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Biodiversity of Microorganisms Isolated from Rhizosphere Soils of Pachamalai Hills, Tamilnadu, India

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ABSTRACT

An attempt was made to isolate and to identify the soil microbial populations from the rhizosphere at Pachamalai forest area. This is located in the Eastern Ghats of India. There are 18 species of bacteria, 9 species of fungi and 7 species of actinomycetes were observed and identified in the help of Bergey's manual and manual of soil fungi they have characterized. This is the first report of kind from Pachamalai forest. These microbes might have played very important role in sustaining in forest ecosystem in Pachamalai forest of Eastern Ghats.

Key words: Pachamalai hills, rhizosphere, soil microbes, India

INTRODUCTION

Biodiversity of soil microbes has been regarded as human and vegetation life resource, especially the one connected with biological and environment resources. This is the first report about the rhizosphere microflora of the Pachamalai hills is situated to the north of Turaiyur taluk of Tiruchirappalli districts, at latitudes 11° 09' 00" to 11° 27' 00" N and longitudes 78° 28' 00" to 78° 49' 00" E and occupy an area of about 527.61 sq km. Climate is tropical with temperature ranging between 25 to 30°C and a minimum temperature range of 12 to 18°C and annual rainfall of 800- 900 mm in the altitude of 1015 MSL. It has dry mixed deciduous forests. The area is marked by the presence of crystalline rocks of the Archaean age comprising gneisses, charnockites and granites with little soil cover of red loamy and black. The crystalline terrain exhibits multispectral and poly metamorphic complexity. According to Soosairaj *et al.* (2005) there are three types of sedimentary rocks in Pachamalai hills based on their period of origin (Fig. 1).

Pachamalai is hilly with steep and gradual slopes having various typical soil colors which make it possible for the diversity of microbes, especially in rhizosphere area. Environment is still virgin and has not been touched by the cruelty of chemical fertilizers, pesticides and is an advantage to gives a positive impact to vegetation and indigenous microbes. Especially photosphere area, it is rich in biological activities as microbes feed on the carbon compounds exuded by root. Plants may exude compounds that attack certain species to the rhizosphere that protect the root from diseases (Widawati and Suliasih, 2001).

Soil is a unity of subsistence that includes the varieties of microbes, because microbial community is one of the important components of soil, therefore, the microbial activity and species compositions are generally influenced by the physical characteristic and soil chemical properties, climate and vegetation (Jha *et al.*, 1992).

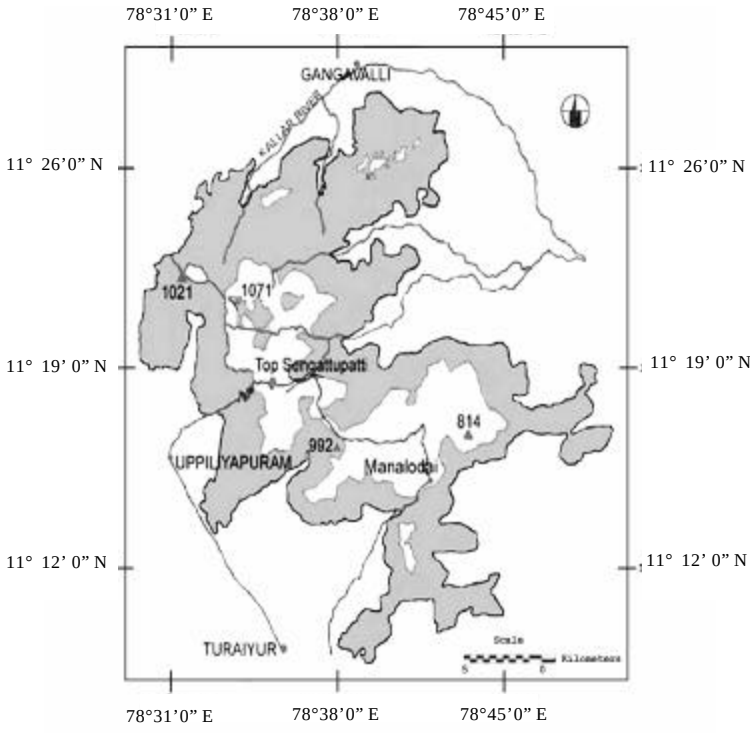

Fig. 1: Location map of the Pachamalai hills, Shaded region indicates protected area and numbers indicate altitude in m (mean sea level)

Soil microbes are one of biota communities, which are very interesting to be studied in order to find out their existence and uses. So, soil microbes have an important role to the subsistence on earth.

Because it has the role on biological and chemical cycling among the flora, fauna and life of microbes itself. Nevertheless, not every soil microbe is suitable and compatible with the habitat and its host and it is well known that they can perform symbiotic and commensalisms. Each type of microbes fills as a unique niche and plays a different role in nutrients cycling and soil structure. Microorganisms living in the soil can be grouped into bacteria, fungi, Actinomycetes, algae and protozoa (Widawati *et al.,* 2004). Some groups of soil microbes are useful as bio-fertilizer and bio-control. They were belong to the genera such as; *Klebsiella, Nitrosomonas, Thiobacillus, Lactobacillus, Azotobacter, Azospirillum, Rhizobium, Bacillus, Pseudomonas* and *Frankia* (one of Actinomycetes group). Another Actinomycetes group is *Streptomyces* which is potential as a source of various bioactive compounds used in pharmaceutical industry, agriculture and for other purpose. *Streptomyces* were found to have high biodiversity and can be used as source of germplasm. This work was also done to find candidate of bio-pesticide from *Streptomyces* that can be applied together with *Rhizobium* and phosphate solubihzing bacteria as biofertilizer. Thus the soil microbes perform a wide range of function in the ecosystem.

The present investigation revealed that biodiversity of soil microbes from rhizosphere at Pachamalai hills area.

MATERIALS AND METHODS

The soil was collected randomly (sampling square method/stratification) from (during November 2009) 12 places from rhizosphere area of Pachamalai Hills of Sengatupatti Reserved Forest (SRF). Soil sample number 1 to 5 collected area was dominated by *Albizia amara* (Roxb.) Boivin. Where as number 6-12 collected areas was dominated by *Commiphora caudate* Engl. and *Drypetes sepiaria* (Wight and Arn.). The soil sample was taken from rhizosphere ranging from 0-15 cm depth. There are many different color types, physical element and soil chemistry. It founded that five type soil samples from Pachamalai Hills of SRF. One kilo gram soil sample from 12 sites at Pachamalai Hills of SRF was kept in black plastic bags (still in fresh condition) and in the Soil Microbiological Laboratory, these samples were air dried before the analysis of physical element and soil chemistry. The fresh soil samples were used for isolation of microbial population. The population of microbes was determined by serial dilution plate count method (Thompson, 1989; Diaz-Ravina *et al*., 1992). Isolation, identification and counting the population of microbes were done by using a selective medium such as nutrient agar, eiosin methylene blue agar, *pseudomonas* isolating agar, luria bertani, rose bengal chloramphenicol agar and *actinomycetes* isolating agar. Identification of soil microbes were estimated by their morphological, physiological, microscopic and biochemical tests with Bergey's Manual.

Isolation, identification and population procedure of bacteria: Ten grams of fresh soil sample was suspended into 90 mL distilled water solution. Mix on wrist action shaker for one hour to provide mechanical desegregation of bacterial cells. Subsequent dilutions were prepared by shaking the suspension for 10 sec to resuspend the soil, manually then, transfer 1 mL of aliquot with a sterile pipette to 9 mL sterile distilled water in a test tube. This suspension was shacked manually for 10 sec and subsequent serial dilutions were prepared above 10^{-1} to 10^{-7}. 0.2 mL of soil suspension from each serial dilution were spread on to selective nutrient agar medium. The number of bacteria colony was estimated after 3-7 days of incubation at 28°C by plate count method. The colonies were picked up and transferred to the same selective medium with 8 strains per petri dish. Different colony strains were transferred to nutrient agar (Oxoid) or Luria Bertani medium (culture collection medium). Eiosin methylene blue agar and *Pseudomonas* isolating agar. The isolated bacterial strains were identified by using their morphological characteristics, cell shapes by gram stain and based on their living cells with standard procedure (Holt, 1994).

Isolation, identification and population procedure of fungi: Ten grams soil samples were suspended in 90 mL of distilled water (in Erlenmeyer glass), then mix by using wrist action shaker for one hour at 120 rpm. The soil extract was diluted from 10^{3} to 10^{5}. 0.2 mL of oil sample suspension from each serial dilution were spread on Rose bengal chloramphenicol agar medium (Oxoid). The cultures were incubated at 28°C for 15 days. The colonies were picked up and the transferred to the same isolation medium. The isolated mycelium was transfered to a drop of lactophenol cotton blue and mounted on a glass slide. Finally, the slide was examined under microscope to identify the fungal strains with the help of Manual of Soil fungi (Gilman, 1957).

Isolation, identification and population procedure of *Actinomycetes*: Soil samples were dried at room temperature for 3 to 5 days. Then they were heated at 90 to 110°C for 10 to 60 min.

The soil samples were spread on the surface of *Actinomycetes* isolating agar medium (Difco). They were incubated at 28°C for 7 to 14 days and then the colonies were transferred to the same agar medium with 8 colonies/Petri dish). The isolates were subcultured to same isolating medium. The isolated Actinomycetes were identified with morphological characteristics i.e., observation of colony (growth, color of aerial, substrate mycelium and diffusible pigment) and microscopic morphologies (spore, sporangium, aerial mycelium and substrate mycelium).

RESULTS AND DISCUSSION

The typical characteristics of soil samples from different sites and microbial population at Pachamalai Hills of Sengatupatti Reserved Forest. such as bacteria, fungi and Actinomycetes were studied and summarized in Table 3-5. In aerobic conditions, bacteria dominated the area and carried out some microbiological activities in the soils. Because fungi and actinomycetes could not grow well without oxygen (Widawati and Suliasih, 2001).

Physico chemical analyses showed that pH range of soil conditions ranging from 6. 2 to 7.8 in Pachamalai Sengatupatti Reserved Forest. The soil textures were clay soil, clay loamy soil and sandy clay. The soil textures were determined depending upon the percentage of sand, dust and clay (Table 1). In the case of sandy clay or dust clay, its particles came together to form an aggregate. The stability of an aggregates depending upon both the content of organic matters in each type of the soil samples (Table 1) and the nature conditions of microbes which tied the soil particles to become one. Soil textures are important for microbes and vegetative population to survive in their habitat. Analysis of soil chemical characteristics is shown in Table 2.

The soil sample No. 2 with Dodona viscosa showed normal pH (7.0) followed by element contents of N, P, K, C and highest amount of C content then other soil samples. This shows that most Pachamalai Sengatupatti Reserved Forest area is fertile.

Table 3-5 illustrate the microbes identified at Pachamalai hills as follows as eighteen bacterias namely *Azotobacter* sp. *Actinotobacter* sp. *Bacillus* sp. *Citrobacter* sp. *Flavobacterium* sp. *Klebsiella* sp. *Nitrosomonas* sp. *Aeromonas* sp. *Alcaligenes* sp. *Micrococcus* sp. *Planococcus citrus, Pseudomonas* sp. *Rhizobium* sp. *Thiobacillus* sp. *Azospirillum* sp. *Escherichia coli, Flavobacterium breve* and *Staphylococcus* sp. nine fungus (*Aspergillus niger, Penicillium* sp. *Aspergillus flavus,*

Table 1: Analysis of soil physical characters from 12 sites in Pachamalai Sengattupatti reserved forest

			Soil textures (%)		
Soil samples	Soil colors	Vegetation	Sand	Clay	Dust
1	Brown reddish	*Albizia amara*	17.23	42.19	40.56
2	Red	*Dodonia viscosa*	11.38	67.51	21.11
3	Black	*Albizia amara*	20.01	37.78	42.21
4	Red	*Albizia amara*	12.78	62.45	24.77
5	Brown reddish	*Chloroxylon swietenia*	16.98	40.24	42.78
6	Dark brown	*Commiphora caudate*	06.89	53.09	40.02
7	Brown	*Drypetes sepiaria*	20.51	19.05	60.44
8	Red	*Commiphora caudate*	13.24	61.75	25.01
9	Brown	*Drypetes sepiaria*	24.62	18.58	56.80
10	Dark brown	*Dodonia viscosa*	08.32	54.81	36.87
11	Brown	*Chloroxylon swietenia*	29.19	15.98	54.83
12	Brown reddish	*Chloroxylon swietenia*	17.65	42.66	39.69

Trichoderma sp. *Mucor, Monilia* sp. *Cephalosporium* sp. *Verticillium* sp. and *Candida* sp.) and seven actinomycetes (*Streptomyces* sp. *Streptosporangium* sp. *Thermomonospora* sp. *Nocardia* sp. *Thermoactinomyces* sp. *Micromonospor* sp. and *Mycobacterium* sp.). Thus, soil acidity, soil fertility, soil textures, vegetation type's elevation of area and soil colors (Table 1), can influence the variety and population of microbes in Rhizosphere. According to Widawati and Suliasih (2001) the number of microbes at halimun mountain was influenced by the different vegetation type, soil pH and the

Table 2: Analysis of soil chemical characteristics from 12 sites of Pachamalai Sengattupatti reserved forest

Soil Samples	N(%)	P(ppm)	K (me 100 g^{-1})	C(%)	C/N	Ca (Me 100 g^{-1})	pH
1	0.28 (m)	3.9 (l)	0.48 (m)	2.54 (m)	14.48 (m)	9.37 (m)	7.4 Alkaline
2	0.25 (m)	4.9 (m)	0.42 (m)	2.21 (m)	11.37 (m)	29.34 (v.h)	7.0 Neutral
3	0.31 (m)	2.6 (l)	0.31 (l)	2.85 (m)	2.54 (l)	8.58 (m)	7.4 Alkaline
4	0.29 (m)	2.5 (l)	0.23 (l)	3.14 (h)	12.80 (m)	9.75 (m)	7.8 Alkaline
5	0.21 (m)	3.8 (l)	0.29 (m)	2.67 (m)	16.74 (h)	8.13 (m)	6.5 Acidic
6	0.30 (m)	2.4 (l)	0.15 (l)	2.33 (m)	13.46 (m)	9.40 (m)	7.2 Alkaline
7	0.25 (m)	3.0 (l)	0.25 (l)	3.54 (h)	12.79 (m)	26.00 (v.h)	6.7 Acidic
8	0.22 (m)	3.5 (l)	0.36 (l)	2.64 (m)	11.31 (m)	9.34 (m)	6.2 Acidic
9	0.31 (m)	4.6 (m)	0.45 (m)	2.61 (m)	10.35 (l)	9.55 (m)	6.9 Acidic
10	0.27 (m)	3.2 (l)	0.18 (m)	0.36 (l)	12.00 (m)	9.04 (m)	7.3 Alkaline
11	0.31 (m)	3.9 (l)	0.15 (l)	2.73 (m)	16.25 (h)	9.31 (m)	7.5 Alkaline
12	0.26 (m)	3.5 (l)	0.49 (m)	2.49 (m)	16.80 (h)	8.03 (m)	6.4 Acidic

Annotation: v.l = very low; l = low; m = moderate; h = high; v.h =very high

Table 3: Identification and population of soil bacteria from 12 sites in Pachamalai Sengattupatti reserved forest

	Number of soil samples											
Microbes identified from soil samples	1	2	3	4	5	6	7	8	9	10	11	12
	Number of bacterias (cell g^{-1} soil)											
Azotobacter sp.	2+	1+	2+	-	-	-	2+	-	1+	-	3+	-
Acinetobacter sp.	1+	2+	-	-	3+	-	1+	2+	-	1+	-	-
Bacillus sp.	2+	2+	3+	2+	1+	2+	2+	2+	3+	2+	3+	3+
Citrobacter sp.	3+	-	-	1+	-	1+	-	2+	-	1+	-	1+
Flavobacterium sp.	1+	-	-	-	2+	-	1+	-	-	1+	-	1+
Klebsiella sp.	-	-	-	1+	-	-	1+	2+	2+	-	-	2+
Nitrosomonas sp.	1+	2+	1+	3+	1+	-	-	2+	2+	-	1+	2+
Aeromonas sp.	2+	1+	1+	-	-	-	1+	1+	-	-	-	2+
Alcaligenes sp.	1+	-	-	2+	2+	1+	-	-	-	1+	-	-
Micrococcus sp.	-	2+	-	2+	-	2+	-	1+	-	-	-	-
Planococcus citrus	2+	-	-	3+	-	2+	-	-	2+	-	-	1+
Pseudomonas sp.	-	-	-	-	-	2+	-	1+	-	-	2+	-
Rhizobium sp.	2+	3+	2+	2+	3+	1+	1+	1+	1+	3+	3+	2+
Thiobacillus sp.	2+	2+	3+	1+	1+	1+	1+	1+	2+	2+	3+	1+
Azospirillum sp.	2+	2+	1+	2+	1+	1+	3+	2+	2+	2+	1+	1+
Escherichia coli	-	-	-	1+	-	-	1+	-	-	-	1+	-
Flavobacterium breve	1+	-	1+	3+	-	2+	-	3+	-	3+	-	2+
Staphylococcus sp.	2+	-	2+	-	-	3+	-	-	1+	-	1+	1+
Total	24	17	16	23	14	18	14	20	16	18	20	18

Annotations: 1+ = low (10^4-10^5), 2+ = moderate (10^6), 3+ = high (10^7-10^8). From 1-5 samples collected area was dominated by *Albizia amara* From 6-12 samples collected area was dominated by *Commiphora caudate* and *Drypetes sepiaria*

elevation of area. The composition of population and soil microbes activity were influenced by the different climate and vegetations (Jha *et al.,* 1992). On the other hand, the activities of microorganisms are constantly changing with temperature, moisture, pH, food supply and other environmental conditions. So, different species prefer different conditions. So, microbes are generally assumed that of the major microbial group's soil, fungi are tolerant of acidity, whereas most bacteria and Actinomycetes are relative in tolerant.

The microbial populations are mostly present in rhizospere soil than non rhizospere soil samples. This can be seen in soil sample No. 7 which contained limestone (Table 3). There might have been no associate connection between microbes and local vegetation that the existence of microbes in the soil is not good. The association occurs between vegetation and microbes, plant root exudates macro and micro element to release it into the soil rhizospere to create a new environment (niche) for the growth of microorganisms (Stafford *et al.,* 2005). More number of microbial populations was present in soil sample area (1, 4, 8 and 11). It was supposed that connection between vegetation types and microbes had taken place. Therefore, each type of microbe filled a special niche and played a different role in the nutrient cycle. Microbes, which were potential as bio-fertilizers were often found in rhizosphere. The following microbes were identified from Pachamalai hills namely, *Azospirillum, Azotobacter, Pseudomonas, Aspergillus* and *Streptomyces* and which potential to become bio-fertilizers or bio-controls. Because Rhizosphere is rich with biological activity as microbes feed on the carbon compounds exuded by root, while organic and inorganic materials released by the plants into the areas (in the form of exudates), will be useful for life continuity of soil microbes (Rosch *et al.*, 2002).

Bacteria: Bacterial strains are the most dominant microorganisms in soil samples. They may cover half of the biomass in the soil. Table 3 revealed that different bacteria population from 12 sites of Pachamalai hills. In acid soil type with low to moderate nutrient content, bacterial populations spread unevenly. In five soil types at 12 rhizosphere, 18 bacterias were identified and two of them were identified at species level. *Azospirillum* sp. *Bacillus* sp. *Pseudomonas* sp. *Rhizobium* sp. *Thiobacillus* sp. *Flavobacterium breve* and *Nitrosomonas* dominated the area. These bacterias are generally found in the soil regardless of the soil condition (Kundu *et al.*, 2009). Widawati *et al.* (2004) proposed that *E. coli* was rarely found in the soil, except as a contaminant or a waste. Present study also proved that *E. coli* was present in the soil samples from three sites.

Bacteria's ability to survive in favorable ecosystem is due to their character to form Spores which have thick strong sheathes to make it easier for them to survive in a savage environment. Bacteria can also stand extreme climate condition although temperature, humidity, pH, agriculture practice, fertilizers, pesticide and the addition of organic matter can influence their population (Widawati *et al.,* 2004). In Pachamalai hills, eighteen bacterial strains were present which uses for plants as biofertilizers to maintain the ecosystems.

Fungi: The number of fungi in the soil is fewer than those of bacteria. All fungi have mycelium thread, which are organized from individual hypha. So, a fungi colony can dominate all soil types (Haili *et al.*, 2008).

There are nine fungal nine genus present in 5 soil types from 12 sites at Pachamalai Sengatupatti Reserved Forest. Among nine genus of fungi, two strains namely *Aspergillus niger* and *Aspergillus flavus* were dominated in several places.

The fungus is one of the most important microbes in the soil ecosystem dynamics, because they function in the decomposition, mineralization and organize the migration of soil elements to plant root (Widawati and Suliash, 2001). The results of fungi population count have the same total

Table 4: Identification and populations of fungal strains from 12 sites at Pachamalai Sengattupatti reserved forest

Soil samples	Vegetation	Fungal strains	Population
1	*Albizia amara*	*Penicillium* sp.	+
2	*Dodonia viscosa*	*Candida*	+
3	*Albizia amara*	*Aspergillus niger*	+
4	*Albizia amara*	*Mucor*	+
5	*Chloroxylon swietenia*	*Monilia* sp.	+
6	*Commiphora caudate*	*Tricoderma*	+
7	*Drypetes sepiaria.*	*Penicillium* sp.	+
8	*Commiphora caudate*	*Aspergillus flavus*	+
9	*Drypetes sepiaria.*	*Cephalospharium* sp.	+
10	*Dodonia viscose*	*Verticillum* sp.	+
11	*Chloroxylon swietenia*	*Mucor*	+
12	*Chloroxylon swietenia*	*Candida*	+

average (no obvious difference) (Table 4). Thus, although a fungi colony is microbes which is more resistant to soil acidity, their live hood still depends on the availability of organic materials and is much influenced by climate, especially soil moisture content (Widawati *et al.*, 2004).

In the acid soil area, dominated by *Myristica cylindrical* plants, eight fungus were found and dominated by *A.niger*. That species and other genus like *Cuninghamella* and *Penicillium* have a wide distribution, especially in the tropic and subtopic areas (Nilima *et al.*, 2007). Buee *et al.* (2007) stated that even though fungi were resistant to soil acidity, also they were not resistant to drought and poor nutrition in the soil. It was the same with Pachamalai Sengatupatti reserved forest soil condition. The soil is so acid with low nutrient contents that the number of fungi found was fewer than those of other microbes like bacterias. According Widawati *et al.* (2004) said that all environment factors which influenced bacteria and actinomycetes migration also influenced the migration of fungi in the soil.

Actinomycetes: Actinomycetes (order Actinomycetales) are a group of prokaryotic organisms belonging to gram-positive bacteria. Many of them show a branched filamentous growth and generally form spores and some actinomycetes form sporangia and zoospores. It mainly inhibits the soil and plays an important ecological role in recycling substances in the natural world (Buee *et al.*, 2007). Seven genus of actinomycetes were identified from 12 soil samples namely, *Mycobacterium, Nocardia, Micromonospora, Thermoactinomyces Streptosporangium, Thermonospora* and *Streptomyces* (Table 5 and 6). Considering actinomycetes in the soil were quite plentiful and surprising that genus variety. It is possible that Pachamalai Sengatupatti reserved forest soil ecosystem is not fitness for actinomycetes, because of the presence of acid pH and low soil nutrient. Most actinomycetes are not tolerant to soil acidity. The deeper the soil, the higher was the percentage of actinomycetes in the total microbes population (Widawati *et al.*, 2004). The increase of the discomposed organic matter would also increase the number of actinomycetes.

The identified actinomycetes were common genus namely *Streptomyces* (almost 70%), *Nocardia* and *Micromonospora* (Table 5), while *Streptomyces* genus was often found in the heap of garbage with the temperatures of 55 to 65°C. *Streptomyces* species are very common in soil samples and responsible for decomposition and degradation of natural and synthetic organics. Sembiring (2003) noted that the genus of *Streptomyces* accommodates an unusually high degree of natural diversity with more than 500 validly described species. Nevertheless, a steady flow of new *Streptomyces* species are being described to accommodate either organisms isolated from diverse habitat.

Table 5: Identification and populations of actinomycetes from 12 sites at pachamalai sengattupatti reserved forest

Soil Samples	Vegetation	Genus	Population
1	*Albizia amara*	*Streptosporangium*	+
2	*Dodonia viscosa*	*Streptomyces*	+
3	*Albizia amara*	*Mycobacterium*	+
4	*Albizia amara*	*Thermoactenomyces*	+
5	*Chloroxylon swietenia*	*Thermomonospora*	+
6	*Commiphora caudate*	*Nocardia*	+
7	*Drypetes sepiaria.*	*Thermomonospora*	+
8	*Commiphora caudate*	*Micromonospora*	+
9	*Drypetes sepiaria.*	*Micromonospora*	+
10	*Dodonia viscosa*	*Thermomonospora*	+
11	*Chloroxylon swietenia*	*Thermoactenomyces*	+
12	*Chloroxylon swietenia*	*Streptosporangium*	+

Table 6: Representative characteristics of actinomycetes

		Morphology				
Genus	Isolates No.	Vegetative cell	Aerialmycelium	Sporangium	Spore	Motility
Thermomonospora	5, 7, 10	Mycelia	+	-	1	-
Thermoactenomyces	4, 11	Mycelia	+	-	1	-
Streptosporangium	1, 12	Mycelia	+	+	Multi	-
Streptomyces	2	Mycelia	+	-	Long	-
Nocardia	6	Mycelia	+	-	-	-
Mycobacterium	3	Rod	-	-	-	-
Micromonospora	8, 9	Mycelia	+	-	-	-

CONCLUSIONS

The result of isolation, identification and population studies of soil microbes in rhizosphere from 12 samples at Pachamalai Sengatupatti Reserved Forest showed that 18 bacterial populations (*Azotobacter* sp. *Accinetobacter* sp. *Bacillus* sp. *Citrobacter* sp. *Flavobacterium* sp. *Klebsiella* sp. *Nitrosomonas* sp. *Aeromonas* sp. *Alcaligenes* sp. *Micrococcus* sp. *Planococcus citrus*, *Pseudomonas* sp. *Rhizobium* sp. *Thiobacillus* sp. *Azospirillum* sp. *Escherichia coli*, *Flavobacterium breve* and *Staphylococcus* sp.) nine fungal populations (*Aspergillus niger*, *Penicillium* sp. *Aspergillus flavus*, *Trichoderma* sp., *Mucor* sp. *Monilia* sp. *Cephalospharium* sp. and *Candida* sp.) and seven actinomycetes populations (*Streptomyces*, *Streptosporangium*, *Thermomonospora*, *Nocardia*, *Thermoactinomyces*, *Micromonospora* and *Mycobacterium).* The population of *Bacillus* (10^8-10^9 CFU mL^{-1}), *Rhizobium* (10^6-10^7 CFU mL^{-1}), *Azospirillum* (10^6-10^7 CFU mL^{-1}) and *Thiobacillus* (10^4-10^9 CFU mL^{-1}) were found in all soil samples.

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