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EXPLORATION OF ANTAGONISTIC ACTINOBACTERIA FROM AMIRTHI FOREST

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ABSTRACT

Actinomycetes are a group of bacteria that find widely in nature. Actinomycetes are a major microbial population in the soil. Aim of this study is to isolate variety of Actinomycetes from the soil of Amirthi forest, Vellore, South India and to evaluate there antagonistic activity. In the current study ten soil samples were collected from different area of the Amirthi forest. Actinomycetes were isolated by serial dilution and plating method on starch casein agar. Total 50 species were isolated on the bases of colony catachrestic on starch casein agar. All the isolates were primary screened for antibacterial activity by cross streak method. Potent strains were further screened for antibacterial activity by agar well diffusion method on MH Agar.

Keywords: Amirthi forest, Actinomycetes

INTRODUCTION

The Actinomycetes are Gram positive, free living, saprophytic bacteria and ubiquitous in nature. Majority of them are found in soil, fresh waters, and surface of water bodies and also in sea water. Odor of freshly turned soil comes from volatile compounds produced by these bacteria. Colonies have pastel colors, soil-like odor, & are hard & stick into agar. In fresh water bodies the odor is also to some extent contributed by Actinomycetes[1].

Actinomycetes population has been identified as one of the major group of soil population, which may vary with the soil type. Soil rich in organic matter is highly suitable for the growth of Actinomycetes. Primary and secondary metabolites produce by these organisms are highly potent and biologically active and remain a powerful source for pharmaceutical discovery. Actinomycetes are the largest antibiotic producing genus in the microbial world discovered so far. Recent reports show that this group of microorganism still remains an important source of antibiotics. As a result of the increasing prevalence of antibiotic resistant pathogens and the pharmacological limitation of antibiotics, there is an exigency for new antimicrobial substances from bacteria and fungi. Most Actinomycetes produce a diverse array of antibiotics including aminoglycosides, anthracyclins, peptides, polyenes, polyether and tetracyclines.

They are producers of approximately two thirds of all bioactive compounds known and they produce a great variety of compounds which have clinical application on the basis of their activity against different kinds of organisms and cells as antibacterial (macrolides, avermectins), ant tumor (anthracyclines, angucyclines, aureole acid group) and also compounds showing immunosuppressant activity (rapamycin, FK506)[2].

Amirthi forest is situated under the Javvadu/Javadi Hills of Tellai across Amirthi River in Vellore, TN, India. It contains a wide variety of flora and fauna. The soil is highly rich in organic matter and suitable for the growth of microorganisms. The aim of this work was to obtain detailed data on the abundance and antimicrobial properties of Actinomycetes isolated from soils of Amirthi forest

MATERIALS AND METHODS

Collection of soil sample

Ten soil samples were collected from Amirthi forest during November 2006 at the depth of 5-15 cm in sterile plastic bags. All samples were transported to VIT University Laboratory, labeled and refrigerated for further investigation. .

Isolation of Actinomycetes

Isolation of actinomycetes was performed by serial dilution and plating technique using starch casein agar medium[3, 4]. One gram of soil sample was taken in 9 ml of distilled water and mixed properly. Serial dilution was made up to 10^{-7}. 1 ml of the diluted sample was inoculates in the starch casein agar medium plates from each dilution. The Petri plates were rotated clockwise and anticlockwise to spread the sample uniformly. Plates were incubated at room temperature (28 to 30ºC) for 1-3 weeks[5].

Test organisms

In vitro antibacterial activities were performed against the *Staphylococcus aureus, Pseudomonas auriginosa, Enterococci, Escherichia coli, Salmonella. typhi, Klebsiella pneumoniae, and Proteus mirabilis.*

Characterization and Identification of Actinomycetes

Microscopic observation

Gram staining, acid fast staining was performed to check the morphology of the cells and spore chain

morphology was identified by cover slip culture technique.

Morphological identification

Isolates of Actinomycetes were observe under a high power magnifying lens and colony morphology was noted with respect to color, aerial mycelium, size and nature of colony, reverse side color and feeling the consistency with a sterile loop.

Biochemical characterization

Actinomycetes isolates were biochemically characterized by Catalase Test, Oxidase Test, Nitrate reduction test, IMVIC test, Carbohydrate Fermentation test.

Fermentation process

Actinomycetes isolates were inoculated in 100 ml starch casein agar medium in a 250 ml capacity Erlenmeyer flask. Flasks were lodged on the flask shaker at a speed of 110 rpm at room temperature for 7 days. After fermentation, the medium was harvested and centrifuged to remove cells and debris. Filtrate is collected in a sterilized screw cap bottle.

Isolation of antibacterial metabolites

Antibacterial compound was recovered from the filtrate by solvent extraction method. The filtrate was mixed with Ethyl acetate, chloroform, Ethanol and Butanol in the ratio of 1:1 (v/v) and shaken vigorously for 1 hour in a solvent extraction funnel. The solvent phase that contains antibacterial compound was separated from the aqueous phase. Solvent phase was used to determine antimicrobial activity.

Determination of the antibacterial activity

Antibacterial activity of the partially purified extract was determined by the agar well diffusion method. Concentration of all test organisms was adjusted at 0.5 Mcfaland turbidity standards and lawns cultured on MHA plates by using sterilize cotton swabs. Plates were bored by using sterilized gel borer and 100 µl of each extract was poured in the wells. Plates were incubated at 37°C for 48 hours.

Table 1: Antibacterial activity of actinobacteria

	ZONE OF INHIBITION (MM)												
Species	**AF1**	**AF2**	**AF3**	**AF4**	**AF5**	**AF6**		**AF7**		**AF8**		**AF9**	
	B	**B**	**B**	**B**	**B**	**B**	**EA**	**B**	**EA**	**B**	**EA**	**B**	**EA**
K. pnemoniae	11	10	11	08	-	18	10	20	8	15	12	18	15
E .Coli	8	10	10	-	-	16	15	18	-	19	-	18	-
P. mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeureginosa	9	8	10	11	-	16	-	15	-	10	-	11	-
S. typhi	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterococci	-	-			13	-	-	-	-	-	-	-	-
S. aureus	8	12	7	6	-	-	-	-	-	-	-	-	-

B= Butanol extract; EA= Ethyl acetate extract: Each value is the mean of three replicate

RESULT AND DISCUSSION

There is no report regarding isolation of Actinomycetes from Amirthy forest. In this present study, Fifty Actinomycetes strains were isolated from Amirthy forest based on the colony morphology on starch casein agar medium. The antagonistic activity of all the isolates was tested by cross streak method, 9 strains show the antibacterial activity (Table 1) against the tested Gram positive and Gram negative Organisms.

All potent strains were mass multiplied in liquid medium and the Ethyl acetate, chloroform, Ethanol and Butanol extracts were prepared. All the extracts were checked for there antibacterial activity by agar well diffusion method. Two strains showed high inhibition against tested Gram negative Organisms (*Staphylococci aureus*, Enterococci, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeuroginosa*, *Proteus mirabilis*.

None of the Amirthi Isolate suppressed *P. mirabilis, Salmonella typhi, Enterococci.* However, they showed a significant activity against Gram negative organisms. The highest inhibition was shown against *E. coli* and in the most of the cases mycelial extracts and the liquid culture filtrates are active.The Actinomycetes exist in various habitats in nature. The terrestrial ones from the soil have been extensively used for the production of secondary metabolites useful to human.

The antibiotic activity spectrum of the nine strains was extended by testing their suppression potential against Gram positive and Gram negative bacteria. The active strains against all the tests were 25.2% of them and only four strains showed higher results. A promising producer of bioactive compounds isolated from a Brazilian tropical soil was tested for its range of antimicrobial activities. Strain 606, classified as Streptomyces sp., and could not be identified up to species level, suggesting a possible new taxon. The supernatant and 10 extracts and fractions, obtained by extraction and chromatographic techniques, presented antimicrobial activity using antibiograms. The methanol fraction was highly active against pathogenic bacteria, phytopathogenic fungi and the human pathogenic yeast *Candida albicans*. It also possessed high antiviral activity inhibiting the propagation of an acyclovir-resistant herpes simplex virus type 1 strain on HEp-2 cells at non-cytotoxic concentration. The strong cytotoxic effect suggests an antitumour action[6].

Table 2: Cultural characters of actinomycete isolates

Isolates	CHARACTERS		
	Aerial mycelium	**Substrate mycelium**	**Pigment**
AF1	Black	Black	-
AF2	White Grey	Brown	-
AF3	Dark Brown	Brown	Yellow
AF4	Black	Black	-
AF5	Brown	Brown	Yellow
AF6	Black	Black	-
AF7	Black	Black	-
AF8	Brown	Brown	Yellow
AF9	White Grey	Brown	-

Table 3: Utilization of carbon sources of actinomycetes isolates

Characteristics	ACTINOMYCETES ISOLATES								
	AF1	**AF2**	**AF3**	**AF4**	**AF5**	**AF6**	**AF7**	**AF8**	**AF9**
Galactose	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	+	+	-	-
Fructose	+	-	+	+	-	+	+	+	+
Dextran	+	+	+	+	-	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+
Adonitol	+	+	+	+	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-	-	-
Iso-Butanol	-	-	-	-	-	-	-	-	-

+ = Presence of growth; - = Absence of growth

The genus of strains with good antagonistic activity against the pathogens was identified using morphological (Table 2&3), biochemical (Table 4) and physiological studies (Table 5) and Bergey's manual of determinative bacteriology[7]. AF1, AF2, AF3 have been identified as *Streptomyces sps.*, AF4, AF5, AF6 have been identified as *Saccharopolyspora sps.* and AF7, AF8, AF9 have been identified as *Saccharomonospora sps.*

Table 4: Biochemical characterization of actinomycetes isolates

Biochemical Test	Actinomycetes isolates								
	AF1	**AF2**	**AF3**	**AF4**	**AF5**	**AF6**	**AF7**	**AF8**	**AF9**
Hydrolysis of casein	-	-	-	-	-	-	-	-	-
Hydrolysis of starch	-	-	-	-	-	-	-	-	-
Hydrolysis of xanthine	+	+	+	+	+	+	+	+	+
Hydrolysis of elastin	+	+	+	+	+	+	+	+	+
Acid from sucrose	-	-	-	-	-	-	-	-	+
Acid from xylose	-	+	-	-	-	-	-	+	+
Nitrate reduction	-	+	+	-	+	-	+	+	+
Indole	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	+	-	-	-	+
VP	-	-	-	-	-	+	-	-	+
Citrate	+	+	+	+	+	+	+	+	+
TSI (Slant)	AK	AC	AK	AK	AC	AK	AK	AK	AC
TSI (Butt)	AC	AC	AK	AC	AC	AC	AC	AC	AC

+ = Positive; - = Negative; AK= Alkaline; AC= acid

Table 5: Physiological characterization of actinomycetes isolates

Growth at	ACTINOMYCETES ISOLATES								
	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF8	AF9
25°C	+	+	+	+	+	+	+	+	+
37°C	-	-	-	+	-	-	-	-	-
1.5% Nacl	+	+	+	+	+	+	+	+	+
3% Nacl	+	+	+	+	-	+	+	+	+
7% Nacl	-	-	-	-	-	-	-	-	-
15% Nacl	-	-	-	-	-	-	-	-	-
20% Nacl	-	-	-	-	-	-	-	-	-

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