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Antagonistic effect of marine Actinobacteria on MDR Uropathogens

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Abstract

Actinobacteria are the antagonistic bacteria able to release antagonistic compounds which inhibits the growth of other microbial species. This study was carried out to identify biologically potential Actinobacteria from natural source. This study also confirms antimicrobial nature of the Actinobacteria. Twenty six marine water samples was collected from different time and points in the Bay of Bengal and isolated thirteen actinobacterial isolates using different culture medium. All actinobacterial isolates were identified by macroscopic and microscopic observation. The isolated actinobacteria were screened for their antimicrobial activity against UTI pathogens. The marine isolate Stretomycetes sp. identified from marine water were found to be more efficient in the production of secondary metabolites. This will pave the way for further investigations to study their potential importance in combating pathogenic bacteria.

Keywords: Actinobacteria, streptomyces, antagonism, MDR pathogens.

Introduction

Actinobacteria are a group of bacteria which shows multiple potentialities as it inhibit the growth of other microbial species through a process called antagonism. They also produce numerous pharmacologically active substances essential for health. During the last few decades multiple numbers of antimicrobial substances are screened from actinobacteria1. Antagonism is one of the best known phenomena exhibited by a group of bacteria called Actinobacteria. Antimicrobial substances secreted by these bacteria are liable for this activity. Over 5000 antimicrobial substances are identified from these groups of microorganisms[2]. Streptomyces, Actinomyces, Micromonospora, Actinomadura, Actinoverticillium and Thermoactinomycetes are the major antagonistic Actinobacteria. Among these, 60% of antimicrobial substances were made from Streptomyces[3]. Current antibiotics were failed due to the development of multidrug resistance among microorganisms. These microbes may cause more vulnerable infections. To overcome these, people are in search of newer antimicrobial substances from the nature. Microbes and plants are the best source of these substances. Among microbes antagonistic actionobacteria is the best choice. Approximately two thirds of all antimicrobial substances are made from these groups of microorganisms[4]. Actinobacteria show a considerable proportion of the population of soil, lakes and river muds. Atinobacteria have been isolated from dry ecosystem since time immemorial. The first report of actinobacteria being recovered from marine sediments appeared several decades ago. Very few reports suggested that marine actinobacteria in sediments of the ocean are the best source for isolation of bioactive compounds. Studies are established that actinobacteria isolated from the marine environment are metabolically active and have adapted to life in the sea[5]. Actinobacteria isolated from marine ecosystem shows different features than the isolates from the dry ecosyetem (Terrestial). They may also produce effective antagonistic compounds. Hence in this study, antagonistic activity of marine actionbacteria is studied to understand its efficiency and the antimicrobial substance production.

Material and Methods

Sample processing: Seawater samples were collected from Bay of Bengal, India. Sampling area covers from off shore area of Bay of Bengal at Puducherry, UT of India. The collected water samples were stored in sterile polypropylene bottles and preserved in the laboratory for future use.

Isolation of marine actinobacteria: Serial dilution along with pourplating was done to isolate Actinobacteria[6]. The serial dilution was done by taking one ml of water sample and mixed with 1ml sterile distilled water in a test tube. The sample was serially diluted up to 1:10000 dilution by transferring 1ml aliquots to a series of test tubes each containing 9ml sterile distilled water and from respective dilution. One ml of diluted sample was transferred to the sterile petri plates and poured starch casein agar (SCA). Different media like actinomycetes isolation agar, ISP2 and ISP6 are used also used for isolation of actinobacteria from marine samples. The isolation media was supplemented with the antibiotics cycloheximide (25mg/ml) and nalidixic acid (25mg/ml) [7]. The plates were incubated at 28±1ºC for 7 to 21 days. The colonies were identified by their cultural characters.

Characterization of marine actinomycetes: A standard method of Slide culture technique was performed to characterize aerial mycelium, spore arrangements[8].

Screening of anti microbial activity: Identified actinobacteria were incubated at 30±2ºC on a rotary shaker at 220 rpm for 10 days for complete recovery of antagonistic substances. The culture was collected and centrifuged at 6000 rpm for 15 min. Cell free extract was collected after filtering through millipore filter (0.45µm). Well diffusion method was used for assaying the antimicrobial activity against multi drug resistant *E. coli* and *K. pneumoniae.*

Results and Discussion

Twenty six marine water samples was collected from different time and points in the Bay of Bengal and isolated thirteen actinobacterial isolates using different culture medium. All actinobacterial isolates were identified by growth characteristics and Gram staining. Based on these characteristics actinobacteria were identified as *Stretomyces sp* and *Nocadia sp.* (table-2). Out of thirteen selected and identified actinobacteria Stretomycetes sp showed significant prevalence and antimicrobial activity against multidrug resistant UTI pathogens. Five pathogenic strains isolated from cases of UTI infection (three *Escherichia coli* (E-51, E-44, E-71) and two *Klebsiella sp.,(*K-52 and K-53) were used as a test organism for antagonistic study. Among the actinobacteria tested, test organism RW2-3 and RW2-5 strains produced the best activity against all the test organisms (table-3). When 100µl / disc concentrations of the actinobacteria grown fermentation medium were tested for antibacterial activity against MDR urinaryisolates, the results indicated that all the actinobacterial strains showed good antibacterial activity. All the strains showed a zone of inhibition against urinary pathogens. Best antimicrobial activity was exhibited by RW2-3 and RW2-5 strains and these strains were considered as a *Streptomyces sp*. In the present study, zone of inhibition ranges from 8mm - 15mm with the best activity against E. coli 51 and E. coli 44 by RW2-3 strain (table-4).

Similar kind of antagonistic activity also reported by different authors from India but they used different pathogens[9-11]. Several studies reported that, the actinobacteria are worth mentioning as antibiotic producers making three quarters of all known products and are especially prolific[12-17]. According to Mohana and Radhakrishnan[18] actinomycetes isolates are often encounter activity against gram positive bacteria than gram negative bacteria, which is due to secondary metabolites, whereas our results shows different report. In the present study all the test urinary MDR pathogens were belonging to gram negative group (*E.coli and K. pneumoniae*). Marine *Steptomycetes sp* exhibited the highest antibacterial activity against *E .coli* and *K. pneumoniae*.

Conclusion

The present study concluded that the marine actinobacterial isolate, *Streptomyces sp* is dominant in marine water and is considered as a budding resource of novel bioactive compounds. A further study on the molecular characterization of the isolates and purification of bioactive compounds is in progress.

Table-1
Isolation of Actinobacteria from Marine for the Production of Antimicrobial Compound

S. No	Strain No.	Nature of isolate
1.	SW02-001	Nil
2.	RW02-002	7 types of colonies were observed (3 white, 3 gray, 1green spot colour colonies)
3.	MHT05-001	-
4.	MLT05-002	White colour
5	MLT5-003	-
6	MLT5-004	-
7	MLT5-004	-
8	MLT-001	-
9	MLT-002	White colour
10	MLT-003	-
11	MLT-004	-
12	MLT-005	-
13	M10F05-003	-
14	ELT05-004	-
15	EHT-005	White colour
16	E10F05-006	-
17	E20F -007	White colour
18	MLT01-009	-
19	MLT02-009	-
20	MHT01-010	White colour
21	M10F01-011	White colour
22	M10F01-012	-
23	ELT01-012	-
24	EHT01-013	-
25	EHT02-014	-
26	EHT03-015	-

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Table-2
Characteristic features of actinobacterial isolates

S. No	Test isolate	Aerial mycelium	Substrate mycelium	pigment	Spore chain morphology	Gram staining	Glucose	Fructose	Lactose	Maltose	Mannital	identification
1	RW2-1	gray	yellow	no	spirals	Gram positive rod	+	+	–	–	–	*Streptomyces sp.,*
2	RW2-2	gray	yellowish brown	no	spirals	Gram positive rod	+	+	–	–	+	*Streptomyces sp.*
3	RW2-3	light brown	yellowish brown	no	Simple spirals	Gram positive rod	+	-	-	+	+	*Streptomyces sp.*
4	RW2-4	white	white	no	retinaculiaperti	Gram positive rod	+	–	+	–	–	*Streptomyces sp.*
5	RW2-5	yellowish brown	yellowish brown	no	Flexible	Gram positive rod	+	+	–	+	+	*Streptomyces sp.*
6	RW2-6	Yellowish brown	yellowish brown	no	spirals	Gram positive rod	+	–	+	–	–	*Streptomyces sp.*
7	RW2-7	yellow	yellow	no	spirals	Gram positive rod	+	–	+	–	+	*Streptomyces sp.*
8	E20F-007	gray	Ash-yellow	no	spirals	Gram positive rod	+	+	–	–	+	*Streptomyces sp.*
9	MLT-002	light	yellow	no	spirals	Gram positive rod	+	–	–	–	–	*Streptomyces sp.*
10	EHT-005	cream	light	no	Spirals	Gram positive rod	+	–	–	+	+	*Streptomyces sp.*
11	MHT01-011	gray	brown	no	spirals	Gram positive rod	+	+	–	–	+	Nocardia sp
12	M10F01-010	gray	Yellow-ash	no	Spirals	Gram positive rod	+	–	–	–	–	Nocardia sp
13	EHTOI	gray	Yellow green	no	flaxibile	Gram positive rod	+	–	–	–	–	*Streptomyces sp.,*

Table-3
Antagonistic activity of actinobacteria multidrug resistance pathogens

S. No	Test organism	*E.coli 51*	*E.coli 44*	*E. coli 71*	*K.pneumoniae 52*	*K.pneumoniae 53*
1	RW2-1	+	-	-	-	+
2	RW2-2	+	-	-	-	-
3	RW2-3	+	+	+	+	+
4	RW2-4	+	-	+	-	+
5	RW2-5	+	+	+	+	+
6	RW2-6	+	-	+	-	-
7	RW2-7	+	-	+	-	+
8	E20F-007	+	-	+	+	-
9	MLT-002	-	-	+	-	-
10	EHT-005	+	-	+	+	-
11	MHT01-011	-	-	+	+	+
12	M10F01-010	-	-	+	-	-
13	EHTOI	-	-	+	+	-

Table-4
Antimicrobial activity of the product obtain from marine sample against Uropathogens

S. No	Isolate number	Organisms Used / Zone of inhibition in mm				
		E-51	**E-44**	**E-71**	**K-52**	**K- 53**
1	RW2-1	08	-	09	10	11
2	RW2-2	-	-	09	10	12
3	RW2-3	15	15	12	11	12
4	RW2-4	-	-	11	12	11
5	RW2-5	14	15	13	12	12
6	RW2-6	10	12	12	11	10
7	RW2-7	13	11	13	14	11
8	E20F-007	09	11	12	12	11
9	MLT-002	13	12	13	11	-
10	EHT-005	13	12	11	13	09
11	MHT01-011	-	09	09	08	-
12	M 10F01-010	10	10	09	11	-
13	EHTOI	-	09	09	11	-

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