



ISOLATION OF CELLULAR FATTY ACID DISTRIBUTED IN UNKNOWN RHIZOBIAL STRAINS

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ABSTRACT

Fatty acid (UFA) biosynthesis is essential for the maintenance of membrane structure and function in many groups of anaerobic bacteria. In this study isolation of cellular fatty acid distributed in unknown rhizobial strains collected from five different soil samples by pour plate method using YEMA medium. The five soil sample are namely Klt 1, Alg 2, Bdl 3, Rf 4 and Mdk 5. The isolated rhizobial sample was identified based on morphological and biochemical characterization. Then the purified culture was mass multiplied and used for isolation of fatty acid. The fatty acid profile of five different rhizobial isolates were identified by GC and the result of fatty acid was totally seven fatty acids from Rhizobial isolates. Among them the Myrestic acid (C:14) were present all the isolates respectively.

Key words: Myrestic acid; Rhizobial isolates; fatty acid; YEMA medium.



Council for Innovative Research

Peer Review Research Publishing System

Journal of Advances in Natural Sciences

Vol 2, No. 2

editorjansonline@gmail.com

www.cirworld.com



worldwide (Postaga,1998). Considering the potential of legume-Rhizobium associations, rhizobial inoculants have been used to improve plant and soil health for more than a century now. Inherent with the use of bioinoculants is the problem of variability in field performance and successful establishment of introduced strains on account of competition with the indigenous rhizobacterial population (Grahm, 1988).

In rhizobia reduce atmospheric nitrogen to ammonia using the enzyme nitrogenase and supply this essential nutrient to the host plant cells. Soil system supports a conglomerate of microorganisms with a high degree of diversity and their interactions are extremely complex. This sometimes creates a hostile environment for the inocula. The introduced microorganisms in the soil are also subjected to abiotic stress (Barnet *et al.*, 1991). The ability of the inoculated strain of *Rhizobium* to outperform the indigenous population is termed as nodulation competitiveness.

In the present study, the attempt has been made to isolate and identify various rhizobial strains collected from five different soil samples from Thanjavur district. The biochemical characteristics were studied from the isolated strains. The isolated strains are mass multiplied to study the distribution of fatty acid present in the unknown rhizobial sample.

MATERIAL AND METHODS

Collection of soil sample

The soil sample are collected from five different localities Kalathur (Klt 1), Alangudi (Alg 2), Budalur (Bdl 3), Reserved forest (Rf 4) and Madhukur (Mdk 5) on Thanjavur District based on soil atlas.

Isolation and screening of *Rhizobium* from the soil sample

The *Rhizobium* from the soil sample was isolated, purified and screened by pour plate method using YEMA medium. The pH of medium was incubated at 28±2°C for 2 to 3 days. The YEMA medium allowed *Rhizobium* to grow and develop in to colonies. The *Rhizobium* colonies appeared as white translucent, elevated and gummy colonies on YEMA medium. They were removed and purified by repeated streaking and maintained on YEMA slants. They were name as 1,2,3,4, and 5. The agarobacterial colonies appeared as red colonies elevated colonies on YEMA medium.

Morphological characteristics

The cell dimensions and morphology were determined on living cells by Light microscope. Gram's staining technique carried out for differentiation.

Specific test for identification of *Rhizobium*

The specific tests like Growth in YEMA medium with congo red (Hahn, 1966), Hofer's alkaline broth test (Hofer,1941), Lactose agar test (Date and Hurse,1992),Reaction of litmus milk (Vincent,1970) and Staining of Poly beta-hydroxy butyrate (PHB)(Burdon,1946).The biochemical tests were also conducted to identify the bacteria.

Analysis of Fatty acid profile by GLC

The fatty acid was analyzed by the GLC using DEGS column and a flame ionization detector. From the peak area of fatty acid, the amount of fatty acid was calculated using respective standards.

RESULT AND DISCUSSION

Rhizobia are classically defined as symbiotic bacteria capable of eliciting and invading root or stem nodules on leguminous plants, where they differentiate into N_2 fixing bacteroids. A remarkable feature of rhizobial ecology is the ability of these bacteria to change their life style in adaptation to the highly contrasting environments they can inhabit (Wommmer *et al.*, 1988).

In this study, Rhizobium was isolated by five different soil samples (Klt 1), (Alg 2), (Bdl 3), (Rf 4) and (Mdk 5) collected from various place of Thanjavur district by pour plate method using YEMA medium. All the isolates were purified and showed positive result in PHB test and failed to congo red in YEMA coloration was not found in lactose agar test, 3-Ketolactose test and no growth was found in Hofer's alkaline agar and litmus milk reaction test of rhizobial isolates (Table-1).Many workers surveyed many strains of rhizobia and used many cultural test, pointed out that the rhizobial typically show slow growth on peptone glucose agar, form little or no H_2S from bismuth sulphite and no precipitate in glyceryl phosphate agar, not absorbing congo red and did not utilize citrate (Kleczkonwska *et al.*, 1968; Jordon,1984).

The isolated culture were identified by various morphological and biochemical methods as showed in table-2. The result clearly indicate all the Rhizobium were gram-negative, rod shaped and motile in nature ,the cell diameter of all rhizobial isolates ranged from 0.5 to 3µm respectively. The result of biochemical tests showed all the positive result in all the rhizobial isolates and negative result in methyl red test, VP test, triple sugar iron and oxidase test. The isolated culture was mass multiplied using YEMA broth and culture was used by seed treatment.

Tighe *et al.*, (2000) reported that the fatty acid profile of Meso Rhizobium, Bady Rhizobium and Sino rhizobium were evaluated using GC. The clustering of rhizobial species based on fatty acid analysis is usual because several separate clusters are observed from it (Jarvis *et al.*, 1989)

Fatty acid of five Rhizobial sample was investigated by GC and the result of fatty acid was totally seven fatty acids from Rhizobial isolates. One fatty acid was belonged to saturated and the remaining six were found to be



unsaturated. Among them the Myrestic acid (C:14) were present all the isolates. The maximum number of fatty acids (6) was detected from ALg2, Bld3 and MDk5 followed by Rf4 different fatty acids respectively.

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Table-1: Biochemical characteristics of *Rhizobial* isolates

S.No.	Biochemical test	Rhizobial isolates from different soil sample				
		Klt 1	Alg 2	Bld 3	Rf 4	Mdk 5
1	Mac conkey agar	+	+	+	+	+
2	Indole	+	+	+	+	+
3	Voges-proskauer	-	-	-	-	-
4	Citrate utilization	+	+	+	+	+
5	Methyl red	-	-	-	-	-
6	Triple sugar iron	-	-	-	-	-
7	Starch	+	+	+	+	+
8	Urea hydrolysis	+	+	+	+	+
9	Catalase	+	+	+	+	+
10	Oxidase	-	-	-	-	-

+ denote positive

- denote negative

Kalathur (Klt 1)

Alangudi (Alg 2)



Budalur (Bdl 3)

Reserved forest (Rf 4)

Madhukur (Mdk 5)

Table-2: Estimation of fatty acid profile in different Rhizobial isolates(mg/g/of lipid)

S.No	Name of fatty acid	Klt 1	Alg 2	Bdl 3	Rf 4	Mdk 5
1	Capric acid (C:10)	1.29	1.07	-	0.86	0.44
2	Lauric acid (C:12)	6.35	-	5.82	7.35	-
3	Tridecanoic acid (C:13)	5.24	4.06	0.43	-	0.08
4	Myrestic acid (C:14)	0.76	0.54	3.43	2.65	0.12
5	Panta decanoic acid (C:15)	0.14	0.31	-	-	0.02
6	Palmitic acid (C:16)	-	5.83	4.35	-	5.79
7	Hepta decanoic acid (C:17)	-	0.14	0.57	0.47	0.24

