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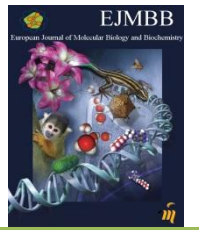

MORPHOLOGICAL AND BIOCHEMICAL CHRACTERIZATION OF THREE DIFFERENT FRANKIA ISOLATED FROM *Casuarina equisetifolia*

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ABSTRACT
The plants nodulated by *Frankia* strains are known as actinorhizal plants. Non-legume nitrogen-fixing shrubs are frequently among the first colonizers on disturbed forest and range sites especially when nitrogen availability is one of the principal limiting factors. Nodulation of these species is initiated through root infection by *Frankia* species. Nodule formation, abundance, and functionality on individual plants is strongly influenced by soil moisture, soil nutrient balance, and age and health of the individual shrub, thus the character of nitrogen-fixation as an ecological process across a shrub population, and through time, may be tremendously varied. We determined the morphological and Biochemical characteristics on three different *Frankia* isolates namely SI, SII and SIII were isolated from nodules of of *Casuarina* plants were collected from three different sites of Nagappattinam districts, Tamilnadu.

INTRODUCTION

The symbiotic nitrogen-fixing actinomycetes of the genus Frankia have been problematic to study because they are difficult to isolate and slow-growing in pure culture .Actinorhizal plants are defined by their ability to form N2- fixing root nodule symbioses with actinomycetes from the genus Frankia . The symbiosis uncouples the plants from a need for soil nitrogen. As a result, the plants and their infective symbionts have radiated into a remarkable variety of niches that include dry tropical soils, temperate wetlands, northern forests, sand dunes, chaparral and matorral, subarctic bogs and tundra, and glacial til. 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.

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These associations cater to the nutritional needs of the biosphere and are responsible for generating almost 50% of the fixed nitrogen annually. The Actinomycete genus *Frankia* belongs to the recently emended 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.*

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 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].

Effects of environmental conditions, plant species and isolates of *Frankia* on the establishment of the symbiosis are relatively easy to assess under laboratory conditions and thus a considerable amount of information is available on isolates of *Frankia* and on their interaction with host plant species. Quantitative analyses of specific *Frankia* populations originating from soil and their interaction with plants and site conditions, however, are methodologically extremely challenging due to problems encountered with the isolation and identification of *Frankia* strains [5].This study aims to characterize 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.

Characterization of isolates

Preliminary characterization was performed using morphological and cultural characteristics. The cultural of the diagnostic sugar *2-0*-mehtyl-mannose and (iii) the features were observed on a number of standards media after 7 days incubation at 28 °C. Morphological identification of the isolates was done under the dissecting and compound microscope to observe colony and growth characteristics. Further characterization was done through biochemical to support the findings of the morphological characterization. Various biochemical methods as given by Bergey's Manual of Determinative Bacteriology [6]. Carbohydrate fermentation was carried according to the method of Harold and Williams[7-8]

RESULTS AND DISCUSSION

Morphological studies of the isolates were done using the dissecting (x160) and inverted microscopes (x1000). All the eleven isolates were Gram positive and grew well on differential agar media. Growth on the media was moderate to abundant for most of the three. All the isolates displayed a branched network of mycelia. Morphological studies under both dissecting and compound microscopes showed that all the isolates form various growth characteristics on the culture media. The isolates exhibited a range of chemotaxonomic and phenotypic properties typical of members of the genus *Frankia*. They formed an extensively branched substrate mycelium, aerial hyphae which carried smooth-surfaced spores in spiral spore chains (Table 1). These results were in line with previous studies by [9] which indicated that actinomycetes have been isolated from reserved areas in Pakistan. The isolates were taken through a series of biochemical tests to determine their characteristics. None of the isolates liquefied gelatin but all of them was positive in all the tests tested except indole, VP and nitrate reduction (Table 1). They grew well on minimal media, utilizing arginine as a nitrogen source and glycerol as a carbon source. They were also found to hydrolyze starch, a characteristic that may confirm their role in the decomposition of organic matter in the habitats [10]. Based on previous results [11-13] the isolates described in this study may also have cellulolytic and lignin solubilizing activities but more physiological studies should be included in the tests to give more insight into their roles in the natural habitats.

Table 1. Morphological characteristics of the isolates as observed under dissecting microscope (x160 and enlarged two-fold) and compound microscope (x1000 andenlarged two-fold).

Isolate	Characteristics			
	Colony color	Growth form	Pigment color	Spore forms
SI	White	Abundant, tough, leathery and round	Yellow pigment	Branched network on either side of aerial mycelia (Spiral)
SII	Red	Abundant, round and wrinkled with raised margins	Red pigment	Spiral at theterminal of aerial mycelia(Spiral)
SIII	Red	Abundant round, smooth and rhizoid	Red pigment	Spiral at theterminal of aerial mycelia(Spiral)

Table.2. Biochemical characterization of three different isolates

S.NO	Biochemical Tests	Isolates		
		SI	SII	SIII
1	Indole	-	-	-
2	Methyl red	+	+	+
3	Voges-Proskauer	-	-	-
4	Citrate utilization	+	+	+
5	Triple sugar iron	+	+	+
6	Starch hydrolysis	+	+	+
7	Urea hydrolysis	+	+	+
8	H_2S Production	+	+	+
9	Nitrate reduction	-	-	-
10	Cytochrome oxidase	+	+	+
11	Catalase	+	+	+
12	**Carbohydrate utilization**			
	Adonitol	+	+	+
	Arabinose	+	+	+
	Cellobiose	+	+	+
	Dextrose	+	+	+
	Dulcitol	+	+	+
	Fructose	+	+	+
	Galactose	+	+	+
	Inositol	+	+	+
	Insulin	+	+	+
	Lactose	-	-	-
	Maltose	+	+	+
	Mannitol	-	-	-
	Mannose	-	-	-
	Melibiose	+	+	+
	Raffinose	+	+	+
	Rhamnose	+	+	+
	Salicin	-	-	-
	Sorbitol	-	-	-
	Sucrose	+	+	+
	Trehalose	+	+	+
	Xylose	+	+	+

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