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Study of legume-symbiosis bacteria (*Rhizobia*) away from host plant zone

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Abstract

Experiment was conducted to study the migration of root nodulating bacteria from their host by different ways such as air, water, etc. The samples were collected from different ways and distances from legume growing site *viz*; 100, 150 and 200 meters. Five root nodulating bacteria from samples were isolated by using yeast extract mannitol agar media with congo red dye. These isolates were further characterized on the basis of morphological, biochemical characters and tested for symbiosis and nodulation on legume plants in laboratory. The results revealed that three isolates were authenticated for symbiosis with legumes and nodulation. M-2 from stagnant water, 150m away from host plant site isolate developed nodulation on *Trigonella foenum-graecum* and M-3 from air, 100m away from host plant site developed nodules on *Trifolium alexandrinum* while one isolate M-5 from running water, 200m away from host plant site developed nodules on *Vigna radiate.* Hence, running water and air are important sources for migration of root nodulating bacteria from one place to another.

Key words : Legume, Migration, *Rhizobium*, *Trifolium alexandrinum, Trigonella foenum-graecum, Vigna radiata*

Many leguminous plants are able to utilize atmospheric nitrogen through an association with *Rhizobia* bacteria that are hosted by the root system of certain nitrogen plants. This self sufficiency achieved by symbiotic fixation of nitrogen from atmosphere in the form of NH_4^+ under the soil. Their colonization in new locations depends on both host plants and environmental factors. Migration of bacteria from one place to another is take place by means of running water, air, soil, river etc. Studies have been done for migration of bacteria using chemotaxis a soft agar assay, soil matric etc (Tambalo *et al.,* 6). The degree of motility and the presence of flagella are important factors influencing the movement of *Rhizobia* (Issa *et al*., 3). Running water is an important source for long distance transportation of some Rhizobia especially in virgin area (Ali *et al*., 1, Wang *et al*.,7). Rhizobia moves passively but their movement is effected by biotic and abiotic factors. In present investigation migration studies of rhizobia was done in stagnant water, air and running water at different distances from the legume host plant such as *Trigonella foenum-graecum. Trifolium alexandrinum, Vigna radiata* and *Pisum sativum*

Samples were collected from different sources and their distance from experimental area, National Research Centre on Seed Spices, Tabiji, Ajmer, for migration study of root nodulating bacteria in air, running water and stagnant water. Each sample was collected in triplicate.

For collection of bacteria from air a muslin cloth was hanged on tree located at 100 m and 150 m away from experimental sites for 72 hrs. After 72 hrs sample were collected. For collection of bacteria from stagnant water mud samples were collected from 100 and 150 m away from host plant site in sterilized bottle. Samples were taken from running water, a drainage system away from the experimental sites in sterilized bottle (Table 1). The experimental area where to collect sample was un-inoculated with any *Rhizobium* sp. before taking sample.

Root nodulating bacteria were isolated from collected samples by using serial dilution method on yeast extract mannitol agar media with congo red dye (CRYEMA). Then plates were then incubated in inverted position at 27± 1°C. The creamish, gummy, translucent, colonies appeared. For purification single colony were isolated and streaked on yeast extract mannitol agar media with congo red dye plates (Ali *et al*., 1). After purification, authentication was done under controlled environmental condition. Seed were surface-sterilized with 0.2% $HgCl_2$ and germinated onto nitrogen-free agar slopes in 20 × 150 mm test tubes under controlled environmental conditions such as pH, humidity, temperature etc. (Pryor and Lowther, 5). One ml (10^7 cfu ml^{-1}) inoculums of each isolates were inoculated to the root of the respective legume. Each isolate in triplicates with negative control without inoculated. After sixty days of inoculation nodulation was checked in each replication.

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The *Rhizobia* was isolated and purified from the collected samples of different sources. The purified cultures were characterized morphologically and biochemically (Table 3 and 4) and authenticated by root inoculation of host plants. The results revealed that out of five collected samples only two isolates i.e. M-2 and M-3 were able to develop nodules with *Trigonella foenum-graecum* and *Trifolium alexandrinum* while one isolate M-5 was nodulated with *Vigna radiata* and no nodulation observed with *Pisum sativum* (Table 2). The results indicated that *Rhizobia* can survive in fresh water and can be transported by running water as well as by air at the distance of 100m. Ali S.F *et al.,* 2010 also reported that water is an important source for migration of some *Rhizobia*. Bashan (2) found that plants inoculated with *Azospirillum brasilense* in aseptic and controlled environmental condition but caused contamination the inoculated plants by air showed the migration of bacteria by air. The present investigation concluded that running water as well as air (100m distance) is the major source for migration of root nodulating bacteria from one place to another from host plant.

Table 1. Sources and distances from legume experiment site for sampling

S. No.	Sources	Distance from legume	Isolates
1	Stagnant water	100 meter	M1
2	Stagnant water	150 meter	M2
3	Air	100 meter	M3
4	Air	150 meter	M4
5	Running water	200 meter	M5

Key: M-migration and Number- code of isolates

Table 2. Authentication of *Rhizobia* isolates from migration study

***Rhizobia* isolates from migration study**	**Legume Crops**			
	Trigonella foenum graecum	***Pisum sativum***	***Vigna radiata***	***Trifolium alexandrinum***
M-1	-	-	-	-
M-2	+	-	-	+
M-3	+	-	-	+
M-4	-	-	-	-
M-5	-	-	+	-

Key: (+) Positive Nodulation; (-) Negative nodulation

Table 3. Morphological characters of *Rhizobia* isolates obtained from migration.

Characteristic	*Rhizobia* isolates				
	M-1	M-2	M-3	M-4	M-5
Colony shape			Round		
Colony colour	W	W	Y	W	W
Colony Texture	C	C	C	CG	C
Day of appearance	3d	3d	4d	3d	3d
Gram staining			-ve, rod		
Capsule staining	+	+	+	+	+
Motility	M	M	M	M	M
YEMA with BTB dye	+Y	+Y	+Y	+Y	+Y
GPA with BCP	-	-	-	+Y	-

Note: CYEMA (Yeast Extract Mannitol Agar medium incorporated with 0.0025% Congo red dye); YEMA with BTB (Yeast Extract Mannitol Agar medium incorporated with Bromo Thymol Blue dye); GPA with BCP (Glucose Peptone Agar medium incorporated with Bromo Cresol Purple dye)

Key : White (W); Yellow (Y); Circular (c); Glistening (G); No Growth (-); Good growth (+); Motility (M); YEMA with BTB-Colour changed from green to yellow (Y); GPA with BCP from purple to yellow

Table 4. Biochemical activity of Rhizobia isolates obtained from migration

Biochemical Activities	*Rhizobia* Isolates				
	M-1	M-2	M-3	M-4	M-5
Catalase Activity	+	+	+	+	+
Oxidase Activity	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+
Citrate Utilization Test	+	+	+	-	+
Nitrate reduction Test	+	+	+	+	+
H_2S Production	-	-	-	-	-
Methyl Red Test	+	-	+	+	+

Key: +ve = Positive test – ve = Negative test

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