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ANTIMICROBIAL ACTIVITY OF SELECTED ACTINOMYCETES FROM SOIL SAMPLES

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ABSTRACT

This work reporting isolation and identification of high antimicrobial activity producing actinomycetes strains from soil, against the common human pathogens like *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeroginosa, Candida albicans, Actinomycetes* strains were isolated from 18 different soil samples of Palakkad and Coimbatore. Out of 65 isolated actinomycetes species, 26 of them showed antagonistic activity against Gram positive, Gram negative bacteria and yeast. Of these 15 isolates were found to exhibit antimicrobial activity in secondary screening. According to antimicrobial activity and spectrum of broadness, 3 of the isolates were selected and characterized by colony morphology, cover slip culture technique and Grams reaction. Hence, the results indicate that the study on these actinomycetes can be useful in the development of new substance for pharmaceutical purpose.

Key words : Soil samples, Actinomycetes, Antimicrobial activity, Screening, Antibiotics,

INTRODUCTION

The demand for new antibiotics continues to be of extreme importance in research because of the rapid spread of antibiotic resistant pathogens causing life-threating infections. The history of new drug discovery processs shows that novel skeletons have in the majority of cases come from natural sources (Bevan *et al* 1995). This involves the screening of microorganisms and plant extracts (Shadomy 1987). The majority of antibiotics in use today were discovered in the 1950's, 1000 antibiotics are known today and most of them (58%) are produced by Actinomycetales especially the genus *Streptomyces* (Edward 1980). The exponential emergence of microorganisms becoming resistant to the clinically available antibiotics already marketed, the need for discovering novel drugs is real. For example, the occurrence of methicillin-resistant *S. aureus* in hospitals has risen from less than 3% in the early 1980s to as much as 40% now. It has

been reported that Coagulase Negative *Staphylococcus* sp. (CoNS) are becoming increasingly important in nosocominal infections and that they may cause serious infections (Kloos *et al* 1994). Therapeutic options for CoNS infections caused by methicillinoxacillin-resistant strains are limited to vancomycin-based regimens. However, vancomycin therapy of Staphylococcal infections has been associated with a slow and inadequate response in many instances (Levine *et al* 1991). Effective treatment of the infections caused by these organisms is yet to be established. Thus, the need for the discovery and development of new and effective antibiotics is a priority.

Actinomycetes hold a prominent position as targets in screening programme due to their diversity and their proven ability to produce novel antibiotic and other non antibiotic lead molecules of pharmaceutical interest. Since the discovery of actinomycin, the first antibiotic from an actinomycete, many commercially important bioactive compounds and antitumour agents have been produced using actinomycetes (Tanaka & Omura 1990). The objective of the present study was to isolate and identify high antimicrobial activity producing actinomycetes from soil.

MATERIALS AND METHODS

Isolation of Actinomycetes: Eighteen different soil samples were collected from various places in and around the areas of Palakkad and Coimbatore. The collected sample were weighed as 1 gram and mixed with sterile 100 ml distilled water. The mixture was serially diluted up to 10^{-8} dilution. From each dilution 0.1 ml was taken and inoculated onto sterile starch casein nitrate agar (SCN) medium (pH-7.0) using L-rod method (Kannan 2002). The inoculated plates were incubated at 27°C for 4 days. Individual colonies with characteristics of Actinomycetes morphology were isolated and pure cultures of respective isolates were maintained on SCN plates and preserved at 4°C.

Test microorgainsms: The following test microorganisms procured from microbial Type Culture Collection and Gene bank (IMTECH, Chandigarh,India) were used during the investigation: *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 739), *Pseudomonas aeroginosa* (MTCC 2453), *Klebsiella pneumonia* (MTCC 4030) and *Candida albicans* (MTCC 227). The fungi were grown at 28°C on potato dextrose agar medium and the bacterial cultures were grown at 37°C on Nutrient agar medium. All the cultures were stored at 4°C and sub-cultured as needed.

Spot inoculation on Agar medium: The actinomycete isolates were spot inoculated on Muller Hinton Agar. (Shomurat *et al* 1979). The plates were incubated at 28°C for 5 days. Colonies were then covered with a 0.6 % agar layer of potato dextrose medium (for fungi) and nutrient agar medium (for bacteria) previously seeded with the test microorganisms. The bacterial plates were incubated at 37°C for 24 hours and fungal plates were incubated at 28°C for 5 days. The zone of inhibition of the test microorganisms was observed after incubation.

Submerge culture: After preliminary testing of the isolates for their antimicrobial potentiality, further studies for the production of antibiotics in liquid medium was carried out using shake flask. The actinomycetes isolates found to be active in the preliminary screening were inoculated into the flask containing SS broth as the production medium and were incubated at 28°C in an orbital shaker(220 rpm) for 7 days. The broth was centrifuged at 10,000 rpm for 10 minutes and the supernatant was tested for extracellular antimicrobial activity by standard well diffusion method (Gramer 1976).

Well diffusion method: The secondary screening was determined by agar well diffusion method (Gramer 1976). By using a sterile cork borer, wells (6 mm) were punctured in fresh test microbial lawn cultures (0.5 Mc Farland turbidity standards) on Muller Hinton Agar plates. Then 100 µl of the supernatant culture broths were administered in each well. The plates were incubated at 37°C for 24 hours and fungal plates were incubated at 28°C for 5 days. Bioactivity was determined by measuring the diameter of inhibitory zones (mm) of test organisms around the well after incubation.

Characterization of the active isolates: The active isolates selected from secondary screening were characterized morphologically to the genus level by both macroscopic and microscopic methods. The microscopic characterization was done by cover slip culture technique. (Kawato & Sinobul 1979).

RESULTS AND DISCUSSION

From 18 different soil samples, 65 actinomycetes were isolated and these actinomycetes, subjected for primary screening process, only 26 isolates showed the activity against test organisms. Out of 26 isolates that were subjected to submerge culture, 15 isolates were found to exhibit antimicrobial activity, while other 11 isolates did not exhibit activity in broth culture. The diameter of zone of inhibition of the respective test microorganisms showed by the culture broth of 15 active isolates is represented in Table 1.

Of these, 3 isolates found to exhibit a broad spectrum of activity against most of the tested microorganisms. According to Bushell (1993), during the screening of the novel secondary metabolite, actinomycetes isolates which show antibiotic activity on agar but not in liquid culture. The results of primary and secondary screening revels that most of the active isolates were active against Gram positive and Gram negative bacteria. This is because of the morphological differences between these microorganisms and Gram negative bacteria having an outer polysaccharide membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, the Gram positive should more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer & Gerhardt 1971)

With reference to the results obtained, 3 active isolates were identified up to the genus level and found to be *Streptomyces* sp based on macroscopic and microscopic methods (Table 2). According to Kutzner (1972) for proper

identification of genera and species of actinomycetes, besides morphological properties, other physiological and biochemical properties such as cell wall chemotype, whole- cell sugar pattern, peptidoglycan type, phopholipids type and G+C % of DNA should be determined.

Table 1 Antimicrobial activities of active isolates (MM)

Isolate no	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Pseudomonas aeroginosa*	*Candida albicans*
A1	-	12	-	9	30
A5	-	-	11	-	24
A6b	-	-	14	-	29
A7c	-	14	-	-	31
A7f	11	-	28	7	31
S8e	-	11	-	5	-
S9a	22	11	20	-	34
S9b	-	-	14	-	-
S12c	26	24	30	-	32
S13	-	-	-	3	-
S15b	-	-	-	-	16
S16a	26	18	32	-	30
SFe	16	-	-	-	-
S17f	-	-	-	-	18
S18d	16	18	-	-	-

Table 2 Morphological characteristics of selected active isolates from secondary screening

Morphological feature	Isolate: A7f	Isolate: S12c	Isolate: S16a
Colony morphology	Smooth	Rough	Smooth
Gram nature	Gram positive	Gram positive	Gram positive
Colour of aerial mycelia	Pink	White	White
Spore chain	Irregularly branched	Branched mycelium	Branched mycelium
Reverse side colour	White	Yellow	Yellow to white

The emergence and dissemination of antibacterial resistance is well documented as a serious problem world wide (Cohen 2000; Gold & Moellenng 1996; WHO 2001). The perspective of rapid emergence of drug resistance among bacterial pathogens shows that the potencies of prevalent antibiotics are deceasing steadily. The search for novel metabolites especially from actinomycetes requires a large number of isolates in order to discover a novel

compound of pharmaceutical interest. The soil has to be further explored to get a new antibiotic against emerging drug resistant pathogens.

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