

Isolation and Characterization of Microorganisms from Rhizosphere Soil and Root Samples of *Melia dubia* trees

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Abstract

Melia dubia belongs to the family Meliaceae and commercially known as Malabar Neem and is locally called as Malaivembu. Being an indigenous species it has great potential to meet the demands of pulpwood and other needs. Microorganisms play an important role in restoring the physico – chemical and biological properties of soil. Microorganisms in the rhizosphere zone contribute for the growth and development of trees. Rhizosphere soil samples were collected from phenotypically superior trees when they were in fruiting stage. The soil samples were collected from tree rhizosphere as well as from germinating seeds. Dominant microorganisms of both the soil samples were compared and screened. Rhizosphere soils were dominated by certain groups of bacteria and actinomycetes. It was found to harbor 3 types of bacteria and two different kinds of actinomycetes. In addition to the dominant bacteria and actinomycetes, other beneficial microorganisms such as diazotrophs, phosphate solubilizers and mobilizers associated with rhizosphere were isolated and characterized by conducting growth studies. Studies on cell morphology of the dominant bacterial isolates revealed that one isolate as gram positive coccus and other two as gram negative bacilli. Isolates grow appreciably at pH 7 they utilized Glucose, Sucrose and Lactose as carbon sources and Peptone as nitrogen source. A possibility to design microbial consortia to improve then growth and development of *M. dubia* has been attempted.

Key words: *M. dubia*, diazotrophs, phosphate solubilizers and mobilizers, rhizosphere

Introduction

Melia dubia belongs to the family Meliaceae and commercially known as Malabar Neem and is locally called as Malai Vembu. It is a large deciduous and fast growing tree with wide spreading branches, straight and tall bole. It is indigenous to the Western Ghats of southern India and is common in moist deciduous forests of Kerala (Gamble, 1922). It also occurs in the tropical moist deciduous forests of the Sikkim Himalayas, North Bengal, Upper Assam and the Khasi hills of Orissa. *Melia dubia* grows on a variety of soils; however, it grows well in deep, fertile and sandy loam soils. *Melia dubia* has been screened as one of the best alternate pulpwood species (Bharti, 2006). Being an indigenous species it has great potential to meet the demands of pulpwood and other needs.

Microorganisms play an important role in restoring the physico – chemical and biological properties of soil.

The application of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic microorganisms to seed, soil or composting areas, effectively increase the number of such microorganisms and accelerate certain microbial processes to augment the extent of availability of nutrients in the form which can be assimilated by plants (Somani,1987). Microbial inoculants contain beneficial microbes such as *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Phosphobactrium* and Vesicular Arbuscular Mycorrhizal fungi (VAM). The asymbiotic dinitrogen fixing bacteria *Azospirillum* improve the plant growth and yield with nitrogen fixation and growth promoting substances (Sumner, 1990). Phosphate solubilizing bacteria solubilize insoluble phosphorus by producing organic acids, which are taken up by plants (Rodriguez and Fraga, 1999). Similarly VAM fungi enhance the uptake and translocation of phosphorus and nitrogen from the soil to the roots (George *et al.*, 1995).

Materials and methods

The present study was carried out to find out suitable microbial inoculants to develop microbial consortia for *Melia dubia* inoculants. The experiment was conducted at the Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam which is located at 11°19'N latitude and 77°56'E longitude and at an altitude of 300 m above MSL.

Survey and isolation of microorganisms from phenotypically superior *Melia dubia* trees

Rhizosphere soil has been collected from the phenotypically superior *Melia dubia* trees at Thalamalai, Erode district, Tamil Nadu. From the rhizosphere soil microorganism's viz., *Bacteria*, *Actinomycetes*, *Azospirillum*, *Azotobacter*, *Beijerinckia* and Phosphobacteria are isolated.

One gram of soil sample was taken and serial dilutions were carried out in sterile water. Dilutions viz., 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} were prepared and are used for the isolation of microorganisms. In order to isolate above microorganisms the following growth media has been used. Nutrient Agar medium, Ken Knight's Agar medium, Nitrogen free malic acid medium, *Beijerinckia* medium, Waksman No. 77 medium and Sperber's hydroxy apatite medium (Media composition given in Annexure) were used to isolate bacteria, Actinomycetes, *Azospirillum*, *Beijerinckia*, *Azotobacter* and phosphate solubilizing microorganisms respectively.

Screening and characterization of dominant cultures

Dominant *Bacterial* and *Actinomycetes* are purified and characterized by following methods. Selected cultures were grown in nutrient broth with different pH at room temperature for two to three days and then the growth was observed.

1. Nutrient broth – pH 5.0
2. Nutrient broth – pH 7.0
3. Nutrient broth – pH 8.0

In order to select the optimum temperature for growth of selected isolates, the organisms were incubated at different temperature and its growth was observed after five days.

1. Growth at 4°C temperature.
2. Growth at 30°C temperature.
3. Growth at 45°C temperature.

Another set of experiments were carried out to determine the ability to utilize various carbon sources viz., glucose, sucrose, lactose, succinic acid and tannic acid.

1. Nutrient broth with glucose
2. Nutrient broth with sucrose
3. Nutrient broth with lactose
4. Nutrient broth with succinic acid
5. Nutrient broth with tannic acid

The test tubes were incubated at room temperature and the growth was assessed after five days.

Another set of experiments were carried out to determine the ability to utilize various Nitrogen sources viz., peptone, ammonium sulfate, sodium nitrate and yeast extract.

1. Nutrient broth with Peptone
2. Nutrient broth with Ammonium sulfate
3. Nutrient broth with Sodium Nitrate
4. Nutrient broth with Yeast extract

The test tubes were incubated at room temperature and the growth was assessed after five days.

Result and Discussion

As explained in materials and methods, survey was done in Thalamalai hills of Thalavaadi, Erode district, Tamil Nadu. Rhizosphere soil samples were collected from phenotypically superior trees when they were in fruiting stage. The soil samples were collected from tree rhizosphere as well as from germinating seeds. The samples were brought to the laboratory along with germinating seedlings / wildings and the associated microorganisms

were isolated by standard plate count technique. Dominant microorganisms of both the soil samples were compared and screened for further studies. The results indicated that the rhizosphere soils were dominated by certain groups of bacteria and actinomycetes. It was found to harbor 3 types of bacteria and two different kinds of actinomycetes.

In addition to the dominant bacteria and actinomycetes, other beneficial microorganisms such as diazotrophs, phosphate solubilizers and mobilizers associated with rhizosphere was assessed and used for evaluation studies. The results are given in Table.

Table 1. Isolation of microorganisms from rhizosphere of phenotypically superior *Melia dubia* trees

S.No.	Microorganisms	Population (x X 10 ^x cfu's / g soil)
1	Bacteria	69 x 10 ⁷ cfu's g ⁻¹ soil
2	Fungi	52 x 10 ⁶ cfu's g ⁻¹ soil
3	Actinomycetes	65 x 10 ² cfu's g ⁻¹ soil
4	<i>Azotobacter</i>	78 x 10 ⁴ cfu's g ⁻¹ soil
5	<i>Azospirillum</i>	77 x 10 ⁴ cfu's g ⁻¹ soil
6	<i>Beijerinckia</i>	79 x 10 ⁵ cfu's g ⁻¹ soil
7	Phosphobacteria	27 x 10 ³ cfu's g ⁻¹ soil
8	VAM infection	85 %
9	VAM spore load	29 g ⁻¹ soil

Morphological and physiological characterization of dominant microbial isolates

In order to identify suitable growth condition of bacteria and actinomycetes isolates, microbial growth studies were carried out with different temperatures viz., 4°C, 30°C and 45°C for identifying suitable temperature for growth of microbial isolates. Similarly investigations for suitable pH, carbon sources and nitrogen source were carried out. Based on cell and colony morphological characterization, three strains of bacterial and two strains of actinomycetes isolates were screened and characterized. The results are documented in Table 2 – 7.

Cultural studies were carried out in nutrient broth indicated that the growth of bacterial and actinomycetes isolates was quite high at 30°C and appreciable at 45°C and growth was almost nill at 4°C. Compared to actinomycetes all the three strains of bacterial isolates growth was moderately higher (Table 2). In the same way growth of bacterial isolates was relatively significant at

pH 7 and appreciable growth at pH 8 and no growth was recorded at pH 5 that is acidic condition (Table 3).

Studies were conducted to test the growth performance of different strains of bacterial and actinomycetes isolates with different carbon sources such as glucose, sucrose, tannic acid, succinic acid and lactose. From this experiment it was observed that tannic acid and succinic acid are not suitable sources of carbon for both bacteria and actinomycetes isolates (Table 4).

The same set of experiment was conducted to find out the nitrogen utilizing pattern of isolates. From these investigations, it was observed that peptone and yeast extract were highly suitable sources of nitrogen for both microbial isolates (Table 5).

Studies on cell morphology of the dominant bacterial isolates revealed that one isolate as gram positive coccus and other two as gram negative bacilli. The results are given in Table 6 & 7.

Table 2. Cultural characterization of microbial isolates - Growth at different temperature

S.No.	Microorganisms	4°C	30°C	45°C
1	Bacterial isolates 1	–	+++++	+++
2	Bacterial isolates 2	–	++++	++
3	Bacterial isolates 3	–	+++++	+++
4	Actinomycetes isolates 1	–	+++	+++
5	Actinomycetes isolates 2	–	++++	++

Table 3. Cultural characterization of microbial isolates - Growth at different pH

S. No.	Microorganisms	pH 5	pH 7	pH 8
1	Bacterial isolates 1	–	++++	+++
2	Bacterial isolates 2	–	++++	++
3	Bacterial isolates 3	–	++++	++
4	Actinomycetes isolates 1	–	++++	+++
5	Actinomycetes isolates 2	–	+++	++

Table 4. Cultural characterization of microbial isolates - Growth at different carbon sources

S. No.	Microorganisms	Glucose	Sucrose	Tannic acid	Scenic acid	Lactose
1	Bacterial isolates 1	+++	++	–	–	+
2	Bacterial isolates 2	+++	++	–	–	++
3	Bacterial isolates 3	+++	+++	–	–	+
4	Actinomycetes isolates 1	++	++	–	–	++
5	Actinomycetes isolates 2	+++	+	–	–	+

Table 5. Cultural characterization of microbial isolates - Growth at different nitrogen sources

S. No.	Microorganisms	Peptone	Yeast extract	Sodium Nitrate	Ammonium Sulfate
1	Bacterial isolates 1	++++	+++	+	–
2	Bacterial isolates 2	+++	++	+	–
3	Bacterial isolates 3	+++	+++	+	–
4	Actinomycetes isolates 1	+++	++	+	–
5	Actinomycetes isolates 2	+++	++	+	–

Table 6. Gram staining of different Bacterial isolates

S. No.	Microorganisms	Colour	Gram	shape
1	Bacterial isolates 1	Red	(-)ve	Rod
2	Bacterial isolates 2	Violed	(+)ve	Cocci
3	Bacterial isolates 3	Red	(-)ve	Rod

Table 7. Colony morphology of dominant bacterial and actinomycetes isolates

S. No.	Microorganisms	Colony morphology				
		Size	Shape	Margin	Elevation	Translucency
1	Bacterial isolates 1	++	Irregular	Lobate	Concave	Opaque
2	Bacterial isolates 2	+	Regular	Undulate	Flat	Translucent
3	Bacterial isolates 3	+++	Irregular	Serrate	Flat	Opaque
4	Actinomycetes isolates 1	++	Irregular	Undulate	Convex	Opaque
5	Actinomycetes isolates 2	+	Irregular	Undulate	Raised	Opaque

The role of rhizosphere microorganisms in perennials would be entirely or partly different from the rhizosphere microorganisms of annuals. In fact if the tree ecosystem is undisturbed or natural, then they develop a peculiar rhizosphere ecosystem. The activity of rhizosphere microorganisms in such environments might be mainly on biogeochemical cycling of nutrients. Many observations and rhizosphere soil studies reveal that these microorganisms are as well involved in improving germination of tree seeds of recalcitrance in nature. One such study conducted by Ravi (2010) also indicated the role of dominant rhizosphere microorganisms along with microwave treatment of fruits in eliciting germination of *Melia dubia*.

The benefits of using rhizosphere soil in improving seed germination and subsequent plant growth were observed in different tree species (Durgapal et al., 2002; Bisht et al., 2003; Tamta et al., 2008). These studies indicate that a small volume of rhizosphere soil may act as a consortium of complex rhizosphere microflora required for seed germination and plant growth. The use of this simple technology however needs certain precautions before its implementation on a larger scale. While the rhizosphere soil is known to possess beneficial rhizoflora, it may also harbor some pathogens. Another possible difficulty may occur if the rhizosphere soil contains certain anti germination factors. Presence of such factors, probably of microbial origin has been reported in the case of *Taxus baccata* sub species *wallichiana* (Pandey et al., 2002).

Hence it is imperative to isolate dominant rhizoflora of the same taxa and to study their impact on germination of seeds or rooting of cuttings and further plant growth at nursery level.

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