Antagonistic Activity of Marine Actinomycetes against Human Pathogens

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Abstract

Antagonism refers to the action of any organism that suppresses the normal growth and activity of a pathogen. Actinomycetes are well known for the production of life-saving drugs such as antibiotics. Many pathogens have antibiotic resistance capacity which requires an extensive study on determining an alternative source from natural products. The main objective of the present study was isolation and characterization of Actinomycetes from the marine sample, which having antagonistic activity against selected human pathogenic strains.

Keywords

Actinomycetes, antagonistic activity, human pathogens.

I. INTRODUCTION

Oceans are the highly complex environment with extreme conditions which are habitats of diversified of microbes for the isolation of new novel products from marine microorganisms. Usually, Marine-derived antibiotics are very efficient against pathogens than terrestrially derived antibiotics (Fenical et al .,1999). Actinomycetes are the dominant groups of the marine population together with bacteria and fungi. They are gram-positive, free-living, saprophytic bacteria and a major source for the production of antibiotics and in the recycling of organic matter (Lacey et al., 1978). Actinomycetes are the most important biotechnologically valuable prokaryotes able to produce a wide range of biologically active metabolites such as antibacterial, antifungal, anticancer activities. Biologically active compounds with antagonistic properties from various groups of organisms are being used for therapy. In recent years there were increases in the reports of the emergence of human pathogens with multiple drug-resistance. The drugresistant human pathogens possess a great threat in the treatment of diseases. This problem forced the scientific community to keep on exploring for novel bioactive compounds with anti-microbial compounds. In the present study, we have reported the isolation and identification of marine actinomycetes from Thoothukudi coastal area with moderate antibacterial, antifungal activities against human pathogens.

II. MATERIALS AND METHODS

A) Study area

Thoothukudi district is located on the South East of Tamil Nadu state. The city lies on the Coromandel coast of Bay of Bengal. It is located about 590 km (367miles) south of Chennai and 190 km (118 miles) northeast of Thiruvananthapuram and by Tirunelveli district on the West and South West.

B) Collection of marine samples :

In this study, the marine water sample was collected from the coastal area of India named as pearl city beach, Thoothukudi. Samples were collected from 5 to 15 cm depth and kept in sterile polythene bags and preserved in the laboratory for further studies.

C) *Physicochemical Parameter Analysis:*

Physical parameters such as Atmospheric temperature, pH, were analyzed using the standard methods (Strickland and Parson, 1972) Chemical parameters like Sodium, Chloride, Sulphate, Calcium, Magnesium, Potassium was analyzed using standard methods (Vogel, 1978).

D) Isolation of Marine Actinomycetes:

Starch casein agar was prepared using sterilized seawater for the isolation and enumeration of *Actinomycetes*. The culture media was prepared and sterilized at 1210C in 15 lbs pressure. The isolation media was supplemented with the antibiotics cycloheximide (25mg/ml) and nalidixic acid (25mg/ml). (Kumar and kannabiran, 2010).The isolation was done by the serial dilution and pour plate technique. The plates were incubated at 300C for 7 – 10 days. The colonies were identified by their cultural and morphological characters under the light microscope.

E) Preparation of pure culture of Actinomycetes species:

(leaving out mixed culture colony) and streaked in the medium in a zigzag manner. Then test tubes were screwcapped and incubated at 30°C for 7 – 10 days.

III. IDENTIFICATION OF THE ACTINOMYCETES ISOLATES

A) *Morphological And Biochemical Studies:*

1.gram staining:

A thin smear of the Actinomycetes colony was prepared on a clean slide. The slide was fixed by using the flame. The smear was stained with crystal violet for 30 sec, then rinsed with water and drained. Next, the smear was covered with iodine for 30 sec, then rinsed with water. Then decolorized with ethanol 95% and washed with water. Finally, it was counterstained with safranin for 30 sec. The slide was dried and examined under oil immersion (100x).

2.screening for enzymatic activity and biochemical characters:

The ability of the isolates to produce extracellular enzymes such as amylase, protease, lipase, gelatinase were determined by various hydrolysis such as starch, casein, gelatin, lipid hydrolysis. Various biochemical tests were performed for the identification of potent *Actinomycetes* are as follows IMViC, H2S as per standard.

B) Screening Of Actinomycetes for Antibacterial Activity

The Starch Caesin Agar (SCA) medium was prepared in test tubes in the form of slant and the isolated colony obtained in the Petri plates were taken

1. Cross Streak Method:

The pure culture of Actinomycetes was inoculated on StarchCaesin Agar plates and incubated at 30oC for 6 days. Afteradequate growth of isolates, the test bacterial pathogens werestreaked perpendicular to the central strip of *Actinomycetes*culture and incubated. Then the zone of inhibition wasmeasured and recorded. (Mohan Remya and RamasamyVijayakumar, 2008)

C) Screening Of Actinomycetes for Antifungal Activity:

1. Cross streak method:

The pure culture of Actinomycetes was inoculated on Starch Caesin Agar plates and incubated at 30oC for 6 days. After adequate growth of isolates, the test fungal pathogens were streaked perpendicular to the central strip of *Actinomycetes* culture. The plates were then incubated at 30oC for a period of 3-4 days. After incubation, the zone of inhibition was measured and recorded.

2.Antimicrobial Susceptibility Assay:

The susceptibility of antibiotics test was carried out by following standard procedure against test organisms on the Mueller Hinton agar plates using the antibiotics Penicillin, Erythromycin & Ampicillin (Dubey and Maheshwari, 2002). The results were recorded and compared with the antimicrobial activity of the three isolates.

IV. RESULT

Gram Staining

Table: 1 Gram staining of the Actinomycetes isolates

S.No	Sample Name	Cell shape	Gram staining
1	KSR 01	Bacilli, filamentous	Gram-positive
2	KSR 02	Bacilli, filamentous	Gram-positive
3	KSR 03	Bacilli, filamentous	Gram-positive

Table 2: Biochemical Characteristics of Actinomycetes

Sample	Indole	M.R	V.P	Citrate	H2S	Starch	Casein	Lipid	Gelatin
KSR 01	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
KSR 02	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
KSR 03	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve

Table 3: Antagonistic Activity Against Human Bacterial Pathogens:

S.NO	SAMPLE	MEDIA	Zone of inhibition (mm)				
			S.aureus	B.cereus	K.pneumoniae	P.aeruginosa	E.coli
1	KSR 01	SCA	24	17	16	18	24

2	KSR 02	SCA	22	15	18	17	23
3	KSR 03	SCA	17	20	22	25	19

Table 4: Antagonistic Activity Against Human Fungal Pathogens:

S.No	Sample	Media	Zone of inhibition (mm)				
			C.albicans	A.fumigatus	C.neoformans	C.neoformans A.flavis	Histoplasma
1	KSR 01	SCA	18	22	13	23	15
2	KSR 02	SCA	28	13	22	15	13
3	KSR 03	SCA	27	19	17	20	18

V. RESULTS AND DISCUSSION

Marine sample was collected from the coastal areas of Thoothukudi. Analysis of the physicochemical parameters of the marine soil sample indicated is as follows. Atmospheric Temperature-34◦C, pH 7.5. Colonies with different morphology in Starch Caesin Nitrate (SCN) medium were chosen for the isolation of actinomycetes. The isolated strains were gram's stained and the results were tabulated (Table 1). The isolated pure cultures were subjected to various biochemical characterization and the results were indicated in Table 2.Actinomycetes culture was inoculated into the SCN agar plates and incubated at 30◦C, then the bacterial pathogens were streaked perpendicularly to the isolates and the zone of inhibition was observed among the isolates. (Mohan Remya and Ramasamy Vijayakumar, 2008). The crude extract of actinomycetes isolates KSR 01 showed potent activity against all the test organisms especially S. aureus and E. coli whereas the actinomycetes isolate KSR 02, KSR 03 inhibited the growth of all test organisms and promotes high activity against K. pneumonia and P.aeruginosa. The results were tabulated (Table 3). Actinomycetes culture was inoculated into the SCN agar plates and incubated at 30◦C, then the fungal pathogens were streaked perpendicularly to the isolates and the zone of inhibition was observed among the isolates. (Mohan Remya and Ramasamy Vijayakumar, 2008). The crude extract of actinomycetes isolates KSR 01 showed potent activity against all the test organisms especially A. fumigates and A. flavis whereas the actinomycetes isolate KSR 02, KSR 03 inhibited the growth of all test organisms and gives potential activity against C. albicans. The results were tabulated (Table 4). This indicates the presence of the bioactive component in the cultured actinomycetes strains.

A) Discussion

Marine Actinomycetes are potential producers of economically valuable secondary metabolites with bioactive components. Shirling and Gottlieb (1996) reported that the marine isolates possess simple spirals, simple flexible and simple reticulum aperture type of spore chains. Shantikumar Singh *et al.* (2006) reported 37 actinomycetes from lake sediments, out of them, 21 exhibits antibacterial, 12 exhibits antifungal activity. Kathiresan *et al.* (2005) isolated 160 marine actinomycetes from the marine sample and 31% of them are proved to be potential against plant pathogenic fungus. In the present study, the cultures grew well in SCN agar and produced aerial and substrate mycelium in the agar medium. The cross streak assay reveals that the marine actinomycetes KSR 01, KSR02, KSR03 were effective against human bacterial and fungal pathogens and was found to be a potential strain. Thus the present study reveals that certain marine actinomycetes from the coastal area of Thoothukudi may be a potent source of novel antimicrobial compounds.

VI. CONCLUSION

In this present study, the three isolates KSR 01, KSR 02, KSR 03 showed a wide range of inhibition zone in the secondary screening against pathogenic bacteria such as E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus cereus and fungi such as Aspergillus fumigatus, A.flavis, C. albicans and C. neoformans and Histoplasma sp. The crude extracts of bioactive compound tested for antibacterial and antifungal activity by cross streak method. Thus, the results of this investigation revealed that the marine Actinomycetes collected from the coastal areas of Thoothukudi might be a potent source of novel antibiotics. On conducting various tests for bioactive compound production, it was found that they produced bioactive compounds were active against certain bacteria and fungi. Further analysis is needed in future to explore the type of bioactive compounds produced by the isolated Actinomycetes, the knowledge of which can lead to the discovery of various novel products that may of medicinal as well as industrial use.

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CONFLICTS OF INTEREST

1. The authors declare that there is no conflict of interest.

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