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DIVERSITY AND ANTAGONISTIC ACTIVITY OF ACTINOMYCETE STRAINS FROM MYRISTICA SWAMP SOILS AGAINST HUMAN PATHOGENS

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Abstract

Under the present investigation *Actinomycetes* were isolated from the soils of Myristica swamps of southern Western Ghats and the antagonistic activity against different human bacterial pathogens was evaluated. Results of the present study revealed that *Actinomycetes* population in the soils of Myristica swamp was spatially and seasonally varied. *Actinomycetes* load was varied from 24×10^4 to 71×10^3, from 129×10^3 to 40×10^3 and from 31×10^4 to 84×10^3 in post monsoon, monsoon and pre monsoon respectively. A total of 23 *Actinomycetes* strains belonging to six genera were isolated from swamp soils. Identification of the isolates showed that most of the isolates belonged to the genus *Streptomyces* (11), followed by *Nocardia* (6), *Micromonospora* (3), *Pseudonocardia* (1), *Streptosporangium* (1), and *Nocardiopsis* (1). Antagonistic studies revealed that 91.3% of *Actinomycete* isolates were active against one or more tested pathogens, of that 56.52% exhibited activity against Gram negative and 86.95% showed activity against Gram positive bacteria. 39.13% isolates were active against all the bacterial pathogens selected and its inhibition zone diameter was also high. 69.5% of *Actinomycetes* were exhibited antibacterial activity against *Listeria* followed by *Bacillus cereus* (65.21%), *Staphylococcus* (60.86%), *Vibrio cholera* (52.17%), *Salmonella* (52.17%) and *E. coli* (39.13%). The results indicate that the Myristica swamp soils of Southern Western Ghats might be a remarkable reserve of *Actinomycetes* with potential antagonistic activity.

Key words: Myristica swamp, Soil, *Actinomycetes,* Pathogens, Antibacterial Activity

INTRODUCTION

Actinomycetes are aerobic, gram-positive bacteria. They are one of the main group of soil population and are very widely distributed (Kuster, 1968). *Actinomycetes* have typical biological aspects such as mycelial forms of growth that accumulates in sporulation. Diversity and load of *Actinomycetes* in a particular soil would be very much influenced by soil temperature, soil type, soil pH, organic matter content, vegetation, aeration, moisture content etc. Based on several studies among bacteria, the *Actinomycetes* are striking as antibiotic producers, making three quarters of all known products; the *Streptomyces* are especially productive (Saadoun and Gharaibeh, 2003). Many of our best known and most valuable antibiotics are produced from *Actinomycetes* and these include novobiocin, amphotericin, vancomycin, neomycin, gentamycin, chloramphenicol, tetracycline, erythromycin, nystatin, etc. (Oskay et al. 2004).

According to the World Health Organization (WHO), over-usage and the inappropriate and indiscriminate uses of antibiotics have led to the establishment of antibiotic resistance in various bacterial pathogens. Currently, the drug - resistant strains of pathogen come out more rapidly than the rate of invention of new drugs and antibiotics. Rising numbers of antibiotic unresponsive infectious disease agents tackle patients globally [Levy, 2002; Livermore, 2003]. So, we require to isolating and monitoring more and more *Actinomycetes* from different sources in hope to discover new *Actinomycetes* strains that can create antibiotics that have not been discovered so far and active against drug - resistant pathogens.

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Hence, there is a renewed attention in discovering novel classes of antibiotics that have different mechanisms of action (Spizek and Tichy, 1995; Barsby et al. 2001).

Myristica swamps were first reported by Krishnamoorthy (1960) from the Kerala region of South Western Ghats. These swamps were found in the valleys of Shendurney, Kulathupuzha and Anchal forest ranges in the southern Western Ghats. The distinctive characteristic of the *Myristica* swamps is the abundance of trees belonging to the family Myristicaceae. It has been projected in the previous studies (Chandran et al. 1999; Varghese, 1992) that the Myristica swamps require particular non biotic environment such as flat bottomed or gently sloping valleys in between heavily forested hills of evergreen forests, deep soil in the adjoining hills with rock below which will allow water to be stored above the rock layer, slow seepage of water from the side hills into the valley throughout the year, heavy annual rainfall averaging 3000 mm and temperature ranging from 20 to 30° C. Hence these swamps are highly restricted in distribution. There is no published literature on the diversity and antibiotic potential of *Actinomycetes* in the soils of Myristica Swamps. Therefore the present study is aimed at isolating *Actinomycetes* from the soil samples of Myristica swamps and ascertains their antibacterial properties.

MATERIAL AND METHODS

Locale of the study: Shendurney Wildlife Sanctuary, Southern parts of Western Ghats lies between 8°50'N - 8°55'N latitude and 77°5'E - 77°15'E longitude in Kollam District, Kerala, India. Most of the land in this area is covered by evergreen forest and patches of Myristica swamps and the annual rainfall is 320cm. The mean temperature in summer and winter are 35°C and 16°C respectively. For the present study we selected 4 sites in Myristica Swamps for soil collection.

Sample Collection: Soil samples were collected from prefixed four sites (MS1 – MS4) of Myristica swamp at a depth of 15 to 20cm from the surface. Each of the sampling sites, subsamples of soils were collected from different spot, joint as one and homogenized so as to get representative samples. Sampling was carried out through post-monsoon, pre-monsoon and monsoon seasons. Collection was carried out by a spade that was carefully cleaned and disinfected between sampling so as to avoid cross-contamination.

Isolation, Enumeration and maintenance of Actinomycete: Standard serial dilution plate technique was used for the isolation and enumeration of *Actinomycetes*. Ten grams of soil was transferred to 90ml sterile distilled water and stirred vigorously. Different aqueous dilutions, 10^{-1} to 10^{-5} of the suspensions were prepared and spread plated on Kusters Agar. Nystatin (50 g/ml) or Amphotericin (75 g/ml) and Streptomycin (25 g/ml) were added to the isolation media in order to prevent fungal and bacterial contamination respectively. The plates were incubated at room temperature for 2 to 3 weeks. After incubation *Actinomycetes* colonies were counted and the load of *Actinomycetes* was expressed as number of colony forming units (CFU) per gram of soil. After counting, separate colonies were streaked on to Kusters Agar plates and incubated at room temperature for 4-6 days to obtain pure cultures.

Identification of Actinomycetes: *Actinomycete* isolates which are maintained as pure cultures on Kusters Agar were characterized by morphological tests as per Bergeys Manual of Determinative Bacteriology (Holt et al. 1994) and physiological tests (Gordon, 1967). The morphology of *Actinomycetes* strains was examined using slide culture technique (Holt et al. 1994). Sterile cover slips were placed at an angle in the *Actinomycetes* growth medium (Kusters Agar) and the mycelia adhering to cover slips were transferred to a slide and examined at 40x and 100x magnification using a light microscope (Olympus CH 20i).

Assessment of antagonistic activity: Young cultures of the selected pathogens (*Bacillus cereus, Staphylococcus, Listeria, Vibrio cholera, Salmonella* and *E. coli*) were prepared in nutrient broth. A lawn culture of different pathogens were prepared by swabbing young culture (16-18 h) in glycerol yeast agar and waited for 15 minutes to absorb the culture to the medium. Agar wells (3 mm diameter) were punched in the plates using a sterile gel puncture. Thirty microlitres of a four day old culture of *Actinomycetes* strains in broth was pipetted in to the wells and plates were incubated for 24 h at room temperature. After incubation, zone of inhibition around the wells were recorded in mm.

RESULTS AND DISCUSSION

Spatial and seasonal variation of Actinomycetes population: *Actinomycetes* population in the soils of Myristica swamp showed spatial and seasonal variations. *Actinomycetes* load was 17×10^3 - 40×10^2, 113×10^2 - 30×10^2 and 21×10^3 - 73×10^2 in post monsoon, monsoon and pre monsoon respectively (Table 1 and Fig. 1). Load of *Actinomycetes* population was lesser in monsoon season possibly due to struggle for nutrients with plants. Protozoan predation of microorganisms also decreases the number (Bhatt and Pandya, 2006). *Actinomycetes* population was high in pre monsoon followed by post monsoon and monsoon seasons. Result showed that *Actinomycetes* were mostly vigorous through pre monsoon season due to enhanced soil temperature, which might favour the microbial activity. In our previous studies (Varghese et al. 2012a, 2012b) in shola and grassland soils of tropical montane forest of Southern Western Ghats also reported that the load of *Actinomycetes* was high during pre monsoon season. Spatial variation of *Actinomycetes* in a particular soil was mainly attributed to soil temperature, soil type, soil pH, organic matter content, cultivation, aeration, moisture content etc.

Table 1 Load of *Actinomycetes* population in Myristica swamps soils during the study period

Sampling sites	Post Monsoon	Monsoon	Pre monsoon
MS1	17×10^3	113×10^2	21×10^3
MS2	40×10^2	49×10^2	91×10^2
MS3	42×10^2	30×10^2	73×10^2
MS4	47×10^2	37×10^2	86×10^2

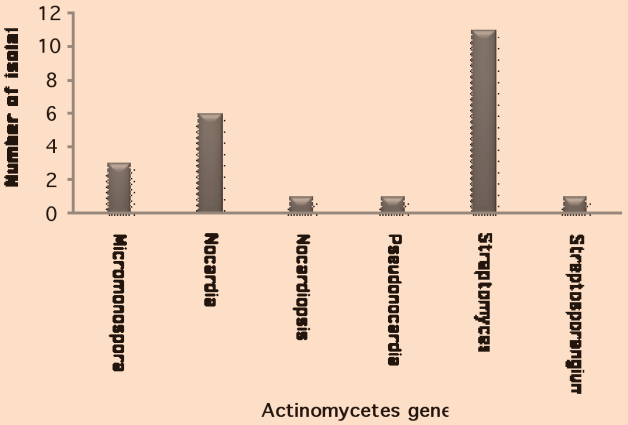

Fig. 1 Generic diversity of *Actinomycetes* in Myristica swamp soils

Diversity of Actinomycetes: Characterization of actinomycetes isolates revealed that out of twenty-three isolates collected, most of the isolates belonged to the genus *Streptomyces* (11), *Nocardia* (6), *Micromonospora* (3), *Pseudonocardia* (1), *Streptosporangium* (1), and *Nocardiopsis* (1), (Fig. 1). In our earlier studies (Varghese et al. 2012a, 2012b) reported most of the genera identified during this study from the shola and grassland soils of southern Western Ghats. Different *Actinomycete* genera were reported from the soils of rainforests of Singapore, among which *Streptomyces*, *Micromonospora*, *Actinoplanes*, *Actinomadura*, *Nonomuria*, *Nocardia* and *Streptosporangium* were the most abundant (Wang et al. 1999). Balagurunathan et al. (1996) reported many of the genera identified during this study from south Indian soil.

Antibacterial potential of Actinomycetes: Antibacterial studies revealed that 91.3% of *Actinomycete* isolates were active against one or more tested pathogens, of that 56.52% exhibited activity against Gram negative and 86.95% showed activity against Gram positive bacteria. 39.13% Isolates were active against all the pathogens selected and its inhibition zone diameter was also high. 69.5% of *Actinomycetes* were exhibited antibacterial activity against *Listeria* followed by *Bacillus cereus* (65.21%), *Staphylococcus* (60.86%), *Vibrio cholera* (52.17%), *Salmonella* (52.17%) and *E. coli* (39.13%). Diameter of inhibition zone (mm) against test microorganisms are presented in Table 2 and the percentage of antibacterial

Table 2 Antibacterial activity exhibited by actinomycete isolates

Isolate No.	Diameter of inhibition zone (mm) against test microorganisms					
	Listeria	*Vibrio cholera*	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*E. coli*
Ms1	21	17	19	24	12	22
Ms2	16	0	11	0	0	0
Ms 3	24	25	21	19	19	32
Ms 4	0	0	13	0	0	0
Ms 5	11	0	0	0	0	0
Ms 6	19	0	16	21	0	0
Ms 7	0	0	0	0	0	0
Ms 8	15	26	0	17	21	0
Ms 9	28	19	23	22	29	28
Ms 10	0	11	0	0	0	0
Ms 11	18	21	22	14	12	21
Ms 12	23	15	18	28	21	27
Ms 13	31	21	29	33	31	21
Ms 14	12	0	0	14	0	0
Ms 15	0	0	0	0	0	0
Ms 16	0	0	0	11	0	0
Ms 17	30	24	32	29	16	0
Ms 18	0	0	11	0	0	0
Ms 19	29	17	24	23	31	23
Ms 20	14	0	0	0	0	0
Ms 21	0	0	13	0	11	0
Ms 22	27	13	26	18	16	17
Ms 23	31	24	28	27	31	20

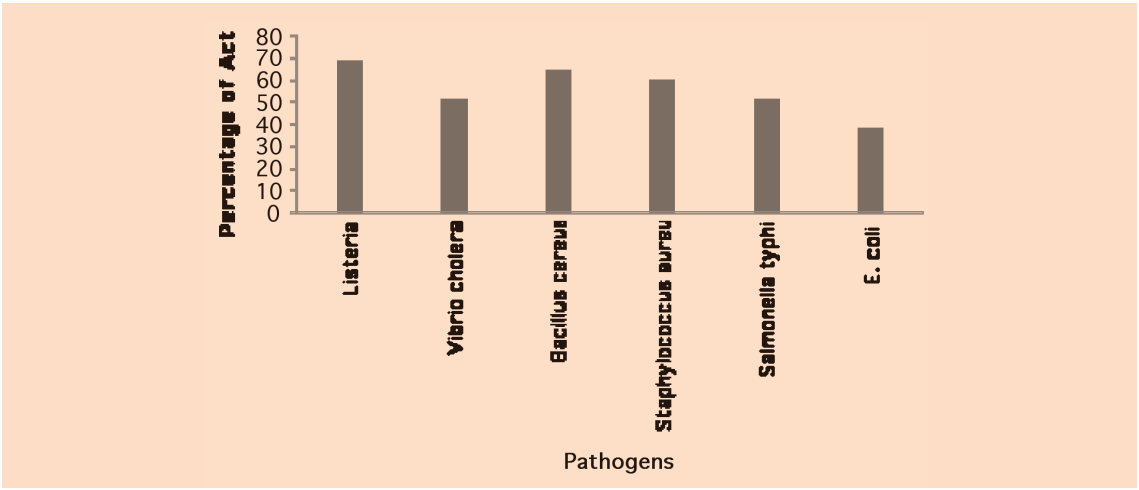

Fig. 2 Percentages of antibacterial activity exhibited by *Actinomycete* isolates from Myristica swamp soils against specific pathogens

activity exhibited by actinomycete isolates are presented in Figure 2. Antibacterial activity studies revealed that majority of the isolates were active against Gram positive bacteria than Gram negative bacteria. In several earlier reports (Sahin, 2002; Thakur et al. 2007; Varghese et al. 2012a; 2012b), high percentage of activity was exhibited against Gram positive bacteria while Gram negative bacteria were less inhibited. The reason for different sensitivity of Gram positive and Gram positive could be ascribed to the morphological differences between these organisms. Gram negative bacteria have an outer polysaccharide membrane carrying the structural lipo polysaccharide components. This makes the cell wall of Gram negative bacteria impermeable to certain solutes, whereas the Gram positive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier. This makes Gram positive bacteria more sensitive to antibiotics produced by *Actinomycetes* (Moncheva et al. 2002). Most of the isolates were also not active against *E. coli*. The present result about *E. coli* is in tune with the previous reports (Anansiriwattana et al. 2006; Oskay et al. 2004).

CONCLUSIONS

Load of Actinomycetes in the Myristica Swamp soils were 17×10^3 - 40×10^2, 113×10^2 - 30×10^2 and 21×10^3 - 73×10^2 in post monsoon, monsoon and pre monsoon respectively. Most of the actinomycetes isolates belonged to the genus *Streptomyces*, *Nocardia*, *Micromonospora*, *Pseudonocardia*, *Streptosporangium*, and *Nocardiopsis*. Antibacterial studies revealed that 91.3% of *Actinomycete* isolates were active against one or more tested pathogens, of that 56.52% exhibited activity against Gram negative and 86.95% showed activity against Gram positive bacteria. Soils of Myristica swamps of southern Western Ghats have excellent diversity of *Actinomycetes* population with potential antagonistic activity against human pathogens. New antibiotics are a thrust area; the extraction of antibacterial substances from *Actinomycetes* of Myristica swamp soils assumes importance.

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