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Original Article

Marine Actinomycetes: A New Source Of Compounds Against Uropathogens

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ABSTRACT

Actinomycetes are virtually unlimited sources of novel compounds with many therapeutic applications and hold a prominent position due to their diversity and proven ability to produce novel bioactive compounds. The present study "Marine actinomycetes: a new source of compounds against uropathogens" is aimed to prove the antagonistic activity of marine actinomycetes against uropathogens. 13 marine actinomycetes were isolated from various marine sediment samples collected from different stations of Menamkulam coastal region, part of Arabian Sea on the western coast of India. The sample collection and isolation of actinomycetes were done by following the standard microbiological methods and the isolated colonies were characterized based on their morphological parameters.. The morphologically distinct 10 isolates obtained in the present study, the isolates SD96,SD97,SD98,SD99,SD100,SD101,SD102,V107,V108,V109,V110 and V111, among them three isolates, SD99,SD100 and SD102 showed significant antagonism against selected uropathogens collected from a tertiary care center in Kerala. The results were promising and indicated that actinomycetes with distinctive biological activity are abundant in the marine environment along Kerala's coast.

Keywords: Uropathogens, Marine actinomycetes, Antagonism, Therapeutic applications.

INTRODUCTION

Urinary Tract Infections (UTIs) are the most frequently reported infections and drive antibiotic use around the world (Allen et al., 1999, Anthony, 2002). UTIs are the fourth most common type of healthcare-associated infection (Magill et al., 2014). The overall prevalence of UTI in India is 33.54%, in which 66.78% females and 33.22% males (Pardeshi, 2018). High prevalence is observed in females as compared to males (2:1). The prevalence of UTI (both asymptomatic

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bacteriuria and symptomatic infection) in pregnant women in India is reported to range from 3% to 24% (Kant et al., 2017). In general UTI is significantly associated with age, duration of diabetes, and poor glycemic control.

UTI is caused by both Gram-negative and Gram-positive bacteria. The most common causative agent of UTI is uropathogenic *Escherichia coli* (UPEC). Other bacteria commonly associated with UTI are *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Hooton, 2012; Kline et al., 2011; Ronald, 2002; Flores-Mireles, 2015). The lack of effective antibiotics against common uropathogens makes many urological procedures more risky and dangerous. The high prevalence of UTI makes it as a key player in the extensive use of antibiotics which eventually leads to the development of antibiotic resistant strains (Trestioreanu et al., 2010; Costelloe et al., 2010). For the invention of new medications, the marine actinomycetes represent relatively unexplored natural resources. This group's diversity, distribution, and metabolic diversity have not yet been fully investigated. The goal of the current study is to better understand the secondary metabolites produced by marine actinomycetes in order to combat the uropathogen crisis, antibiotic resistance, and other related infectious disorders. A glimmer of hope exists in the recent development in the pharmacology of marine actinomycetes, which discloses new antibacterial formulations based on their secondary metabolites.

MATERIALS AND METHODS

(a) *Marine soil collection*

Samples of marine sediment and seashore soil were taken from various sites along Menamkulam Beach in Kerala, on the western coast of India. The samples were taken aseptically, transferred to the lab in ice boxes, and kept in a refrigerated condition.

(b) *Isolation of marine actinomycetes*

One gram of marine samples (sediment and shore soil) was serially diluted aseptically in sterile saline. In starch casein agar (Himedia, India) that had been prepared with 0.5% sodium chloride, one ml samples from 10^{-2} to 10^{-4} dilutions were pour plated (in triplicates). The plates were incubated inverted at room temperature for 4 to 21 days at 28 degrees Celsius. Following the recommended incubation, the plates were examined for the growth of actinomycete colonies, and the colonies that formed were counted using a colony counter (LAPIZ INDIA). Each sample's actinomycete load was determined using a standardised formula.(Usha etal,2010)

The actinomycete load in each samples were calculated using the formula,

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$$The\ actinomycete\ load\ in\ each\ sample = \frac{Average\ number\ of\ colonies\ \times dilution\ factor}{Volume\ of\ sample}$$

(c) Selection of Actinomycetes Isolates

Predominant and morphologically distinct actinomycetes colonies were selected for further studies based on their macroscopic qualities.

(d) Purification and preservation of Actinomycetes Isolates

Selected actinomycetes isolates were microbiologically purified by repeated sub-culturing on starch casein agar and the purified isolates were stored at four degree Celsius in starch casein agar slants for further studies.

Screening for Antagonistic Activity Study

(a) Primary screening Agar overlay Method

72 hours broth cultures of actininomycetes isolates were streaked as a single line through the centre of the Muller Hinton Agar (Himedia, India) plates (vertically). It was incubated at 28^0 C for three days. After appearing the growth of actinomycetes 12 hours young broth cultures of bacterial pathogens were mixed in soft agar and flow on the actinomycetes isolate. The results were observed and reported.

(b) Secondary Screening - Well Diffusion Method

ISP I Broth 50 ml was used to inoculate actinomycetes isolates (Himedia, India). It was cultured in a shaker cum incubator (REMI INDIA) at 120 rpm for four days at 28^0 C. By centrifuging the cell-free culture filtrate at 6000 rpm for 15 minutes, the filtrate was obtained. Whatmann No. 1 filter paper was used to separate the supernatant, which was then stored for future investigations. On the Muller Hinton Agar plates, 12-hour-old overnight bacterial broth cultures were properly swabbed (horizontally, vertically, clockwise, and anti-clockwise). The plates were left undisturbed in their upright position for 20 minutes. On it, wells with a diameter of 6 mm were bored. After being loaded, 50 µl of cell-free culture filtrate was maintained at room temperature for one hour (for the diffusion of culture filtrate into the agar). The plates were incubated for 24 hours at 37^0 C. After incubation, the plates were examined, and a millimetre ruler was used to determine the zone of growth inhibition from the plate's base. The findings were noted.

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(c) Secondary Screening - Disc Diffusion Method

Using a paper puncher, a disc of Whatmann No. 1 filter paper with a diameter of roughly six millimetres was created. The filter disc was dried and then sterilised before being stored. A sterile filter paper disc was filled with approximately 100 µl of cell-free actinomycetes culture filtrate, which was then dried in a hot air oven for 24 hours at 30^0 C. On the Muller Hinton Agar plates, 12-hour-old overnight bacterial broth cultures were successfully swabbed (horizontally, vertically, clockwise, and anti-clockwise). The plates were left undisturbed in their upright orientation for 20 minutes. Using sterile forceps, the culture filtrate-loaded discs were aseptically positioned on the agar surface. The plates were incubated for 24 hours at 37^0 C. Following incubation, the plates were examined, and a millimetre ruler was used to estimate the area of growth inhibition surrounding the discs. The outcomes were seen and noted.

RESULTS

Isolation of marine actinomycetes

Marine sediment and sea shore soil was collected from three sites along Menamkulam Beach in Thiruvananthapuram, Kerala, India, and yielded a total of ten marine actinomycetes. On starch casein agar plates, the samples were pour-plated after being serially diluted. Following a sufficient incubation period, the plates were inspected, and marine actinomycetes colony formation was noted.

Morphological characterization of marine actinomycetes

Based on their morphological traits, the isolated marine actinomycetes were chosen for future research. It was discovered that different isolates had different colony morphologies. From the current station, small, medium, and large sized colonies were distinguished. The bulk of the isolates had irregularly shaped or rounded colonies. A few had circular colony shape, it was discovered. The isolates range in hue from white to off-white to creamy. While some of the isolates had undulating margins, the majority of them displayed the whole boundary. Almost all of the isolates had elevated elevation. The textures of the isolates ranged from being smooth, dry, and rough, and they were all opaque in transparency. These varied colony traits show the potential diversity of the actinomycetes isolated from different sources.

Antagonistic activity of the marine isolates against selected uropathogens

In primary screening by Agar overlay method, 10 marine actinomycetes SD96,SD97,SD98,SD99,SD100,SD101,SD102,V107,V108,V109,V110 and V111 were screened to detect their antagonistic activity to uropathogens as single line (streaked horizontal to the growth of actinomycetes isolate). The results were observed and reported in Table 2.

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Actinomycetes' growth pattern was not uniform on the agar surface when using the agar overlay method, so the results were qualitatively reported in terms of positive and negative signs in accordance with the diameter of the zone of growth inhibition the isolates of actinomycetes produced towards bacterial pathogens, i.e. single or double plus in case of any slight to moderate response (+/++); effective antagonism by triple plus (+++); and absence of antagonism was denote (–). In the initial screening, the isolates SD99,SD100 and SD102 shown notable antagonistic activity against the chosen uropathogenic organisms. The downstream studies were chosen for these 3 isolates.

Secondary screening of antagonistic activity of the isolates against common uropathogens

By using secondary screening techniques, the antagonistic activity of these strains was further demonstrated. Two techniques, the well diffusion assay and the disc diffusion assay, were used for the secondary screening of the isolates. Tables 2 and 3, respectively, illustrating the well diffusion assay and disc diffusion assay results.

In the initial screening, all 10 isolates showed zones of resistance against two or more pathogenic bacteria. Among them, three isolates showed noticeably broad spectrum activity against each of the uropathogenic bacteria that were picked. All of these findings suggest that marine actinomycetes may serve as a reliable source of novel antimicrobial metabolites, which calls for in-depth research to address the problem of the clinical setting's rising antibiotic resistance with an emphasis on uropathogens..

DISCUSSION

Marine One of the most important subgroups of microorganisms to have been isolated from marine sources worldwide is the actinomycetes. They may be a successful treatment for bacteria that are resistant to a range of medications by creating secondary metabolites. The oceanic environment continues to be a promising source for the discovery of novel actinomycetes despite the plethora of research on terrestrial actinomycetes.. (Chakraborty et al., 2015; Rajivgandhi et al., 2018; Rajan et al., 2014).

The purpose of this study is to identify and describe a secondary metabolite produced by marine actinomycetes that is antagonistic to human uropathogens. Several samples taken from various locations along the Arabian Sea's Menamkulam Beach on India's west coast yielded a total of 10 marine actinomycetes.. The colony morphology of the isolates was diverse showing the potential diversity of the actinomycetes population present along the coastal region of Kerala. The isolates SD99,SD100 and SD102 showed significant antagonism against selected uropathogens collected from a tertiary care center in Kerala.

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Marine Actinomycetes are one of the most significant subgroups of microorganisms that have been isolated from marine sources throughout the world. By producing secondary metabolites, they may be an effective remedy for bacteria that are resistant to a wide variety of drugs. Despite the abundance of investigation on terrestrial actinomycetes, the ocean seems to be a prospective environment for the discovery of novel actinomycetes..

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Conflicts of interest

The authors declare no conflicts of interest.

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Tables

Table 1: The antagonistic activities of the isolates by primarily screening

Sl. No	Actinomycete isolate	Response of UTI pathogens (Qualitative estimation)						
		Escherichia coli	***Klebsiella sp.***	***Klebsiella pneumoniae***	***Pseudomonas sp.***	***Pseudomonas aeruginosa***	***Acinetobacter sp***	***Acinetobacter baumannii***
1	SD96	+++	-	-	+	++	-	+
2	SD97	+	++	+	-	++	+	++
3	SD98	+	+	-	-	+	-	-
4	SD99	+++	+++	+++	+++	++	+++	+++
5	SD100	++	-	+	++	-	-	-
6	SD101	+++	+++	+	+++	+	++	++
7	SD102	-	++	++	+	++	++	++

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

8	V107	+	++	+	++	+	+	++
9	V108	-	-	-	+	+	-	+
10	V109	-	+	+	+	-	-	-
11	V110	+++	+++	+++	+++	+++	+++	+++
12	V111	+++	-	++	++	++	++	++

Slight to moderate response- (+/++); effective antagonism - (+++), Isolates named 'V' are from shore soil and 'SD' are from marine sediment

Table 2: Secondary screening of marine actinomycetes by well diffusion method

Sl. No	Actinomycete isolate	Response of UTI pathogens (Qualitative estimation)						
		Escherichia coli	***Klebsiell asp.***	***Klebsiellapneumoniae***	***Pseudomonas sp.***	***Pseudomonas aeruginosa***	***Acinetobacter sp***	***Acinetobacter baumannii***
1	SD99	20	19	11	18	19	15	11
2	SD100	20	17	19	17	21	19	16
3	SD102	21	18	12	12	18	17	15

Table 3: Secondary screening of marine actinomycetes by disc diffusion method

Sl. No	Actinomycete isolate	Response of UTI pathogens (Qualitative estimation)						
		Escherichia coli	***Klebsiell asp.***	***Klebsiellapneumoniae***	***Pseudomonas sp.***	***Pseudomonas aeruginosa***	***Acinetobacter sp***	***Acinetobacter baumannii***
1	SD99	18	17	16	14	19	16	17
2	SD100	14	16	18	16	15	18	19
3	SD102	18	15	16	15	18	16	16