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Antibiotic Resistance and Taxonomic characterization of Rhizobia isolated from Semi Arid Region of Rajasthan

Biological Science

KEYWORDS: Intrinsic antibiotic reistancr(IAR),Cluster analysis, Genetic Variability, Stress tolerance,Taxonmic identification

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ABSTRACT

Rhizobium strains were isolated from root nodules of Tephrosia purpurea and Trigonella foenumgraecum (fenugreek). 20 strains were individually tested for their antibiotic resistance. Ajmer being a semi arid zone of Rajasthan was selected for the study of antibiotic response. Disk diffusion method was used to antibiotic sensitivity. The intrinsic antibiotic resistant profile method was employed for taxonomic characterization.. The intrinsic resistance towards antibiotics is an important attribute that may contribute to the initial selection of a dominant strain with higher survival and competitiveness. Antibiotic profiles were further used for cluster analysis using past software and the dendrogram thus generated depicted that Rhizobium formed different clusters which classify slow growing Rhizobium and fast growing Rhizobium . Antibiotic Resistance patterns is a simple and useful tool used for screening of Rhizobial and Bradyrhizobial cultures. RTP7 showed sensitivity to all the tested antibiotics thus the least stress tolerant stain .In present study most of the Rhizobium found resistance towards Kanamycin.

Introduction

Rhizobia are gram negative rod shaped bacteria that exhibits symbiosis with legumes, forming root or stem nodules and fixed atmospheric nitrogen (Bala and Giller, 2001; Quatrini *et al.*, 2002). They are gram negative, motile rod shaped bacteria. Rhizobia are somewhat unique among soil microorganisms and their ability to form nitrogen-fixing symbioses with legumes. As antibiotic resistant rhizobium have more chances to survive as compared to susceptible rhizobial strains,thus antibiotic resistant bacteria may be play a role in increasing nitrogen content of semi arid soil. Selection of effective, efficient and compatible antibiotic resistant rhizobial strains could help in ecological rehabilitation of degraded soils and an increase in soil fertility thereby improving the growth of associated plants of this region.

Same bacterial group possess a differential response towards the specific antibiotic as some strains showed resistance while other are susceptible for that particular antibiotic. Intrinsic antibiotic resistance (IAR) may indicate the ability of the *Rhizobium* to overcome the effect of anti-microbial chemical present in the soil. Resistance to streptomycin is one of the widely used markers for rhizobia identification (Brockwell *et al.,* 1977; Borges *et al.,* 1990). Intrinsic antibiotic resistance technique is very useful for comparison of different rhizobial strains (Harwani, 2006) .Detection of antibiotic markers is is greater significant than other procedures because the methodology is simple, reliable and non-expensive (Mueller et al., 1988; Brockman and Bezdicek, 1989; Abaidoo et al., 2002). According to Abaidoo *et al.* (2002), the antibiotic resistance profile method can be used as a simple means of assessing taxonomic identification and grouping of a large number of *Bradyrhizobium* spp.

Fast growing rhizobia are more resistant towards tetracycline, penicillin , vancomycin and streptomycin than the slow growing *Bradyrhizobium*.

Material and Method

Experimental site

Rajasthan state falls under arid and semi arid region , of which Ajmer occupies an area of 8480 km^2, and is located between 25°38': 26°58' north latitude and 73°54': 75°22' east longitude. Ajmer and its adjoining area were selected as the experimental site. Mean annual rainfall of the district is below 500mm, showing a semi-arid climate (Khan, 1999) The Farook *et al.*,2009 reported that average maximum temperature of Ajmer district is 46.0 degrees Celsius.

Collection of root nodules and isolation of Rhizobia

Isolation of rhizobia from root nodules of Trigonella foenumgraecum and Tephrosia purpurea was done by the method of Somasegaran and Hoben (1985). All the 20 rhizobial isolates(10 isolated from Trigonella foenumgraecum called RTF 1 to1**0** and 10 isolated from Tephrosia purpurea called RTF 1 to10) Antibiotic sensitivity Test was performed on all strains. Disk diffusion method was used for antibiotic sensitivity. Antibiotics used were Penicillin, Erythromycin, Neomycin, Kanamycin, Gentamycin, Norfloxcin, Streptomycin, Tetracyclin Teramycin and Chloremphenicol. In agar diffusion tests, cells of strains were evenly spread onto YEMA agar plates to obtain a confluent lawn of bacterial growth. Then, disks containing antibiotics were placed on the surface of the plates and incubated at 37 °C for 5 days. After 5 days diameter of the inhibition zone was measured. In the present study antibiotic patterns of studied organisms were generated by measuring diameter of inhibition zone and the data thus obtained were analysed with unweighted pair match grouping (UPGMA) using past software and expressed in Euclidean distances. The less the Euclidean distance more closer will be the species

Result and discussion

Differences between isolates were verified using antibiotic resistance. As in the present study intrinsic antibiotic resistant profile method was also employed as a simple means of assessing genetic variability by earlier workers (Abaidoo *et al.,* 2002). Kucuk *et al.* (2006) stated that the intrinsic resistance towards antibiotics is an important attribute that may contribute to the initial selection of a dominant strain with higher survival and competitiveness..it is predicated that the Rhizobium that showed a high level of resistance did not take up the antibiotics. In the present study antibiotic patterns of studied organisms were generated and the data were analysed with unweighted pair match grouping (UPGMA) and expressed in Euclidean distances and clusters so formed were consists of two clusters **(Figure 1**) Cluster(I) and Cluster (II). Cluster (I) was separated from Cluster(II) at a distance of less than 20 Euclids. Cluster (I) comprised of fast growing rhizobial strains and Cluster (II) comprises of slow growing rhizobium, belongs to bradyrhizobium group of proteobacteria. Similar findings were also observed by earlier workers as more the Euclidian distance less will be the similarity between the strains. Cluster(I) was subdivided into three subclusters i.e. subclusters (1) and subcluster(2) and subcluster(3) at a distance of 13 Euclids. Cluster (II) was also subdivided into two sub clusters subclusters (1) and subcluster(2) at a distance of less than 5 Euclids. Cluster (I) and Cluster(II) were separated on the basis of resistance of organisms towards neomycine, tetracycline and chloremphenicol antibiotics. It was observed that most of organisms showed sensitivity in Cluster (I) thus the organism in clusterI are less resistant to environmental constrains (Kucuk *et al.*(2006). Subcluster(2) was separated from subclusters (1) and subcluster(3) as organism included in subcluster(2) were resistance to teramycine and organism included in subcluster(3) was RTP7 ,which showed sensitivity to all the tested antibiotics thus, RTP7 was least stress tolerant among all the tested organism..RTF 8 showed maximam sensitivity as compared to

studied organisms of subcluster(2) of Cluster(II).These results showed agreement with previous study of Galina 1991 who observed that Bradyrhizobium isolates were resistant to chloramphenicol, neomycin and tetracycline but they were sensitive to kanamycin. *Rhizobium* isolates were sensitive to tetracycline and streptomycin and resistance to kanamycin (**Table1**). In present study most of the *Rhizobium* found resistance towards Kanamycin. However, many workers confirmed the fact that Bradyrhizobia are generally less sensitive to antibiotics than Rhizobia (Cole and Elkan, 1979.). Similar to present study antibiotic Resistance patterns is also employed as simple and useful tool for screening of Rhizobial and Bradyrhizobial cultures(Chanway and Holl, 1986 ; Galiana, , 1991.; Girgis, and Traoré, 2000.). Kingsley and Bohlool ,1983 observed that even very closely related strains can showed different IAR patterns but can be used for taxonomic identification. As different rhizobium strain showed different IAR pattern inspite of all are belong to fast growing group.

Table 1- Intrinsic antibiotic resistance patterns of rhizobial isolates in terms of inhibition (size of inhibition zone measure in mm)

Organism	Penicilin	Erytomycin	norfloxcin	Kanamycin	Gentamycin	Neomycin	Streptomycin	Tetracycline	Teramycin	Chloremphenicol
RTF4	3	3.2	3	2.5	2.5	0	2.5	0	2.7	0
RTF7	3	3.4	3	1.4	3	0	3.1	0	2.7	0
RTF8	3	3	3	2.5	3	0	4	0	2.4	0
RTP1	3.2	3	2.8	1.2	2.6	0	2.8	0	2.1	0
RTF3	3.1	2.4	3.4	0	3.1	3.1	4.1	3.3	3.1	3
RTF5	3.2	2.5	3	0	2.6	3	3.8	3	3	3.5
RTF6	5.1	4.2	2.3	0	2.3	3	2.1	2.8	3.2	3.4
RTF9	3.2	2.4	3.1	0	3.2	1	2.9	2.2	3.1	3.6
RTF0	3.2	2.4	3	0	2.6	3.2	3.8	3	3	3.6
RTP2	3.4	2.6	3.4	0	3	3.2	4.4	3.4	3.1	3
RTP3	3	2	1.6	0	2.1	2.8	1.2	1.8	2.5	2
RTP4	3	2.2	2.4	0	3	4	2.9	2	0	2.8
RTP5	3	3	2	0	2.6	3.4	1.8	2.4	2.2	1
RTP6	5	4.4	2.5	0	2	3	2.2	2.7	3.2	3.4
RTP7	2.8	3.4	2.2	3	2.5	2.2	2.4	2	2.2	3.4
RTP8	2.6	2.6	3	0	3.4	3.4	3.2	1.8	0	3
RTF2	2.6	2.6	3	0	3.4	3.4	3.2	2	0	3
RTF1	2.8	2.6	3	0	3.4	3.4	3.2	2	0	3
RTP9	3	2.5	2.2	2	2.8	2.8	2.6	1.2	0	4
RTP 10	2.4	2	2.6	0	2.8	1.6	2.8	2.2	3	3.4

Conclusion

Antibiotic profile of organism not only depicts the antibiotic resistance/sensitivity towards the specific antibiotic but also proved helpful in taxonomic identification of rhizobial strains. In present study two separate clusters were formed for slow growing i.e. bradyrhizobium and fast growing rhizobium (also confirmed from other methods employed for identification of organism in the same research project i.e. biochemical characterization,fatty acid profiling and DNA sequencing etc.) Thus studied 20 strains antibiotic pattern proves helpful in taxonomic characterization. It is predicted that rhizobium that showed high level of resistance did not take antibiotics and have more potential for competitiveness and survival in harsh environment condition like semi arid zone thus the present study aids in selection of antibiotic resistant strain which help in establishment of effective symbioses of rhizobium and legumes. This effective symbioses help in reclamation of degraded ecosystem and enhance the nitrogen content of that particular area which proves helpful in combating erosion and desertification by restoring sustainable plant cover in barren lands of semi arid region.

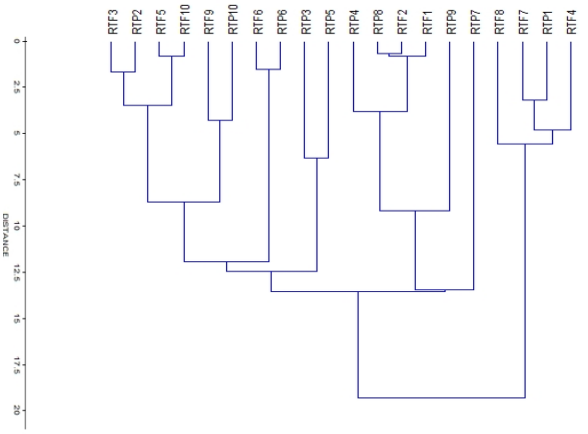

Figure 1- Dendrogram demonstrating the similarity (in Euclids) between Rhizobium strains using antibiotic resistance pattern

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