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Research Article

CHARACTERIZATION OF MARINE ACTINOMYCETES SPECIES ISOLATED FROM AZHIMALA COASTAL REGION, KERALA AND IT'S EFFICACY AGAINST UROPATHOGENIC BACTERIA

V. Robin Perinba Smith*[1], Deepa Mathew P[2]

[1]*Department of Zoology, Scott Christian College, Nagercoil, Kanyakumari District, Tamilnadu, India*

[2]*Research Scholar, Department of Zoology, Scott Christian College, Nagercoil, Kanyakumari District, Tamilnadu, India*

**Corresponding author: deepamathew1947@gmail.com*

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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 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 in the Azhimala coastal region, part of the 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 13 isolates obtained in the present study, the isolates SD65, SD66, SD67, SD68, SD69, SD70, SD71, V66, V67, V68, V69,V70,V71 among them 3 isolates,V67,V68 and V70 showed significant antagonism against selected uropathogens collected from a tertiary care center in Thiruvananthapuram, 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.

1. INTRODUCTION

Urinary Tract Infections (UTIs) are the most frequently reported infections and drive antibiotic use around the world [1, 2]. UTIs are the fourth most common type of healthcare-associated infection [3]. The overall prevalence of UTI in India is 33.54%, in which 66.78% are females and 33.22% are males [4]. High prevalence of UTI is observed in females as compared to males (2:1). The prevalence of UTI (both asymptomatic bacteriuria and symptomatic infection) in pregnant women in India is reported to range from 3% to 24% [5]. 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* [6-9]. 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 [10, 11]. 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.

2. MATERIAL AND METHODS

2.1. Marine sample collection

Samples of marine sediment and seashore soil were taken from various sites along Azhimala 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.

2.2. 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 poured (in triplicates). The plates were incubated inverted at room temperature for 4 to 21 days at 28°C. 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 standardized formula [12].

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

Actinomycetes load in each sample = (Average number of colonies x dilution factor)/Vol. of sample

2.3. Selection of Actinomycetes Isolates

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

2.4. 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.

2.5. Screening for Antagonistic Activity Study

2.5.1. Primary screening Agar overlay Method

Broth cultures (72 hours old) of actinomycetes isolates were streaked as a single line through the centre of the Muller Hinton Agar (Himedia, India) plates (vertically). It was incubated at 28°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.

2.5.2. 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°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°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.

2.5.3. Secondary Screening - Disc Diffusion Method

Whatmann filter paper no.1 was punched to about six mm diameter disc using a paper puncher. The filter disc were sterilized and stored in its dried form. About 100µl of cell free culture filtrate of actinomycetes were loaded over the sterile filter paper disc and kept for drying in hot air oven at 30°C for 24 hours. 12 hours young over-night bacterial broth cultures were effectively swabbed (horizontally, vertically, clock-wise and anti-clock-wise directions) on the Muller Hinton Agar plates. The plates were kept undisturbed for 20 minutes in upright position. The culture filtrate loaded discs were placed aseptically on to the agar surface using a sterile forceps. The plates were incubated at 37°C for 24 hours. After incubation, the plates were observed and the zone of growth inhibition around the discs was measured using a millimeter ruler from the bottom of the plate. The result were observed and recorded.

3. RESULTS

3.1. Isolation of marine actinomycetes

A sum of 13 marine actinomycetes was obtained from marine sedimentsea shore soil collected from 3 stations of Azhimala Beach, Thiruvananthapuram, Kerala, India. The samples were serially diluted and pour plated on starch casein agar plates. After proper incubation period, the plates were observed and noticed colony formation of marine actinomycetes.

3.2. Morphological characterization of marine actinomycetes

The obtained marine actinomycetes isolates were selected for further studies based on their morphological characteristics. The colony morphology of various isolates was found to be diverse. Small, moderate and large sized colonies were identified from the present station. The colony shape of the majority of the isolates was round or irregular. A few were found with circular colony morphology. The color of the isolates varies from white, off white to creamy. Majority of the isolate showed entire margin while a few showed undulate margins. Majority of the isolates showed raised elevation. All the isolates were opaque in transparency and the texture varied from smooth, dry to rough. These diverse colony characteristics observed indicates the potential diversity of the actinomycetes isolated from various sources.

3.3. Antagonistic activity of the marine isolates against selected uropathogens

In primary screening by Agar overlay method,13 marine Actinomycetes SD65, SD66, SD67, SD68, SD69, SD70, SD71, V66, V67, V68, V69,V70 and V71 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 1.

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

Sl. No	Actino-mycete isolate	Response of UTI pathogens (Qualitative estimation)						
		Escherichia coli	*Klebsiella* sp.	*Klebsiellap neumoniae*	*Pseudo-monas* sp.	*Pseudomonas aeruginosa*	*Acinetobacter* sp.	*Acinetobacter baumannii*
1	SD65	+++	-	-	+	++	-	+
2	SD66	+	++	+	-	++	+	++
3	SD67	+	+	-	-	+	-	-
4	SD68	+++	+++	+++	+++	++	+++	+++
5	SD69	++	-	+	++	-	-	-
6	SD70	+++	+++	+	+++	+	++	++
7	SD71	-	++	++	+	++	++	++
8	V66	+	++	+	++	+	+	++
9	V67	-	-	-	+	+	-	+
10	V68	-	+	+	+	-	-	-
11	V69	+++	+++	+++	+++	+++	+++	+++
12	V70	+++	-	++	++	++	++	++
13	V71	++	-	+++	-	+++	++	+

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

In agar overlay method, the growth pattern of actinomycetes was not uniform on agar surface and hence the results were qualitatively reported in terms of positive and negative signs in par with the diameter of zone of growth inhibition produced by the actinomycetes isolates 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 denoted with negative sign (–). The isolates V67, V68 and V70 showed significant antagonism against the selected uropathogenic organisms in the primary screening. These 3 isolates were selected for the downstream studies.

3.4. Secondary screening of antagonistic activity of the isolates against common uropathogens

The antagonistic activity of these strains was further confirmed by secondary screening methods. The secondary screening of the isolates was done by two methods- well diffusion assay as well as disc diffusion assay. The results of well diffusion assay and disc diffusion assay were summarized in table 2 and 3 respectively.

All the 13 isolates displayed zones of inhibition against two or more pathogenic bacteria in the initial screening. There were three isolates among them that displayed noticeably broad spectrum activity against all of the

chosen uropathogenic bacteria. All of these findings point to the possibility of marine actinomycetes as a viable source of novel antimicrobial metabolites, which urges for extensive studies to address the issue of the clinical setting's ever-increasing antibiotic resistance with a focus on uropathogens.

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

Actinomycete isolate	Response of UTI pathogens (Qualitative estimation)						
	Escherichia coli	*Klebsiella* sp.	*Klebsiellapneumoniae*	*Pseudomonas* sp.	*Pseudomonas aeruginosa*	*Acinetobacter* sp.	*Acinetobacter baumannii*
V67	20	19	11	18	19	15	11
V68	20	17	19	17	21	19	16
V70	21	18	12	12	18	17	15

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

Actinomycete isolate	Response of UTI pathogens (Qualitative estimation)						
	Escherichia coli	*Klebsiella* sp.	*Klebsiellapneumoniae*	*Pseudomonas* sp.	*Pseudomonas aeruginosa*	*Acinetobacter* sp	*Acinetobacter baumannii*
V67	18	17	16	14	19	16	17
V68	14	16	18	16	15	18	19
V70	18	15	16	15	18	16	16

4. DISCUSSION

To treat constantly emerging, multi-drug resistant bacteria, the development of novel broad spectrum antibiotics is urgently necessary (MDR). Marine Actinomycetes are among the most significant groups of microorganisms that have been isolated from marine sources around the world. By producing secondary metabolites, they may provide a decisive treatment for bacteria that are resistant to a variety of drugs. Despite the abundance of study on terrestrial actinomycetes, the marine environment remains a promising source for the discovery of new actinomycetes [13-15].

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 Azhimala Beach on India's west coast yielded a total of 13 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 V67, V68 and V70 showed significant antagonism against selected uropathogens collected from a tertiary care center in Thiruvananthapuram, Kerala.

Till now, a few reports are available from actinomycetes to control multidrug-resistant bacteria. Recent reports on antimicrobial activity of *Streptomyces albofaciens* against MRSA showed inhibition zone of 21 mm; in another report, the *Streptomyces* species have a zone of inhibition 22 mm against MRSA [16]. The coastal region of Kerala is a biodiversity hotspot due to its unique ecological characteristic. Many studies are available on terrestrial actinomycetes but a few are available on marine actinomycetes. So the present study is worthwhile to explore the hidden treasures of marine actinomycetes diversity present on the coastal regions of Kerala.

In the primary screening, all the 13 isolates are showing antagonistic activity against two or more pathogenic strains. Among them, 3 isolates showing significantly broad spectrum activity against all the selected pathogenic strains. All these results are depicting the potential of marine actinomycetes as a promising source of novel antimicrobial metabolites which needs further studies to address the ever increasing antibiotic resistance in the clinical scenario with special reference to uropathogens.

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Conflicts of interest

The authors declare no conflicts of interest.

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