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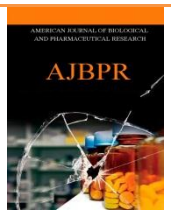

FATTY ACID COMPOSITION OF THREE DIFFERENT FRANKIAL ISOLATES FROM *Casuarina equisetifolia*

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ABSTRACT

Frankia is a genus of Nitrogen fixing, filamentous bacteria that live in symbiosis with actinorhizal plants. In the present investigation, the *Frankia* were isolates from nodules of *Casuarina* plants and were collected from three different sites of Nagappattinam districts, Tamilnadu, namely SI, SII and SIII .In this study totally nineteen different fatty acids recorded in three different isolates. Some of the fatty acid was not detected some other fatty acids showed their increase in quantify over other isolates. The presence of short chain, long chain, saturated and unsaturated fatty acids in three isolates have been detected. A substantial reduction (both in number and quantity) of fatty acids in three isolates (SI, SII and SIII) was recorded.

INTRODUCTION

The growth of all organisms depends on the availability of mineral nutrients, and none is more important than nitrogen which is required in large amounts as an essential component of proteins, nucleic acids and other cellular constituents. Though there is an abundant supply of nitrogen in the atmosphere, molecular nitrogen is metabolically unavailable directly to higher plants and animals. Nitrogen must be converted into ammonium (NH_4+) or nitrate (NO_3-) ions before it can be used by plants and animals. Conversion of molecular nitrogen to NH_4 + forms is also called nitrogen fixation. Only a few microorganisms can 'fix' atmospheric nitrogen, making all other living organisms dependent on them for their requirements of 'fixed' nitrogen. Microorganisms Frankia form symbiotic associations with host plants and utilize fixed carbon supplied by host for fixing atmospheric di-nitrogen (N_2).

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These microorganisms make a substantial contribution of fixed nitrogen to agriculture and forestry. It has been estimated that Frankia contributes about 2–362 kg N/ha/yr while the estimated contribution of rhizobium-legume symbiosis is about 24–584 kg N/ha/yr.

Frankia can grow as microsymbiont in the root nodules of several woody dicotyledonous plants. The Actinomycete genus *Frankia* belongs to the family, Frankiaceae its members are Gram-positive bacteria that nodulate about eight plant families representing about 25 genera of woody, dicotyledonous, perennial angiosperms, collectively called actinorhizal plants [1]. The term actinorhiza is given to root nodules that are formed by *Frankia*.

Symbiotic associations that develop between microorganisms and higher plants have received recognition due to their effects on plant morphogenesis, nutrition, protection against infectious diseases and study of basic cell biology. These associations cater to the nutritional needs of the biosphere and are responsible for generating almost 50% of the fixed nitrogen annually. In the topics, the ability of *Casuarina* species to form

symbiotic N fixing association with *Frankia* is one attribute which makes these tree species potentially important for fuel-wood production, agroforesty and reclamation of infertile soils in the tropics, subtropics and arid zones [2]. During the last decade hundreds of *Frankia* isolates have been obtained from Casuarinaceae nodules using different isolation techniques. All isolates obtained from nodules were assigned to the genus *Frankia* on the basis of (i) morphological features, such as sporangium and vesicle formation in submerged liquid culture, (ii) chemical composition of certain cell constituents such as cell wall type III, phospholipids type PI and the presence of the diagnostic sugar *2-0*-mehtyl-mannose and (iii) the ability to fix nitrogen and to nodulate plants [3]. Many isolates lacking some of these morphological and physiological characteristics of typical *Frankia* have been obtained from actinorhizal nodules [4].

Qualitative and quantitative analyses of total fatty acids composition have been shown to be useful for taxonomic investigations [5]. Analysis of short chain fatty acids has been routinely used in the identification of anaerobic bacteria [6]. Using this information along with physiological and genetical data, Lalonde *et al.*, (1988) proposed the recognition of species *Frankia elaeagni* and *Frankia alni*, with subspecies *pmmerili* and *vandijkii*. However, all these investigations showed a characteristic fatty acid profile for all strains studied. This study aims to characterize fatty acids pattern of three different *Frankia* isolates obtained from root nodules of *Casuarina*.

MATERIALS AND METHODS

Sources of study materials

For this present study the Root Nodules from *Casuarina equisetifolia* were collected from three different sites (SI, SII and SIII) of Nagappattinam districts, Tamilnadu.

Isolation of Frankia

The Frankia used in this study was isolated from *C. equisetifolia* root nodules collected from the study area. The nodules were collected in ice box and stored in frozen condition at −4°C. Afterwards, the nodules were surface sterilized with 30% H_2O_2 and kept in a shaker for 30–40 min. Under aseptic conditions the nodules were rinsed in sterile water and 0.2 g of nodule was ground manually in sterile mortar and pestle. Then the nodule solutions were centrifuged at 1000 rpm for 20 min and the supernatant was filtered through Whatman No.1 filter paper. The suspension was then plated in P medium and incubated at 25°C for 3–4 weeks.

Analysis of fatty acids profile by GLC

Procedure

The cultures were centrifuged at 5000 xg for 10 min. From the pellet 100 mg was taken in separate screw cap test tubes. To each tube 1 ml of reagent 1 was added and tightly sealed with Teflon-lined screw cap. Then the tubes were vortexed for 10 second and kept in a boiling water bath for 5 minutes. Again the tubes were vortexed 10 seconds. Then the tubes were kept in the water bath for the additional 20 minutes. After a total of 30 min of saponification, the tubes were removed from the water bath and cooled to room temperature. To each tube, 2 ml of reagent 2 was added by uncapping the tubes. After vortexing for 10 seconds, the tubes were placed in a water bath set at 80 C for 10 min.

Finally 1.25 ml of reagent 3 was added to each cooled tube. Then test tubes were tightly closed and rotated end-over-end for 10 min. From the tubes, the lower aqueous phase was removed and discarded. To the upper phase, 3 ml of reagent 4 was added and rotated end-over-end for 5 minutes, with the help of clean Pasteur pipette 2/3 of the organic extract from each tube was transferred to GC vials and kept in deep freezer by capping with Teflon-lined septum. From each vial 2µl of sample was analyzed with Hewlett Packard 5890 Gas chromatograph fitted with 10 per cent DEGS column and a flame ionization detector. The carrier gas N_2 was supplied at the rate of 30 ml min^{-1}. The detector gas flow rates were 30 ml of H_2 min^{-1} and 300 ml of air min^{-1}. The chromatograph oven was set at 180°C, with injector and detector temperatures 210°C and 230°C respectively. From the peak area of fatty acid, the amount of fatty acid was calculated using respective standards [7].

RESULTS AND DISCUSSION

Fatty acid profile of three different isolates by gas chromatography and given in the Table.1. Totally nineteen different fatty acids recorded in three different isolates. In all, twelve different fatty acids from SI, fourteen from SII and fifteen from SIII were identified. These include short chain, long chain, saturated and unsaturated fatty acids

The three Frankia isolates (SI, SII and SIII) were varied in quantitative and qualitative of fatty acid composition as shown in Table.1. The fatty acid composition is qualitatively similar for several Frankia strains reported previously [4]. These fatty acids (15:0; 14:1 and 18:1) constituted nearly 80% or more of the total fatty acids of all Frankia isolates. Generally, all tested isolates contained saturated, unsaturated and branched fatty acids. Dominant fatty acids detected were Pentacosanoic acid (C25:0) followed by Eicosenoic acid (C20:1). *Casuarina-Frankia* isolates showed quantitatively and qualitatively significant differences.

Considerable and significant changes in levels of fatty acids, under different environmental conditions such as light and dark [8], aerobic and anaerobic [9-10], salinity [11] and with different effluents [12] have already been reported. Environmental and nutritional conditions leading to enhanced production of some of the fatty acids have also

Table 1. analysis of fatty of three different isolates (mg g^{-1} of lipid)

S.No	Name of the fatty acid	SI	SII	SIII
1	Capric acid (C10:0)	1.0836	-	0.8966
2	Lauric acid (C12:0)	-	0.3088	-
3	Myristic acid (C14:0)	-	-	0.1135
4	Pentadecanoic acid (C15:0)	0.0153	0.0133	0.0146
5	Palmitic acid (C16:0)	0.0607	-	0.5428
6	Heptadecanoic acid (C17:0)	0.1224	0.1881	0.1933
7	Heneicosanoic acid (C21:0)	3.4379	-	0.2476
8	Behenic acid (C22:0)	-	0.0049	0.4194
9	Pentacosanoic acid (C25:0)	3.4768	5.8725	-
10	Myristoleic acid (C14:1)	0.0131	0.1152	0.0429
11	Palmitioleic acid (C16:1)	-	0.5626	0.2020
12	Elaidic acid (C18:1 trans)	0.4829	0.0058	0.1222
13	Oleic acid (C18:1 Cis)	-	0.0064	0.4198
14	Linolenic acid (C18:3 Cis)	0.02849	-	0.6811
15	Eicosenoic acid (C20:1)	3.4407	0.1722	1.9063
16	Eicosadienoic acid (C20:2)	-	0.7249	-
17	Arachidonic acid (C20:4)	0.0114	0.0019	0.0196
18	Docosahexaenoic acid (C22:6)	-	1.7117	0.7259
19	Nervonic acid (C24:1)	0.2730	8.2659	-

(-) Not detected.

been reported in microalgae [13]. Advances in gas chromatography technology now give new power to the analysis of fatty acids, on its application to microbial classification. Moreover, the profile of fatty acids appears to be quite conserved at the genus-level [14-15]. Culturing of *Frankia* under standardized conditions followed by extraction of their fatty acids and gas chromatographic analysis provides data for identification and characterization between *Frankia* strains. However, it could be concluded that the analysis of total cellular fatty acids can be useful and rapid technique in the characterization among closely related *Frankia* strains.

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