGCGATAG$^{3]}$ Yielded clear amplification patterns for isolates of R.leguminosarum. These two primers amplified a total of 110 scorable bands in the molecular weight range of 1970bp to 250bp. Dendrogram and similarity index were constructed based on the RAPD profiles generated by Kit A9 and A12 primer using UPGMA [Bioprofile 1D software].

The primer KitA9 amplified a total of 49 scorable bands and the size of the amplified products varied from 1750bp to 250bp [fig 1a]. The amplification patterns showed a maximum of eight bands in the isolates I [Fig 1a:lane 1], and a minimum of two bands in isolate V [Fig 1a:lane 5] for the primer Kit A9. The dendrogram exhibited three major clusters, comprised of R. Leguminosarum isolates I and III in first cluster isolates II,VI,VIII and IX in second clusters[fig.1b]. The genetic similarity of 100% was recorded among the isolates of VIII and IX [Fig.1c]. The least similarity of 18% was recorded among the isolates isolates of I and II.

The primer KitA12 produced 61 scorable fragments with the molecular weight ranges from 1970bp to 255bp[fig 2a]. The primer generated a maximum of eight bands in isolates II and V[fig.2a lane 9]. The dendrogram constructed from the RAPD profile of KitA12 revealed three distinct and separate clusters [fig.2b]. The three clusters were comprised as isolates II and V in first cluster; and isolates III,IV,VI and X in second cluster; and isolates I,VII,VIII and IX in the cluster. The maximum genetic similarity of 92% was recorded among the isolates of II and V[Fig.2c].

Among these two primers [Kit A9 and A12], Kit A12 produced the most polymorphic amplification patterns that could distinguish almost all the isolates from each other. The RAPD profile of different isolates.

Figure 1a : Random amplified polymorphic DNAs of different isolates of *Rhizobium leguminosarum* generated by the Primer RAPD Kit A9

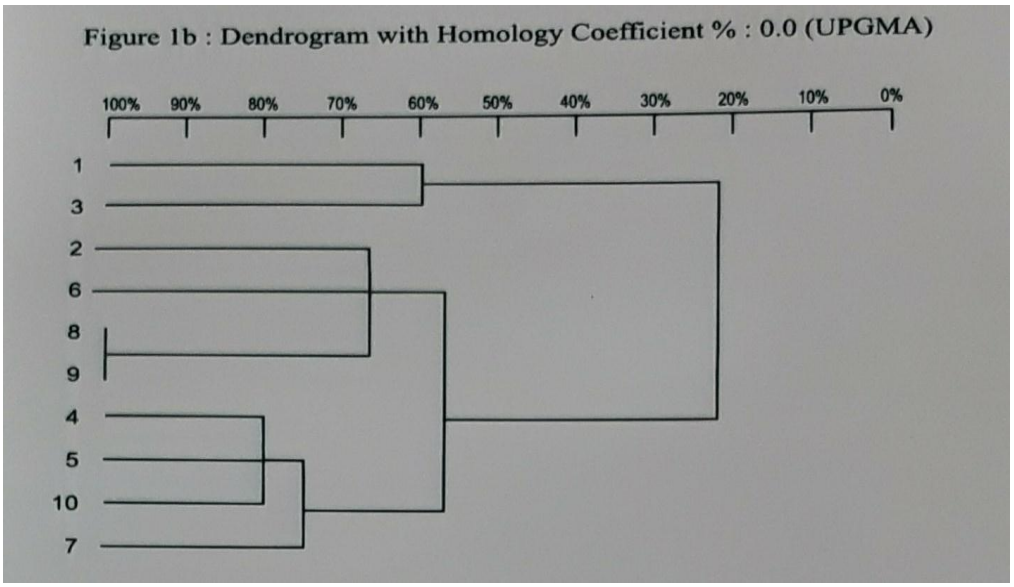
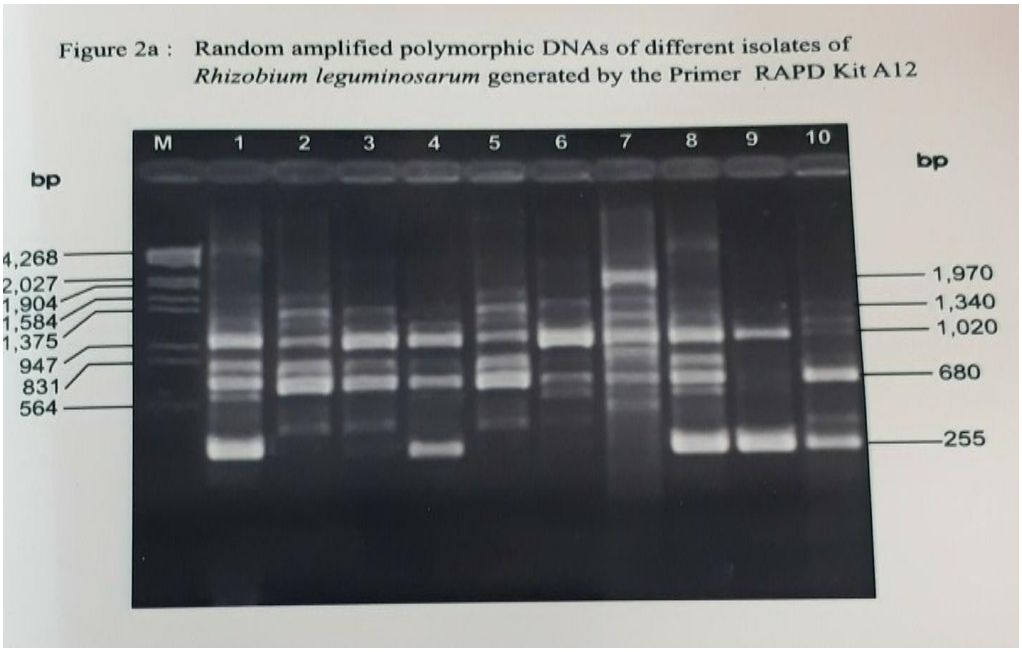


Figure 1c: Similarity index based on RAPD profiles.

	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.18	1.00								
3	0.60	0.36	1.00							
4	0.25	0.44	0.50	1.00						
5	0.22	0.25	0.29	0.80	1.00					
6	0.50	0.67	0.50	0.67	0.40	1.00				
7	0.20	0.73	0.40	0.75	0.57	0.50	1.00			
8	0.25	0.67	0.50	0.67	0.40	0.67	0.75	1.00		
9	0.25	0.67	0.50	0.67	0.40	0.67	0.75	1.00	1.00	
10	0.29	0.50	0.57	0.80	0.50	0.80	0.57	0.80	0.80	1.00



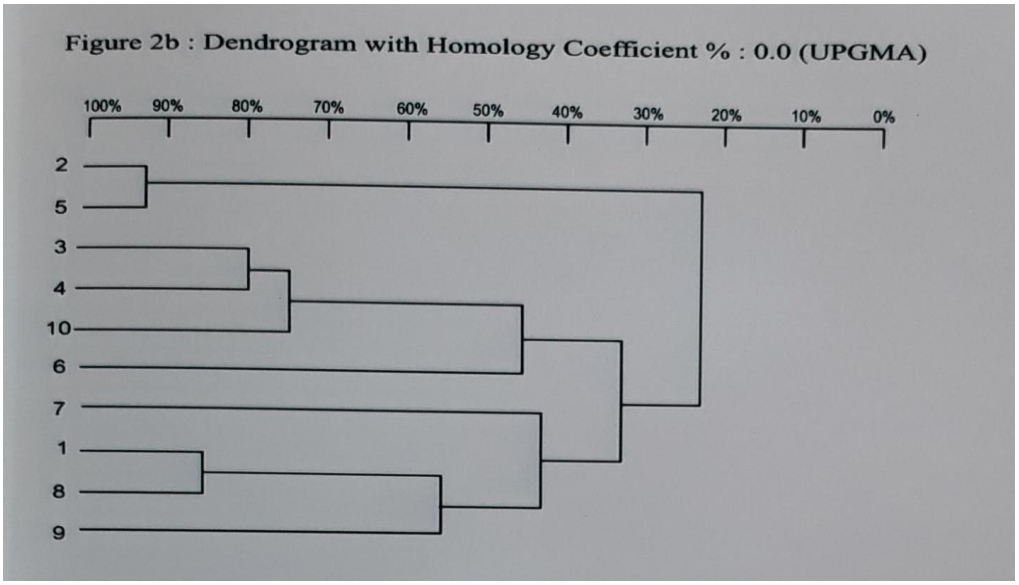


Figure 2c: Similarity index based on RAPD profiles.

	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.22	1.00								
3	0.36	0.22	1.00							
4	0.36	0.22	0.80	1.00						
5	0.18	0.92	0.22	0.22	1.00					
6	0.36	0.22	0.44	0.44	0.22	1.00				
7	0.57	0.22	0.40	0.40	0.22	0.22	1.00			
8	0.87	0.22	0.25	0.75	0.22	0.22	0.40	1.00		
9	0.58	0.25	0.29	0.57	0.25	0.25	0.44	0.57	1.00	
10	0.22	0.67	0.80	0.80	0.66	0.22	0.40	0.50	0.57	1.00

R. leguminosarum generated by Kit A12 primer revealed great degree of genetic poluymorphism among the isolates.

The soil PH has been described as a critical factor in establishing genetic differences among Rhizobium leguminosarum populations (Harrison et al., 1994), moreover, the clay and organic matter contents, together with PH, were found to have a significant effect on the rate of plasmid transfer in soil among Rhizobium fredie strains (Richaume et al., 1989), which influenced the level of genetic diversity of the population. The genome of Rhizobium is complex and contains several reiterated DNA sequences (Girard et al.., 1991; Palacios et al., 1995). The reiterated copies are usually necessary for the expression and effectiveness of the N2 fixation (Flores et al., 1998; Martinez et al., 1990); they also provide sites for recombination that can give rise to genomic rearrangements at high rates (Soberon-Chaves et al., 1986), resulting in alterations on the original characteristics of the cells (Flores et al.,

1988). Paffetti et al., (1996) reported the responsibility of physical and chemical factors of soil for the genetic differences among the strains of Rhizobium meliloti.

Genetic instability of Rhizobium strains as one of the causes that compromise relevant characteristics of these organisms in the nitrogen fixation, both in wild-type strains obtained by conventional selection procedures (Pinto et al., 1998; Sa et al., 1993) and in genetically improved strains (Corich et al., 1996; Wilson and Lindow, 1993). Rhizobium strains capable of nodulating bean display particular genetic characteristics that favor such instability (Raposeiras et al., 2002).

Bacteria are genetically diverse and physiologically heterogenous living nature. Documentation of genetic diversity of agriculturally important bacterial species has potential applications in enhancement of agricultural output. Of which, nitrogen fixers namely Rhizobia are the most important groups of organisms and their association and application potential is mainly based on the genetic nature of the species and its strains. The present study reveals the genotypic patterns and genetic groups of Rhizobium leguminosarum. Further,genetic characterization would give concete information on their association with the host plants based on its genotypic pattern. The genetic diversity of the isolates of R. leguminosarum was found to reside on the whole bacterial genome.

SUMMARY

The root noodles of Arachishypogaeawere collected from ten different localities in Madurai and Virudhunagar districts.

The root nodules were made into extract and the cultures of Rhizobium leguminosarum were established on the YEMA medium.

The genomic DNA isolated from the all ten isolates of R. legumiknosarum and was used for RAPD analysis.

Twenty random decomeric primers were used for RAPD studies. Of these, RAPD Kit A9 and A 12 primers produced clear, reproducible and scorable fragments.

These Kit A9 and A12 primers produced a total of 110 scorable bands in the molecular weight range of approzimately 1970bp to 250bp.

The Kit A12 primer produced the most polymorphic amplification patterns, which could be used to distinguish almost all the isolates from each other. Genotypic patterns and genetic groups of Rhizobium leguminosarum isolates were reported based on RAPD profile and UPGMA cluster analysis.

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