Research Article

Isolation and Characterization of Actinomycetes from the Soil of Devathanam – A Foot-hill of Western Ghats

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ABSTRACT

Actinobacteria are well known as secondary metabolites producers and hence of high pharmacological and commercial interest. In this study, soil samples were collected from Devathanam, a small town situated at the foothills of Western Ghats of Virudhunagar District, India and tests were performed to assess the presence of *Actinomycetes* and their morphology and biochemical activities. Nearly fifteen *Actinomycetes* have been isolated from site of Devathanam. Among which only seven designated as AC_1, AC_2, AC_3, AC_4, AC_5, AC_6, AC_7 were subjected to morphological and biochemical characterization for their identity. The morphological characteristics were studied by Gram staining technique. The isolates showed spiral, coiled and rod shaped spores with branched mycelium resembling the genus *Streptomyces*. *Actinomycete* isolates were subjected to the biochemical tests like hydrolysis of starch and casein hydrolysis by the activity of amylase and caseinase. The antibacterial activity of the *Actinomycete*s isolates was studied by giant colony technique and well plate method. Most of the isolates showed strong antibacterial activity against gram positive and negative bacteria. It is concluded that the soil of Devathanam harbors a variety of *actinomycetes*, which can be used up for further investigation.

Keywords: Actinomycetes, antibacterial activity, isolates, resistance and secondary metabolites, pathogens

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INTRODUCTION

Actinomycetes are the most fruitful source of bioactive secondary metabolites. Actinomycetes are not only good producers of bioactive secondary metabolites but are also excellent producers of enzymes such as protease, chitinase and glucose isomerase which are of industrial use. A number of antibiotics and other bioactive metabolites have been isolated from various microbial sources. Actinomycetes are prokaryotes having high G + C content in their DNA with extreme diverse metabolic activities. The metabolic diversity of the Actinomycetes family is due to their extremely large genome having hundreds of transcription factors that control gene expression. [1]. Several ecological studies on soil actinomycetes collected from various habitats like grasslands, beach sands, under-ground caves, rice-paddies, orchards and sub-glacial ice of Antarctica have been carried out so far. Only few reports are available on forest soil actinomycete communities [2]. These ubiquitous organisms are deemed to have a preference over the soil constituents such as humus, litter, dung and even rock surfaces. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10, 000 of these compounds are produced by Actinomycetes, representing 45% of all bioactive microbial metabolites discovered [3]. Within Actinomycetes, Streptomycetes alone produce 7600 compounds. Many of these secondary metabolites are potent antibiotics which has made streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry [4]. Hence the present study is aimed to screen and isolate potent antibiotic producing Actinomycetes from the collected soil samples.

MATERIAL AND METHODS

Site of Sample collection

Soil samples were collected at a depth of 10 –15 centimeters from Devathanam and collected in sterile containers. The maximum temperature range 30^{0}C-33^{0}C and Minimum temperature range 18-27^{0}C. The containers were tightly closed, and stored at room temperature for 2 days [5].

Isolation of Actinomycetes

Numerous media have been used for the isolation of actinomycetes from soil. Starch casein agar medium has been widely used for isolating soil actionmycetes [6]. In conventional dilution plate technique, 10 g of soil samples were suspended in 100 ml of sterile water and 0.5 ml of suspension from this was spread over starch casein agar medium and incubated for 7–9 days at 28^o C. Individual colonies with characteristics of Actinomycetes morphology were isolated and pure culture of the respective isolates was obtained by repeated streaking on SCN agar plates. The pure isolates were transferred to SCN slants and preserved at 4^o C. These isolates were evaluated for their antimicrobial activity.

Gram staining

Gram staining is based on the ability of microorganisms to retain the purple colour of crystal violet during decolorization with alcohol. Gram negative bacteria are decolorized by the alcohol whereas gram positive bacteria were not decolorized. After decolorization, saffranin, a red counterstain, is used to impart a pink colour to the decolorized Gram negative organisms. The morphological observation was compared with Bergey's manual of Determinative Bacteriology [7] and the organism was identified.

Lipid hydrolysis

An inoculum of Actinomycetes was streaked onto Tween 80 agar plates and incubated at room temperature for 4 to 8 days. A opaque zone was formed around the inoculums [8].

Casein hydrolysis

Skim milk agar plates were prepared and sterilized. The plates were inoculated with the Actinomycetes isolates and incubated at 28 ± 2^o C for 7 days. The formation of zone of clearance around the colonies indicated the positive result.

Giant colony technique for selecting antibiotics producing Actinomycetes

Each colony of the *Actinomycetes* isolates was streaked in a narrow band across the center of the nutritious agar plates. Muller Hinton agar plates were used for bacteria. The plates were incubated at room temperature until the growth of *Actinomycetes* [9].

Preparation of inoculums

The bacterial cultures like *B. subtilis*, *P. aeruginosa*, *S. aureus*, *S. typhi* and *E. coli* were inoculated into the nutrient broth and incubated at 37^o C for 24 h. The test organisms were streaked from the edges of the plate to giant colony without touching the colony. Again the Muller Hinton agar plates were incubated at 37^o C for 24 h. After incubation, the length of growth line was measured in millimeter from the edge of the giant colony to the tip of the growth of the test organism.

Submerge culture

After preliminary testing of the isolates for their antimicrobial activity, further studies for the production of antibiotics in liquid medium were carried out. The *Actinomycetes* isolates which were active in the preliminary screening were inoculated in Muller Hinton broth production medium and incubated at 28^o C in an orbital shaker (220 rpm). To monitor the activity an aliquot of culture broth was withdrawn every 24 h for 10 days and the activity was evaluated. The broth cultures were centrifuged at 10.000 rpm for 10 minutes and the supernatant was tested for extracellular antimicrobial activity by standard well diffusion method.

Testing antibacterial spectrum by well plate method

Test microorganisms used were *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhii* and *Escherichia coli*. For evaluation of antimicrobial activity of broth culture, wells of 6 x 4 mm size made by were puncturing fresh test microbial lawn cultures on Muller Hinton agar medium. Actinomycetes were placed in prepared wells of the Muller Hinton agar medium deep inoculated with the test micro organisms. The bacterial plates were incubated at 37^o C for 24 h. The activity was determined by measuring the

diameter of inhibitory zones formed by test micro organisms around the well after incubation [10].

RESULTS

The diluted soil samples were spread on to the plate and incubated. After isolation, the selected isolates were subjected to further analysis. They were labeled as AC_1, AC_2, AC_3, AC_4, AC_5, AC_6 and AC_7. The actinomycetes isolates were subjected to morphological, biochemical and antibacterial activity analyses. Table 1 represents the morphological characteristics of the *Actinomycetes* isolates as studied by colony characterization (colour of the aerial mycelium and substrate mycelium) and Gram staining. AC_1 and AC_4 showed highly coiled spores. AC_2, AC_3, AC_5, AC_6 and AC_7 isolates showed no spiral spores but it appears that very few spores are formed by the breakdown of mycelium Fig. 1.

Table 2: shows biochemical characterization of *Actinomycetes* isolates subjected to lipase activity and casein hydrolysis.

Table 1: Morphological Characters of *Actinomycetes* Isolates

Strain	Aerial mycelium	Substrate mycelium	Gram straining
AC_1	Violet	White	+
AC_2	White	White	+
AC_3	Brown	Brown	+
AC_4	Yellow	White	+
AC_5	Light brown	Light brown	+
AC_6	Orange	White	+
AC_7	Blue	White	+

Table 2: Biochemical Characteristics of *Actinomycetes* Isolates (+ sign indicates Activity)

Parameters	*Actinomycetes* strains						
	AC_1	AC_2	AC_3	AC_4	AC_5	AC_6	AC_7
Lipid hydrolysis	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+

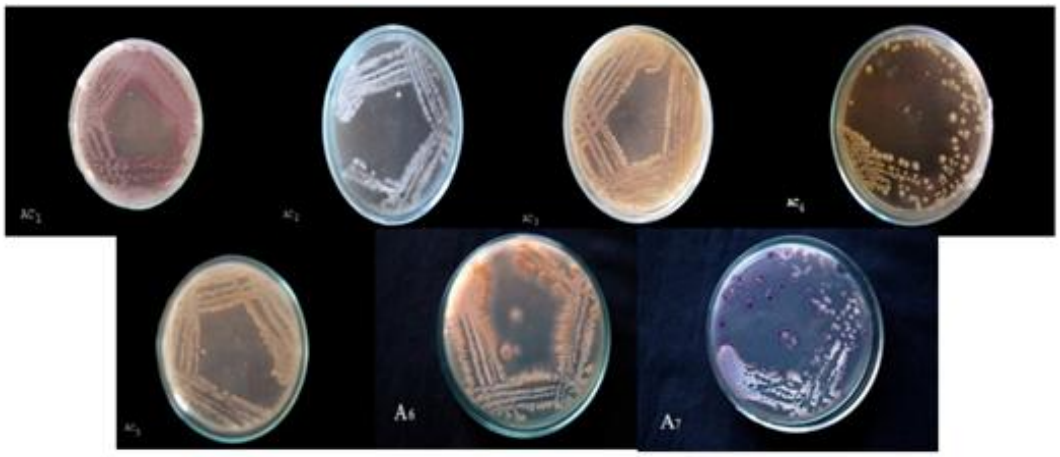

Figure 1: *Actinomycetes* Isolates Grown on Starch Casein Nitrate Agar Medium

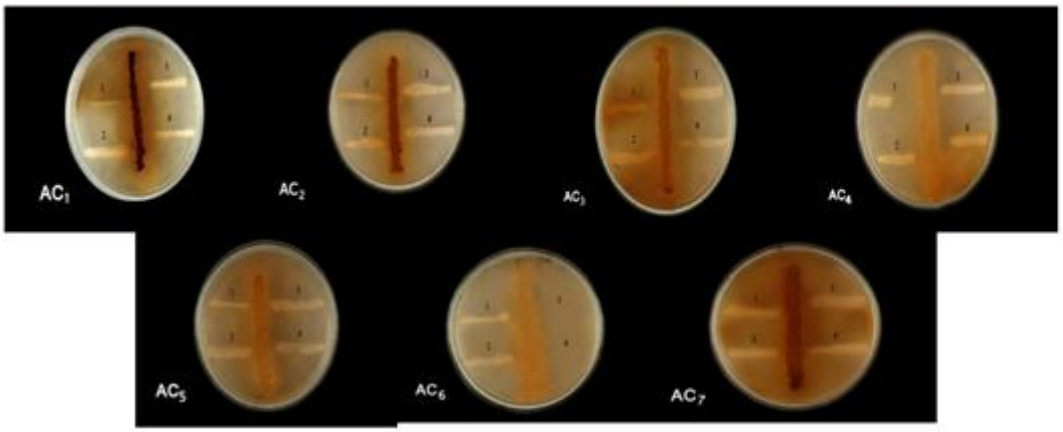

Figure 2: Antibacterial Activity of *Actinomycetes* Isolates by Giant Colony Technique

Table 3: Antibacterial Activity of *Actinomycetes* Isolates by Giant Colony Technique (signs +++ + and - sign indicates moderate, strong and no activity Respectively

Actinomycetes isolates	Test Organisms			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
AC_1	+	+	+	+
AC_2	++	++	++	++
AC_3	++	++	++	++
AC_4	+	++	++	+
AC_5	++	++	++	++
AC_6	++	++	-	-
AC_7	+	+	+	+

The antibacterial activity of *Actinomycetes* isolates determined by giant colony technique is shown in Fig. 2. In giant colony technique, all the isolates showed antibacterial activity except that AC_1, AC_2, AC_3, AC_5 and AC_7 having highest antibacterial activity. Comparatively, *E. coli* was highly sensitive than other bacteria, *Pseudomonas aeruginosa* showed resistance against the *Actinomycetes* tested. The results are tabulated in Table 3.

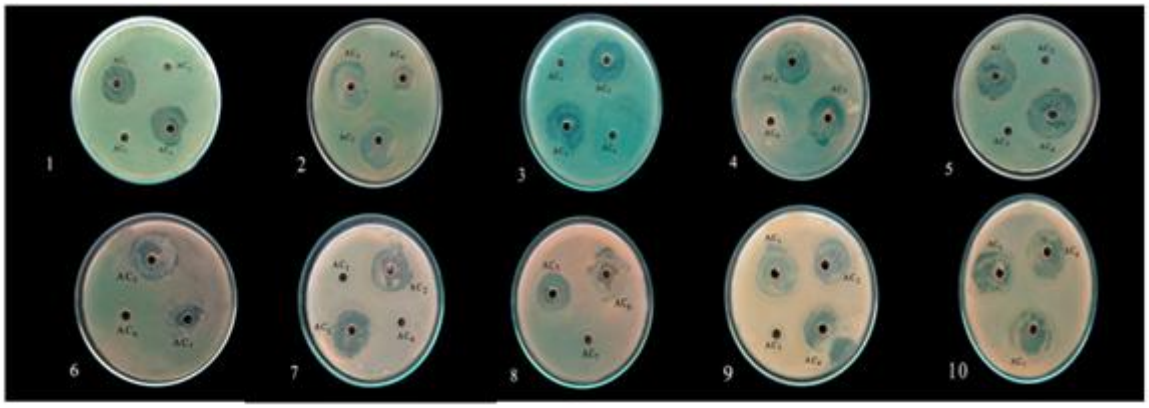

Figure 3: antibacterial activity of *Actinomycetes* isolates against *B. subtilis* (1,2), *S. aureus* (3,4), *E. coli* (5,6), *P. aeruginosa* (7,8), *S. typhi* (9,10)

Table 4: Mycelial extract of Actinomycetes isolates against Test Organisms

Test Organisms	Zone of inhibition(mm)						
	AC_1	AC_2	AC_3	AC_4	AC_5	AC_6	AC_7
B. subtilis	12	Nil	Nil	12	10	Nil	11
S. aureus	Nil	11	9	Nil	12	Nil	11
E. coli	11	Nil	Nil	12	13	Nil	11
P. aeruginosa	Nil	12	11	Nil	10	15	Nil
S. typhi	12	11	Nil	10	10	12	11

DISCUSSION

The present investigation was aimed to isolate *Actinomycetes* from the soil of Devathanam, a small town located the foothills of Western Ghats in Virudhunagar District. The soil contains a diversity community of organisms differentiated by morphology, biochemical, giant colony technique and antibacterial activity. To study the production of secondary metabolites, the isolates of *Actinomycetes* were inoculated into a suitable medium and morphological characters were studied by Gram staining, Acid Fast staining and Coverslip culture technique. Most of the isolates showed good sporulation, with compact chalky like dries colonies of different colony variation and spore arrangements relevant to that of *Streptomyces* [11]. The biochemical studies indicate that all *Actinomycetes*, isolates can be used a potent source for the enzymes like amylase, lipase, caseinase and gelatinase. The enzymes are very much useful for their saprophytic mode of life [12].

Most of the isolates were efficient in hydrolyzing starch, casein, lipid and gelatin etc. The production of H_2S, Urea, acid, and acetone were strictly negative [13]. The Giant colony technique was used to screen the antibiotic producing *Actinomycetes* using Muller Hinton agar medium. Antibacterial activity was exhibited by 80% of the isolates. The lowest activity was exhibited by Gram positive bacteria (40%). The putative isolates of primary screening when subjected to secondary screening showed different activity. Some showed the activity in the secondary screening while some showed less inhibition. The results of primary screening and secondary screening reveals that most of the active isolates were active against *E. coli*, *P. aeruginosa*, and *S. typhi* than Gram positive bacteria. The reason for such differential sensitivity could be ascribed to the morphological differences in these microorganisms; According to [14] the Gram positive could be more susceptible as they have only an outer peptidoglycan layer which is not an effective permeability barrier.

The antibacterial activities were studied by well plate method. All the *Actinomycetes* isolates showed antibacterial activity against five pathogenic organisms. The extract also showed strong activity against *E. coli* (13 mm), *B. subtilis* (12 mm), *Salmonella typhi* (12 mm) and moderate activity against other tested bacterial stain [15]. In our study the *Actinomycetes* were isolated and identified by their morphological and biochemical characteristics. Morphological examination of the seven isolates clearly indicates that they belong to Streptomycetes genera and Streptomycetaceae family (Spore chains with coiling and branching).

CONCLUSION

The present investigation is aimed to isolate *Actinomycetes* from the soil of Devathanam, a small town located the foothills of Western Ghats in Virudhunagar District and screened for the presence of *Actinomycetes*. The presence of seven *Actinomycetes* was confirmed by morphological, biochemical and antibacterial activity. Biochemical characterization of *Actinomycetes* isolates were subjected to lipase activity and casein hydrolysis. By Giant colony techniques, antibacterial activity was investigated. *Actinomycetes* isolate AC_1, AC_2, AC_3, AC_5 and AC_7 showed highest antibacterial activity. Among the tested microorganisms, *E. coli* was highly sensitive than other bacteria,

and *P. aeruginosa* showed resistance to *Actinomycetes* extract.

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