WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 837-843. Research Article ISSN 2277– 7105

ISOLATION AND PARTIAL CHARACTERIZATION OF POTENT SPONGE DERIVED ACTINOMYCETES AGAINST MULTIDRUG RESISTANT BACTERIA

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Article Received on
17 Jan 2015,

Revised on 12 Feb 2015,
Accepted on 08 Mar 2015

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ABSTRACT

The present study aimed to investigate the antimicrobial activity of sponge derived actinobacteria against multidrug resistant pathogenic strains. Forty actinobacterial strains were isolated from five sponges belonging to the class demospongiae isolated along the Andhra Coast. Potential colonies were purified and stored. Primary screening was done using cross-streak method against pathogenic bacteria. Isolates with antagonistic properties were biochemically characterized and inoculated into production media for secondary metabolite extraction. The antimicrobial activity of crude and solvent extracts was performed using Kirby-Bauer method. The six actinomycete strains showing maximum antibacterial activity were isolated further characterized based on their colony characteristics and bioichemical analyses. The ethyl acetate extracts showed high inhibition against the two pathogenic bacteria tested methicillin – Resistant *Staphylococcus aureus* (*S. aureus*) and vancomycin-resistant Enterococci (VRE). The results of the current investigation show a high diversity of actinomycetes associated with marine sponges as well as highlight their potential to produce new drugs molecules.

KEYWORDS: Actinomycetes, Marine Sponges, Antibacterial activity, Multidrug resistance.

INTRODUCTION

Phylum porifera (sponges) consists of benthic (sessile) organisms that occur primarily in marine environments at different depths.[1] As typical filter feeders, sponges are the prime bacterial filters of the sea. They are capable of pumping thousands of liters of water per day,[2] using prokaryotic microorganisms as the main source of food.[3-5] In addition to sponges feeding on microorganisms, the presence of bacteria in high density in internal sponge layers indicates that a selective process favoring particular prokaryotes, involving microbe-sponge interactions, is likely to occur.[6] Indications of the common presence of actinomycetes in sponges in marine habitats have increased efforts to discover new take and thus new metabolites.

Actinomycetes are important sources of new bioactive compounds such as antibiotics and enzymes[7-9] which have diverse clinical effects and are active against many pathogenic organisms. Among all the known microbes, members of the actinomycetes genus especially streptomyces species have been recognized as prolific producer of useful bioactive metabolite with broad spectrum of activities which has antibacterial, antifungal, antibiotic, antiparasitic, antitumor, antiviral, insecticide, herbicide, immunomodulators, antithrombatic agents.[10]

Antimicrobial drugs used for prophylactic or therapeutic purposes in humans, veterinary and agricultural purposes were favoring the survival and spread of resistant organisms.[11] The appearances of multidrug resistant pathogenic strains caused substantial morbility and mortality especially among the elderly and immunocompromised patients. To overcome this situation, there is an interest to improve or discover novel class antibiotics that have different mechanisms of action worldwide.[12,13] Therefore, the objective of the present study was isolation of actinomycetes from different sponges for screening of antibacterial compounds against multidrug resistant bacteria.

MATERIALS AND METHODS

Collection of sponges: Sponges were collected along the Andhra Coast in September, 2013. Sponges were transferred to sterile plastic bags containing sea water and transported to the laboratory. Sponges specimens were rinsed in sterile seawater, cut into pieces of Ca. $1cm^3$, and then thoroughly homogenized in a sterile mortar with 10 volumes of sterile seawater. The supernatant was diluted in ten – fold series (10^{-1}, 10^{-2}, 10^{-3}) and subsequently plated out on agar plates.

Actinomycete Isolation

Different media Zobell marine, Marine agar, glycerol asparagine agar and R2A agar were used for isolation of actinobacteria. All media were supplemented with 0.2 µm pore size filtered cycloheximide (100 µg/ml), nystatin (25 µg/ml) and nalidixic acid (25 µg/ml) to facilitate the isolation of slow-growing actinobacteria. Cycloneximide and nystatin inhibit fungal growth, while nalidixic acid inhibits many fast growing gram – negative bacteria.[14] The inoculated plates were incubated at 28^0C for 2-3 weeks. The suspected colonies for actinomycetes were selectively isolated and transferred to actinomycetes isolation agar medium with the help of loop inoculum method.[15] The different colaration of aerial and substratum mycelia and diffusible pigments at bottom of inoculated plates were observed.[16] Some strains of actinomycetes were selected on the basis of mycelium concentration and diffusible pigments. The morphology of the filaments or mycelium was determined by light microscopy.

Biochemical Characterization

The isolates of actinomycetes were used for biochemical studies. The various biochemical tests were performed for the identification of the potent isolates. All the cultures were incubated at 28 ^{0}C for 24- 72 h.

Extraction of Secondary Metabolites

Submerged fermentation was carried out for extraction of secondary metabolites. 200 ml of starch casein agar medium was prepared and dispensed into 500ml Erlenmeyer Flask, sterilized and cooled. At room temperature the medium was inoculated with 2ml cnspension of isolates and incubated for 14 days in a rotary shaker at 200 rpm/ min at 28 ^{0}C. Then the medium was harvested by centrifugation at 4000 rpm/ min for 15 min. The supernatant was collected and added equal volumes of ethyl acetate (1:1 v/v) then shaken vigorously for 1 hr and repeated twice. The solvent phase was separated from aqueous phase by using a separating funnel and subjected to rotary vacuum evaporator at a water bath temperature of 60^0C at 100rpm/min to remove solvent and to get crude extracts.[17]

Antibacterial studies

The crude extract containing secondary metabolites were screened for antibacterial activity against multidrug resistant bacteria (Methicillin-Resistant *Staphylococcus aureus*, vancomycin – Resistant Enterococci.[18, 19]

The antibiotic sensitively of test organisms was determined by the standard disc diffusion method against a number of antibiotics. All the antibiotic discs were purchased from Hi-Media Pvt.Ltd. The antibacterial activity was performed according to CLSI, USA guidelines on Mueller Hinton Agar well medium using diffusion method.[20, 21]

RESULTS

Among the microorganisms, actinomycetes are best known for their outstanding ability to produce a great variety of medically and economically important secondary metabolites or compounds with antibacterial, antifungal and antifungal properties and encompass a large variety of antibiotics. Yet there are very few reports regarding organisms is increasing and compromising the treatment of a growing number of infectious diseases. As a result, there is an urgent need for developing new drugs which are effective against current antibiotic resistant pathogens.

In the present study 40 actinomycetes were isolated from 5 sponges belonging to the class demospongiae. Among them 40 actinomycetes strains 6 isolates were found to possess antimicrobial activity. And it was found that isolate 3 showed maximum zone of inhibition of 13mm with the test bacteria.

Mycelium color and diffusible pigment of actinomycetes isolates MB 101 produce black mycelium and no pigment. MB 102 produce dark brown mycelium and yellow pigment. MB 103 produce white grey mycelium and no pigment. MB 104 produce grey mycelium and brown pigment. MB 105 produce black mycelium and pigment. MB 106 produces grey mycelium and pink pigment.

All 6 actinomycetes isolates showed positive results for catalase, starch utilization and casein utilization. And showed negative results for indole and triple sugar iron biochemical tests actinomycetes isolated from sponges along Andhra Coast (Kakinada). Therefore sponges were collected along Kakinada coast for isolating actinomycetes. 40 actinobacterial strains were isolated from 5 sponges belonging to class demospongiae. Among the 40 actinobacterial strains 6 strains were selected which showed antimicrobial activity.

The 6 actinomycetes strains were studied based on their colony morphology, mycelium coloration and pigment diffusion (Fig 1). The biochemical properties such as catalase, casein hydrolysis, starch hydrolysis, indole activity and triple sugar iron (TSI) Agar of

actinomycetes isolates were studied. All the isolates showed positive test with catalase, starch and casein utilization. It was observed that none of isolates showed in dole and TSI utilization. The isolates were screened for their inhibitory activity against the human pathogenic bacteria *E. coli*, *S. epidermis* and MRSA (VRE). (Table 1. (Figure 2).

Table – 1 Antibiotic susceptibility pattern of multidrug resistant bacteria

Strain	Methicillin	Vancomycin	Erythromycin	Clindanycin	Ciprofloxacin
MRSA	R	S	R	R	S
VRE	R	R	R	R	S

R: Resistant, S: Sensitive

Table – 2 Isolate MB 103 out of 6 isolates showing maximum antibacterial activities against pathogenic bacteria *E. Coli* (MTCC), *S. aureus* (MTCC), Methicillin resistant – *S. aureus* and Vanocmycin resistant Enterococin

Bacteria	Zone of Inhibition (diameter in mm)					
	MB 101	MB 102	MB 103	MB 104	MB 105	MB 106
E.Coli	-	-	13	-	-	-
S. Aureus	-	-	12	-	-	-
MRSA	-	-	12	-	-	-
VRE	-	-	11	-	-	-

DISCUSSION

Currently, the incidence of multidrug resistance is major problem in treatment of infections. It was found that isolate 3-MB103 had broad spectrum antimicrobial activity as it showed its potential against gram positive and gram negative bacteria and vancomycin resistant enterococci. Therefore, it is suggested that actinobacteria from marine sponges are a potential source of bioactive natural products. Further studies of taxonomic characterization and purification of compounds are underway.

CONCLUSION

In summary, the current investigation show a high diversity of actinomycetes associated with marine sponges as well as highlight their potential to produce new drugs molecules.

ACKNOWLEDGEMENT

The first author acknowledges department of science and technology, government of India for providing financial assistance by sponsoring a project under fast track – young scientist scheme under the file No.SB/FT/LS-247/2012 dated 02-05-2013.

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