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Antimicrobial Activity of Marine Actinomycetes against Human Pathogenic Bacteria.

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Abstract

A total of 114 actinomycetes strains were isolated from the coastal region of Chennai beach and were identified by morphological studies. Actinomycetes were further screened and strains were selected based on the antimicrobial activity. The Cross-streak method was used to check the antimicrobial activity of isolated actinomycetes against test organisms and the crude extraction was used for the production of antibiotics from the isolates. Agar well diffusion was used for antimicrobial activity of the crude extracts against test organisms. Twenty two actinomycetes strains showed antimicrobial activity during primary screening and these isolates were further screened for their secondary metabolites activity on three human bacteria which were *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The Actinomycete strain MB41 was found to be more active against the test pathogenic bacteria.

Keywords: Actinomycetes, Human Pathogenic Bacteria, Antimicrobial activity,

INTRODUCTION

Actinomycetes are the prokaryotes of the gram-positive bacteria but they are distinguished from other bacteria by their morphology. Actinomycetes are widely distributed in natural and man-made environments and are of universal occurrence in nature. They are found in large numbers of soils, fresh wastes, lake, river bottoms, manures, composts, and dust as well as on plant residues and food products. However, the diversity and distribution of actinomycetes that produce secondary metabolites can be determined by different physical, chemical and geographical factors [**1**, **2**]**.** They have different biological activities such as antibacterial, antifungal, antiparasitic, antitumor, anticancer and immune suppressive actions **[3, 4&5].** Microbial bioactive molecules are the source for the development of new medicines. Bio-resources of the ocean have exhibited microbial bioactive molecules over many decades to safeguard of our lives. The bioactive molecules have wide applications in agriculture, veterinary and pharmaceutical Industry. According to the World Health Organization, improper use and consumption of over the counter antibiotics and has led to resistance of many pathogens. Clinically important bacteria such as *Staphylococcus aureus* are becoming resistant to commonly used antibiotics. In recent times new resistant strains emerge quickly while the rate of discovery of new antibiotics is slowing down. Hence many scientists are focusing on screening programs of microorganisms, primarily actinomycetes for their production if antibiotics [**6**]. Researchers are finding new antibiotics and increasing productivity of such agents has gained importance **[7]** as some important drugs are expensive and/or have the side effect to the host, some microbes have no successful antibiotics and others are developing multidrug resistance. This situation requires finding solutions and one solution could be searching and producing new and effective antibiotics from microbes such as actinomycetes. There is no scientific report on antibiotic producing actinomycetes from soil samples collected in Chennai coastal region. Therefore the objective of the present study was to Isolate and screen antibiotic producing actinomycetes from the soil sample in Chennai coastal region. To outcome of this finding may be Important to give direction to researchers and for future treatment of multi-resistant human pathogens.

MATERIALS AND METHODS

Reagents and Chemicals

All the chemical and culture media used in the present study are of AR grade. Starch, Casein, and agar were purchased from Hi-Media Ltd, Mumbai, India. Buffer salts like Tris, ammonium sulfate, calcium carbonate, ferrous sulfate, potassium nitrate, potassium hydrogen phosphate, magnesium sulfate and sodium chloride were purchased from Merck India Limited, Mumbai, India. Corning® 96 Well Clear Polystyrene Microplate was purchased from Corning Incorporated life Sciences, Acton, MA, USA.

Sample collection

The marine soils were collected from Chennai coastal region namely from Marina Beach, Elliot's Beach, and Neelankarai beach. The samples were collected using alcohol rinsed person grab and were transferred to new zip lock bags using a sterile spatula **[8, 9]** and the samples were transported to the laboratory for the isolation of actinomycetes.

Isolation of actinomycetes enrichment

One gram of soil sample was serially diluted in sterile distilled water. One ml of each aliquot was spread on starch casein agar (SCA) medium and used for the isolation of actinomycetes. The antibiotics such as nalidixic acid and nystatin were added into the medium, in order to inhibit bacteria and fungi, respectively. The plates were incubated at 28˚C for 6 to 7 days **[10, 11&9]**. The isolated agar plates were observed for the presence of actinomycete colonies from 3rd day onwards. Single separated colonies were selected and the subculture was maintained in SCA slants at 4˚C for further use.

Identification of marine actinomycetes by cover slip method

The isolated strains were confirmed as actinomycetes by studying their morphology under the microscope. The SCA was poured on sterile slides and allowed for solidifying. Then the organisms were streaked on it and incubated at 37˚C for 48 hrs. After incubation, the coverslip was carefully removed with respect to its orientation and placed upwards on a slide. About 2 drops of methylene blue dye were added and were allowed to stand for a minute after which the colony color of actinomycete strains were isolated and categorized into light whitish, grayish, light-dark gray brownish colours. These isolates were identified and covered with the cover slip and the morphology was observed under the microscope.

Microscopic Examination

The following microscopic observations were recorded using cover slip culture.

- Presence or absence of substrate mycelium
- Fragmentation of substrate mycelium
- Presence of sclerotia or sporangia
- Spore chain morphology

The generic level identification was carried out by using Bergey's Manual of Determinative Bacteriology 8th edition [**12**]**.**

Antimicrobial activity

The actinomycetes strains were identified and isolated from different marine soil samples and were screened for their antimicrobial activities. The test bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* used for screening. Activities were assessed using nutrient agar media. Each plate was streaked with an isolate at the center of a plate and incubated at 37°C for 7 days. Next, the subcultured test organisms were streaked perpendicular to the actinomycete isolate **[1].** Then the plates were incubated for 48 hrs at 37 °C. After incubation, the zone of inhibition was measured and recorded.

Extraction of antimicrobial compounds

The Selected antimicrobial actinomycete isolates were inoculated in to starch casein medium. It was carried out in 200 ml starch casein agar medium pH7.0 in a 250 ml capacity conical flask under sterile conditions. Flask was lodged on the flask shaker at a speed of 200 rpm at room temperature for 7 days. After incubation the medium were filtered through whatman filter paper and equal volume of ethyl acetate was added separately and centrifuged at 8000 rpm for 15 min at 4˚C to extract the antimicrobial compound [**7**]. The compound was tested for their activity against the human pathogens bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) by well diffusion method. After incubation for 48 hrs the zone of inhibition was measured.

RESULTS AND DISCUSSION

Results

The samples were collected from the soils of Chennai beach regions from which 114 isolates of actinomycetes were isolated **[Table 1].** The colony color of actinomycete strains isolated was categorized into light whitish, grayish, light-dark gray brownish [**Figure** 1]. These isolates were identified according to their morphological test [**Figure** 2]. The actinomycetes isolates were cultured on starch casein agar media for five or six days. Twenty two strains were showing antimicrobial activity amongst the 114 isolates. The isolates were screened for their inhibitory activity against the human pathogenic bacteria, *Staphylococcus aureus, Escherichia coli* there was the highly significant difference (14 mm) among antagonistic activity of isolates against *E.coli.* The most promising isolate against *Escherichia coli* was MB41 14mm followed by MB22 13mm. The potent isolate – MB41 - against *Staphylococcus aureus* was resistant against BN14, MB16 & NK05 isolates. The isolate MB41 show maximum antibacterial activities against pathogenic bacteria Escherichia coli, Pseudomonas aeruginosa and *Staphylococcus aureus* out of twenty two isolates **[Table 2].**

Figure 1: Colony color of isolated actinomycete strain MB41-Brownish

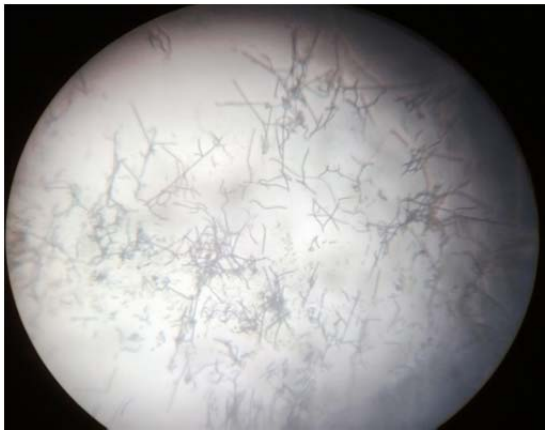

Figure 2: Photograph showing spore chain morphology of actinomycte isolate No: MB41

Table 1: Collection of soil sample for isolation of Actinomycetes

S.No.	No of Isolates	Symbol of strains	Collection area
1	57	MB1-MB57	Marina Beach
2	40	BN1-BN40	Besant Nagar Beach
3	17	NK1-NK17	Neelankarai Beach

Table 2: Antimicrobial activity against human pathogenic Bactria

Isolate Strain	Diameter of zone of Inhibition (mm)		
	E.coli	*S. aureus*	*P. aeruginosa*
MB16	08	11	10
MB20	08	08	10
MB22	13	04	09
MB36	07	00	09
MB41	14	07	08
MB45	08	09	00
MB49	07	08	09
MB53	08	09	07
MB54	00	07	08
BN07	09	00	09
BN14	07	12	06
BN17	08	09	09
BN22	07	05	05
BN30	06	07	00
BN34	07	08	09
BN37	08	08	07
BN39	08	00	05
NK05	09	10	07
NK07	07	09	06
NK11	00	05	06
NK13	08	00	07
NK16	07	08	00

Discussion

Antibiotics are the most important bioactive compounds for the treatment of infectious diseases. Due to the emerging multi-drug resistant pathogens, there is a basic challenge for effective treatment of infectious diseases. Since the burden of multidrug-resistant pathogens in the world, there has been increasing interest in searching effective antibiotics from soil actinomycetes in diversified ecological niches [**13**]**.** In the present study, the screening of actinomycetes in beach soils of Chennai using cross streak methods indicated that twenty two out of 114 actinomycete isolates showed potential antimicrobial activity against one or more test bacteria. The result showed the zone of activity of 14 mm being the highest (MB41). Observation of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities against test organisms from actinomycetes. According to the present result, MB41 showed 14 mm inhibition zone against E. coli which had the greatest inhibition zone when compared to other isolates. These isolate may be used in the application of treatment of different pathogenic microorganisms. Hence it is suggested that intensive studies on the action-bacterial diversity of the session to establish the rich actinomycetes diversity should be undertaken and this could put an important input in pharmaceutical industries.

CONCLUSION

The findings of the study may be useful to the future investigators to identify alternative and new bioactive metabolites like antibiotics to treat the resistant human pathogens.

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