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CHARACTERIZATION AND ANTIMICROBIAL POTENTIAL OF MARINE ACTINOMYCETES FROM ESTUARIES OF UTTARA KANNADA DISTRICT, KARNATAKA

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ABSTRACT

The oceans cover about 70% of the Earth's surface and harbor most of the planets biodiversity. The marine environment has become a prime resource in search and discovery for novel natural products and biological diversity and marine actinomycetes turn out to be important contributors. The diversity of marine actinomycetes is enormous and has immense scope for the discovery of novel bioactive metabolites. The present study describes the antimicrobial potential of crude extracts of marine Actinomycetes species isolated from Aghanashini, Sharavathi and Kali estuaries of Uttara Kannada district, Karnataka, India. The sediment and water samples were collected from different sites of estuaries and subjected for serial dilution and plating. A total of 43 actinomycetes isolates were obtained on Starch Casein Nitrate Agar and Kusters Agar supplemented with 10% sea water. The isolates were identified as Actinomycetes by morphological and biochemical studies. The results revealed diversity of Actinomycetes with varying spore, aerial and substrate mycelium colours such as white, grey, pink, yellow and black. The colonies formed were discrete, powdery, raised and velvety colonies with brown, yellow and maroon pigmentations. The spore chain morphology studies showed different arrangements like rectus, flexibilis, retinaculum aperatum – open loops, hooks and spira – simple spirals. Primary screening for antimicrobial activity was determined by cross streak method against 16 bacterial isolates among which 07 isolates were gram positive and 09 isolates were gram negative, 02 yeasts viz., *Candida albicans* and *Cryptococcus neoformans* and a dermatophyte *Trichosporon*. The actinomycetes isolates from marine environments have shown to be potent in inhibiting the test bacteria and fungi. Further studies on cytotoxic potential of the actinomycetes isolates on cell lines are under progress.

Key words: Actinomycetes, Marine sediments, Estuary, Antimicrobial, Cross streak method.

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INTRODUCTION

Marine environment encompass about 71% of earth's surface. The microorganisms growing in marine environments are metabolically and physiologically diverse from terrestrial organisms. They have a diverse range of metabolic activities and metabolites[1]. Although marine plants and the invertebrates have received considerable attention as a resource for natural product discovery, the microbiological component of this diversity remains unexplored. Marine ecosystem is relatively untapped with regards to isolation of indigenous organisms, although existence of terrestrial origin has been reported [2, 3, 4].

Infectious diseases are leading health problems with high morbidity and mortality in developing countries. The development of resistance to multiple drugs is a major problem in the treatment of infectious diseases caused by pathogenic microorganisms. This multidrug resistance is presently an urgent focus of research and new bioactive compounds are necessary to combat these multidrug resistance pathogens. Marine derived antibiotics are more efficient at fighting microbial infections because the terrestrial bacteria have not developed any resistance against them[5, 6, 7]. Screening of microorganisms for the production of novel antibiotics has been intensively pursued for many years by scientists. Antibiotics have been used in many fields including agriculture and pharmaceutical industry [8,9,10].

In this regard Actinomycetes gain special importance as most potent source of antibiotics, bioactive secondary metabolites and therapeutically relevant natural products[11]. The actinomycetes are gram positive, free living, saprophytic bacteria and ubiquitous in nature. Majority of them are found in soil, fresh waters and surface of water bodies and also in sea water. They produce branching mycelium which is of two kinds *viz*., substrate mycelium and having characteristic long chain of arthrospores within aerial mycelium at a mature stage in their life cycle. These bacteria have high G + C (>55%) content in their DNA. Members of the actinomycetes, which live in marine environment, are poorly understood and only few reports are available pertaining to actinomycetes from marine sources[12, 13]. Recent studies have concluded that selected groups of marine actinomycetes are found to offer a reliable source of new natural products[2]. Streptomycetes and related actinomycetes continue to be useful sources of novel secondary metabolites with a range of biological activities that may ultimately find application as antiinfectives, anticancer agents or other pharmaceutically useful compounds[14, 15].

Uttara Kannada is the land of rivers. Uttara Kannada district (formerly North Kanara) is located between 13º55' to 15º32' North latitude and 74º05' to 75º 05' East longitude. Its geographic area is 10,291 km². The Coastal region consisting of 5 Talukas of Karwar, Ankola, Kumta, Honnavar and Bhatkal with its Coastal Bait of about 144 K.M. This Coastal Area is a fairly level plain, 5 to 10 miles in breadth and mainly consisting of rice fields, coconut gardens and Estuaries (Brakish Water). The Uttara Kannada district is unique in the Western Ghats, with regards to the distribution of biodiversity. This unique feature is probably due to its central location where takes place biogeographical transition between the northern and southern Western Ghats. Five major rivers of Uttara Kannada viz., Kalinadi, Gangavali (Bedti), Aghanashini, Sharavati and Venkatapura have their sources in the Sahyadris and flow west through the district into the Arabian Sea. Some of the magnificent spectacular waterfalls in the district such as the Jog falls, the Lushington falls (Unchalli) and Magod falls are associated with the rivers Sharavati, Aghanashini and Gangavali respectively. Also, where these rivers meet the sea, there are some of the finest estuaries of the west coast[16, 17].

Marine waters and sediments are found to be valuable source for the isolation of novel actinomycetes with the potential to yield useful products. The aim of the present study is concentrated on isolation, characterization, and screening of marine Actinomycetes for its antimicrobial potential from Aghanashini, Sharavathi and Kali estuaries of Uttara Kannada District of Karnataka.

MATERIALS AND METHODS

Collection of water and sediment samples

The water and sediment samples were collected from different sites of Aghanashini, Sharavathi and Kali estuaries. The sediment samples were collected at a depth of 10-25cm from surface and were placed in new sterilized polythene bags using sterile spatula, prior to laboratory analysis. The sediment samples were pretreated with $CaCO_3$ (10:1 w/w) and incubated at 37^0 for 4 days[18, 19]. Water samples were collected in the sterilized glass bottles and transported to laboratory and stored in refrigerator at 4º C until use[20].

Isolation of actinomycetes

The isolation and enumeration of actinomycetes present in the sediment and water samples were performed by serial dilution technique followed by plating on solidified Starch Casein Nitrate agar medium (SCN), Kuster agar medium (KUA), Actinomycetes Isolation Agar (AIA)(Himedia), Oat Meal agar (all the media were supplemented with 10% sea water)[21]. The inoculated plates were incubated at 30±2ºC for 7-14 days. Streak plate method was used to purify the marine actinomycetes colonies. Pure cultures were transferred on slants and preserved at 4ºC for further analysis. The media were supplemented with antibiotics Flucanozole and Griseofulvin to prevent fungal and bacterial contamination[22, 23]. A total of 43 isolates were obtained.

Morphological and staining characteristics

The morphological characteristics of isolates such as colour of aerial and substrate mycelium, production of diffusible pigments were studied. To study spore arrangements cover slip method was carried out. The coverslip method was performed by inoculating the pure cultures of the isolates on thin block of Starch Casein Agar and Kuster Agar which was supplemented with 10% sea water and placed on a grease free clean glass slide. After inoculation sterilised coverslip was placed on the agar block, the entire set up was then placed in a moist chamber and was incubated at 30±2ºC for 3-4 days. Moist condition was maintained by addition of sterile distilled water. After the incubation period, the coverslip were removed from the agar surface and were mounted on another slides by using crystal violet as staining reagent. The observation was done using binocular research microscope under oil immersion objective. The organisms were identified upto their genus level based on their characteristic spore chain arrangement[24]. All the isolates were subjected for Gram's staining and Acid-fast staining[25, 26].

Biochemical Characteristics

Biochemical tests such as Starch hydrolysis, Casein hydrolysis, Gelatin liquefaction, Hydrogen sulfide

production test, Citrate utilization, Methyl Red test, Voges Proskauer test, Urease test, Sugar utilization test (Lactose, Maltose, Starch, Dextrose, Sucrose), Nitrate Reduction test, and Catalase test were performed for the isolates[25, 26].

Antimicrobial activity:

Test bacteria

Gram positive bacteria -*Staphylococcus epidermidis, Streptococcus species, Bacillus cereus, Staphylococcus aureus, Streptococcus lutea, Bacillus subtilis, Streptococcus pyogenes*

Gram negative bacteria - *Vibrio cholerae, Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella aerogenes.*

Test fungi

Yeasts – *Candida albicans, Cryptococcus neoformans*

Dermatophyte –*Trichosporon* sp.

Primary screening of actinomycetes for Antimicrobial activity:

The primary screening was carried out by cross streak method for actinomycetes isolates on SCN and KUA media containing 10% sea water. The media was also amended with peptone and beef-extract to facilitate bacterial growth and dextrose for yeasts and dermatophyte growth. The plates were prepared and the Actinomycetes isolates were inoculated by a single line streak in the centre of the petriplate and were incubated for four days at 30±2°C. After 4 days the plates were then inoculated with the test organism's perpendicular to the growth of actinomycetes isolates and incubated for 24 hours in case of bacteria and 72 hours in case of fungi. The absence of growth or a less dense growth of test organism near the actinomycete isolate was considered positive for production and secretion of antimicrobial metabolite by the isolates[27, 28, 9].

RESULTS

Isolation of Actinomycetes

A total of 43 actinomycetes species were isolated from the water and sediment samples. Starch casein nitrate medium and Kuster agar medium were best medium for isolation that yielded high number of actinomycetes isolates followed by Oat meal agar and Actinomycetes Isolation agar.

Characterization of Actinomycetes:

Morphological and staining characterization:

All the 43 isolates were subjected for further studies. The results revealed a diverse morphological characteristics with varied spore colours, colony morphology, aerial and substrate mycelium colourations. The spore morphology showed different arrays of spore arrangement that varied from flexibilis, rectus, retinaculum aperatum –open loops, simple spira, hooks, compact spirals (Table 1; Figure 1 and 2). Based on the spore chain arrangements the isolates were assigned to be Actinomycetes. All the 43 isolates were found to be gram positive and non-acid fast.

Table 1: Morphological Characteristics of isolated Actinomycetes species

Isolates	Aerial Mycelium	Substrate Mycelium	Spore Arrangement	Diffusible pigment	Spore/ Colony morphology
SMRO 1	Light grey	Grey	Rectus straight	-	Valvety
SMRO 2	Blackish brown	Black	Flexibilis	-	Discrete
SMRO 3	White	Brown	Rectus	-	Powdery
SMRO 4	Green	Red	Rectus	Red	Powdery
SMRO 5	Grey	White	Verticillate	-	Powdery
SMRO 6	Black	Brown	Rectus	-	Raised
SMRO 7	Grey	White	Flexibilis		Valvety
SMRO 8	White	Yellow	Retinaculum spirals & hook	-	Valvety
SMRO 9	Grey	Grey	Flexibilis	-	Powdery
SMRO 10	Grey	Grey	Flexibilis	-	Raised
SMRO 11	Dark brown	Brown	Simple spirals	Brown	Raised
SMRO 12	Light pink	Maroon	Rectus	Pink	Valvety
SMRO 13	White	White	Retinaculum aperatum-hook	-	Raised
SMRO 14	Yellow	Orange	Flexibilis	-	Radial
SMRO 15	Black	Black	Rectus	-	Radial Concentric
SMRO 16	Cream	Grey	Rectus	-	Discrete
SMRO 17	White	Light brown	Rectus	-	Rugose
SMRO 18	White	Cream	Rectus straight	Brown	Raised
SMRO 19	White	White	Flexibilis	Brown	Powdery
SMRO 20	White	White	RA-Open loop	-	Radial
SMRO 21	White	Cream	Rectus	-	Valvety

SMRO 22	White	Brown	Flexibilis	Brown	Rugose
SMRO 23	Grey	Maroon	Rectus	Maroon	Powdery
SMRO 24	White	Cream	RA-extended spirals	-	Powdery
SMRO 25	Cream	Brown	Rectus straight	-	Powdery
SMRO 26	Cream	White	Flexibilis	-	Powdery
SMRO 27	White	Cream	RA- Hook	-	Valvety
SMRO 28	Grey	Deep pink	RA- Open loop	Pink	Powdery
SMRO 29	White	White	Flexibilis	-	Powdery
SMRO 30	Pink	Deep pink	Flexibilis	Pink	Powdery
SMRO 31	Light grey	Brown	Rectus	Brown	Raised
SMRO 32	Grey	Brown	Rectus	Brown	Raised
SMRO 33	Cream	White	RA-Hook	-	Powdery
SMRO 34	White	White	Rectus	-	Powdery
SMRO 35	Grey	Grey	RA-hook	-	Valvety
SMRO 36	White	White	Flexibilis	-	Radial
SMRO 37	Cream	Brown	Rectus	-	Powdery
SMRO 38	Grey	Grey	Rectus	-	Radial
SMRO 39	White	White	Rectus	-	Powdery
SMRO 40	Grey	White	Flexibilis	-	Powdery
SMRO 41	Grey	Grey	Rectus	-	Radial
SMRO 42	Black	Black	Rectus	-	Discrete
SMRO 43	Grey	Yellow	Flexibilis	-	Rugose

Figure 1: Representative Actinomycetes from Estuaries of Uttara Kannada District

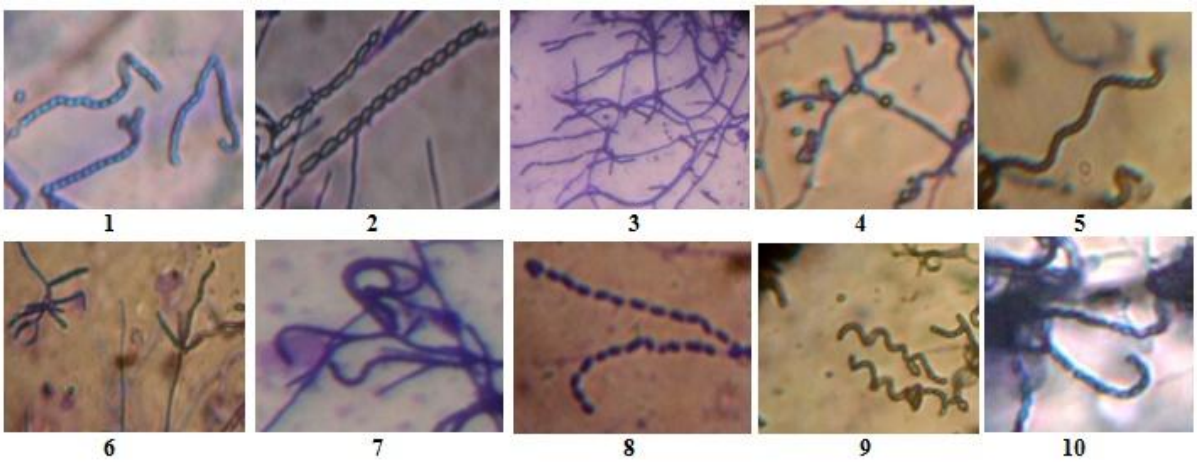

Figure 2: Spore chain arrangement (1-Hooks, 2- Rectus/ Straight, 3- Rectus, 4- Micromonospora, 5- Retinaculum aperatum (RA), 6- Verticillium, 7- Hooks, 8- Flexibilis, 9- Spiral, 10-Hooks)

Biochemical characterization

The biochemical characterization showed varied activities of the isolates. All the 43 isolates were positive for starch hydrolysis. 36 isolates were positive for catalase. 28 isolates were positive for gelatin hydrolysis, 41 isolates were positive for urease test. 32 isolates were positive for H_2S production. 37 isolates were positive for nitrate reduction. 38 isolates were positive for citrate utilization. 38 isolates were positive for casein hydrolysis. All the 43 isolates were negative for methyl red and voges proskauer test (Table 2).The carbohydrate utilization tests showed variable results and showed acid and alkaline production. The acid production was observed by colour change from red to yellow and there was no accumulation of the gas in the Durham's tube. The alkali production was observed by colour change from red to deep pink. 8 isolates showed positive for acid production in Dextrose, 7 isolates in Lactose and Maltose, 6 isolates in Sucrose and only 2 isolates in Starch. Majority of the isolates showed positive results for alkali production in all the tested sugars (Table 3).

Table 2: Biochemical characteristics of Actinomycetes isolates

Isolate No	Starch hydrolysis	Gelatin liquefaction	Casein Hydrolysis	Nitrate reduction	Catalase test	Citrate utilization	MR	VP
SMRO 1	+++	+	++	+++	+	+	-	-
SMRO 2	++	-	+++	++	+	++	-	-
SMRO 3	+++	+	++	+	+	++	-	-
SMRO 4	+	-	-	+	+	+++	-	-
SMRO 5	+++	+	+	+	+	++	-	-
SMRO 6	++	+	++	-	+	+	-	-
SMRO 7	+++	+	++	+++	+	-	-	-
SMRO 8	+++	+	++	+	+	-	-	-
SMRO 9	++	+	++	++	+	++	-	-
SMRO 10	+	+	+++	++	+	+++	-	-
SMRO 11	+	+	++	++	-	-	-	-
SMRO 12	++	+	++	+++	+	++	-	-
SMRO 13	+	-	++	++	+	-	-	-
SMRO 14	+++	-	+	++	+	++	-	-
SMRO 15	++	-	++	++	+	+	-	-
SMRO 16	+++	-	+	-	+	+	-	-
SMRO 17	+	-	++	+++	+	+	-	-
SMRO 18	+++	-	+++	++	+	+++	-	-
SMRO 19	+	+	++	++	+	++	-	-
SMRO 20	++	+	-	+	+	++	-	-
SMRO 21	+++	+	+	+	+	+	-	-
SMRO 22	++	+	++	+	-	+	-	-
SMRO 23	+++	-	++	++	+	++	-	-
SMRO 24	+++	+	++	+++	+	++	-	-
SMRO 25	+++	+	+	++	-	++	-	-
SMRO 26	+++	+	+	+++	-	+	-	-
SMRO 27	++	+	++	++	+	++	-	-
SMRO 28	++	+	+	+	-	-	-	-
SMRO 29	++	-	+	-	+	+	-	-
SMRO 30	+++	+	+++	+	+	+++	-	-
SMRO 31	+++	-	-	++	+	++	-	-
SMRO 32	++	+	++	+++	+	++	-	-
SMRO 33	+++	-	-	++	+	++	-	-
SMRO 34	++	-	+	-	+	+	-	-

SMRO 35	++	+	+++	+++	+	++	-	-
SMRO 36	+++	+	+++	++	+	++	-	-
SMRO 37	+++	+	-	++	+	++	-	-
SMRO 38	++	-	++	+++	+	++	-	-
SMRO 39	++	+	++	+++	+	+	-	-
SMRO 40	++	+	++	+++	+	+++	-	-
SMRO 41	+++	+	++	++	-	+	-	-
SMRO 42	++	-	+++	-	+	+++	-	-
SMRO 43	+++	+	+++	-	+	++	-	-

Table 3: Urease, H_2S and Carbohydrate Utilization Test

Isolate No	Urease test	H_2S test	Sugar Fermentation														
			Dextrose			Lactose			Sucrose			Maltose			Starch		
			A	G	Alk	A	G	Alk	A	G	Alk	A	G	Alk	A	G	Alk
SMRO 1	+	++	-	-	++	-	-	++	-	-	++	+	-	-	-	-	+++
SMRO 2	+	-	-	-	++	+	-	-	-	-	++	-	-	-	-	-	+++
SMRO 3	+	+	-	-	++	-	-	++	-	-	+	-	-	++	-	-	+++
SMRO 4	-	++	++	-	-	+	-	-	+	-	-	+	-	-	+	-	-
SMRO 5	+	+++	-	-	+++	-	-	+++	-	-	+++	-	-	+++	-	-	++
SMRO 6	+	-	-	-	+++	-	-	+++	-	-	-	-	-	-	-	-	+++
SMRO 7	+	+	-	-	+++	-	-	+++	-	-	++	-	-	++	-	-	+++
SMRO 8	+	++	-	-	+++	-	-	+++	-	-	++	-	-	+	-	-	+++
SMRO 9	-	+++	-	-	++	-	-	++	-	-	+++	-	-	++	-	-	+++
SMRO 10	+	-	-	-	+++	-	-	++	-	-	++	-	-	++	-	-	++
SMRO 11	+	++	-	-	++	-	-	+	-	-	+++	-	-	+++	-	-	++
SMRO 12	+	+	+	-	-	-	-	+	-	-	+	-	-	++	-	-	+++
SMRO 13	+	-	-	-	++	-	-	+++	-	-	+++	-	-	+++	-	-	+++
SMRO 14	+	+++	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
SMRO 15	+	++	-	-	++	-	-	++	-	-	++	-	-	++	-	-	+++
SMRO 16	+	+++	-	-	+++	-	-	++	-	-	++	-	-	+++	-	-	+++
SMRO 17	+	++	-	-	++	+	-	-	-	-	+++	+	-	-	-	-	+++
SMRO 18	+	-	-	-	++	-	-	+++	-	-	+++	-	-	++	-	-	+++
SMRO 19	+	++	+	-	-	-	-	+++	-	-	+	-	-	++	-	-	++
SMRO 20	+	+	-	-	+++	-	-	++	-	-	+++	-	-	+++	-	-	+
SMRO 21	+	-	-	-	+++	-	-	++	-	-	+++	-	-	++	-	-	+++
SMRO 22	+	+++	-	-	++	-	-	+	-	-	++	-	-	++	-	-	+++
SMRO 23	+	++	-	-	++	+	-	-	-	-	+++	-	-	+++	-	-	++
SMRO 24	+	+++	-	-	+++	-	-	+++	+	-	-	-	-	++	-	-	+++
SMRO 25	+	+	-	-	++	-	-	++	-	-	+	-	-	++	-	-	+++
SMRO 26	+	-	+	-	-	-	-	+	-	-	-	-	-	++	-	-	+++
SMRO 27	+	++	-	-	++	-	-	++	-	-	+	-	-	+++	-	-	++
SMRO 28	+	++	-	-	+++	-	-	+	-	-	+++	-	-	+	-	-	+++
SMRO 29	+	+	-	-	++	-	-	+++	-	-	+++	-	-	+	-	-	++
SMRO 30	+	-	-	-	+++	-	-	+++	-	-	++	-	-	+++	-	-	++
SMRO 31	+	+	+	-	-	-	-	+++	-	-	++	-	-	++	-	-	+++
SMRO 32	+	++	-	-	++	-	-	++	-	-	+++	-	-	+++	-	-	+++
SMRO 33	+	+++	-	-	+++	-	-	+++	-	-	++	-	-	++	-	-	+++
SMRO 34	+	++	-	-	+++	+	-	-	-	-	++	+	-	-	-	-	++
SMRO 35	+	-	-	-	++	-	-	+++	+	-	-	-	-	+++	-	-	+++
SMRO 36	+	++	+	-	-	-	-	++	-	-	+++	-	-	+	-	-	++
SMRO 37	+	+	-	-	+++	-	-	+	-	-	-	-	-	++	-	-	+++
SMRO 38	+	-	-	-	++	+	-	-	-	-	-	+	-	-	-	-	-
SMRO 39	+	++	+	-	-	-	-	++	+	-	-	-	-	+	-	-	+++
SMRO 40	+	++	-	-	++	-	-	+++	-	-	+++	+	-	-	-	-	+++
SMRO 41	+	-	-	-	++	-	-	+++	+	-	-	-	-	+++	-	-	++
SMRO 42	+	++	-	-	+	-	-	++	-	-	+++	-	-	+	-	-	+++
SMRO 43	+	++	-	-	+++	-	-	+++	-	-	+	-	-	+++	-	-	+++

('-' Negative, '+' Positive, '++' Good, '+++' Very good, A- Acid, Alk- Alkali, G- Gas)

Antimicrobial activity:

Antibacterial activity:

The primary screening of the isolates showed the antagonistic activity against the test organisms to a varied level. The antagonistic activity was measured as the interaction between the actinomycetes isolates and the test organisms. The antibiosis was seen as a clear zone around the actinomycetes isolates inhibiting the growth of test organisms. Majority of the isolates caused inhibition of one or more. Isolate no SMRO 13, SMRO 16, SMRO 20, SMRO 27, SMRO 32, SMRO 36 and SMRO 40 showed marked inhibition. Isolate no SMRO 22, SMRO 33, SMRO 34 and SMRO 43 were least inhibitory. Isolate no SMRO 23 and SMRO 42 were ineffective against all the bacteria tested (Table 4; Figure 3).

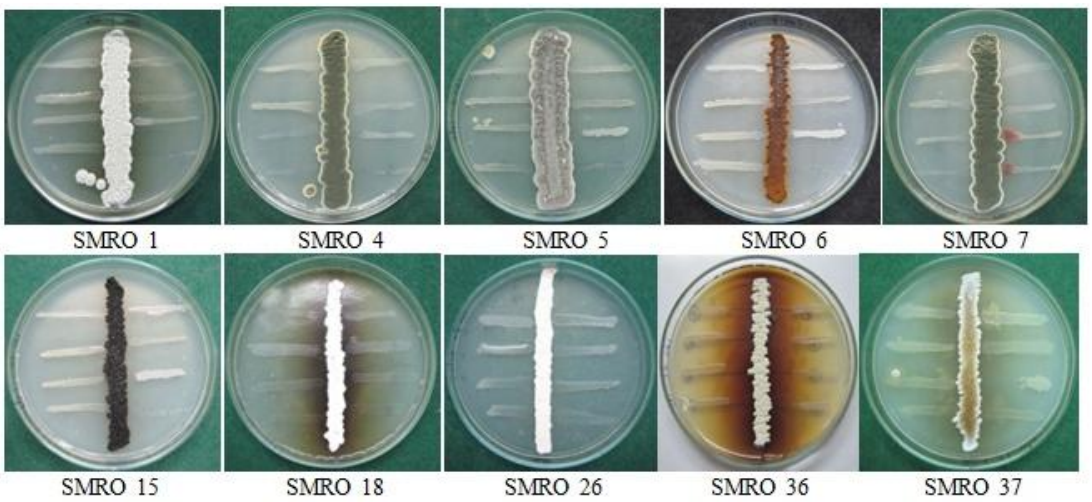

Figure 3: Antibacterial activity of some Actinomycetes isolates

Table 4: Primary screening for antibacterial activity

Isolate No	Extent of Inhibition															
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
SMRO 1	++	++	+	+	++	+	++	+	+	-	-	++	+	+	-	++
SMRO 2	-	-	-	-	-	++	++	+	-	++	-	-	-	+	+	+
SMRO 3	++	++	++	++	++	++	++	++	+	++	+	++	++	++	-	+++
SMRO 4	++	++	++	+++	++	+++	-	++	++	+++	+++	+++	++	++	++	+++
SMRO 5	-	++	++	++	-	++	+	-	-	+	+	+	-	+	+	+
SMRO 6	-	-	-	-	+	+	-	-	-	+	+	+	-	++	-	+++
SMRO 7	++	++	++	+	+	+	++	+	++	+	++	++	-	+	-	++
SMRO 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SMRO 9	+	+	++	+++	+++	+++	+++	++	++	++	+	+++	+++	++	++	++
SMRO 10	+	+	+	+	+	+	+	+	+	-	++	++	+	+	+	+
SMRO 11	-	-	++	++	-	-	+	-	-	-	-	+	-	-	-	-
SMRO 12	-	-	-	-	++	++	+	-	-	-	+	-	-	+++	++	+++
SMRO 13	++	+++	+++	+++	+++	+++	+++	+++	++	++	+++	++	++	+++	+++	+++
SMRO 14	-	-	-	-	+	+	-	-	-	-	+	++	+	+	-	+++
SMRO 15	-	+	++	-	+	+	-	-	+	+	+	+	++	+	+	+
SMRO 16	++	+	+++	+	++	+++	++	++	++	+	+++	+++	++	++	+	++
SMRO 17	+	++	+++	-	-	++	++	+	+	-	+++	+++	-	+++	-	+++
SMRO 18	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+
SMRO 19	+	-	-	-	++	++	+++	+++	-	-	+++	+++	-	++	+++	+++
SMRO 20	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+	+++	+	+++
SMRO 21	+	+	+	-	-	+	+	+	-	-	+	+	-	+	+	+
SMRO 22	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+
SMRO 23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

SMRO 24	-	-	++	++	-	-	-	-	++	+++	-	-	+	+	++	+++
SMRO 25	+	+	-	-	++	+++	-	-	-	-	+	++	+++	+++	+	-
SMRO 26	+++	++	+	+	-	-	+	+	++	++	-	-	++	++	+++	++
SMRO 27	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+	++	+	++
SMRO 28	-	-	-	-	-	++	-	-	-	-	++	-	++	+++	++	-
SMRO 29	+++	+++	-	-	+	+	++	++	-	-	+	+	++	++	+	+
SMRO 30	+	+	++	+++	+++	++	-	-	++	+++	+	+	++	-	-	+
SMRO 31	++	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	-	+++	-	+++
SMRO 32	++	++	+++	++	+++	++	+++	+++	++	+++	+++	+++	+	+++	+	+++
SMRO 33	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
SMRO 34	+	+	-	-	-	-	+	+	-	-	++	-	-	-	+	+
SMRO 35	++	+++	+++	++	+++	+++	+++	+++	+++	-	++	++	-	+++	+++	+++
SMRO 36	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	+++	++	+++	+++	+++
SMRO 37	+	+	+	++	-	-	-	+	-	-	-	+	-	-	++	+
SMRO 38	++	++	+	-	-	+++	+	-	++	+++	++	-	-	+	+	+
SMRO 39	+++	+++	++	-	++	+++	-	-	++	+++	-	++	+++	+++	+	++
SMRO 40	++	++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	++	++	++	+++
SMRO 41	++	++	+	-	-	+++	+	-	++	+++	++	-	-	+	+	+
SMRO 42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SMRO 43	+	-	+	+	+	+	-	-	-	-	+	++	+	-	-	-

('-' Negative, '+' Positive, '++' Good, '+++' Very good; B1- *V. cholerae*, B2- *E. aerogenes*, B3- *S. flexneri*, B4- *S. epidermidis*, B5- *K. aerogenes*,B6- *P. aeruginosa*, B7-*Streptococcus species*, B8-*B. cereus*,B9-*P. mirabilis*, B10- *S. sonnei*, B11- *S. aureus*, B12-*S. lutea*, B13-*K. pneumonia*,B14- *E. coli*, B15-*B.subtilis*, B16-*S.pyogenes*)

Antifungal activity

The primary screening of the isolates showed marked inhibition of the yeasts and dermatophyte. The antagonistic activity was measured as the interaction between the actinomycetes isolates and the test organisms. 32 isolates showed maximum inhibition of *C. neoformans* (74.41%). 27 isolates showed inhibition of *C. albicans* (62.79%). 10 isolates showed least inhibition of *Trichosporon* (23.25%). Isolate no SMRO 8, SMRO 14, SMRO 15, SMRO 18, SMRO 40 and SMRO 41 were ineffective against the yeast and dermatophyte tested (Table 5; Figure 4).

Figure 4: Antifungal activity of some Actinomycetes isolates

Table 5: Primary screening for antifungal activity

Isolate No	Extent of inhibition			Isolate No	Extent of inhibition		
	C. albicans	*C. neoformans*	*Trichosporon*		*C. albicans*	*C. neoformans*	*Trichosporon*
SMRO 1	+++	-	+	SMRO 23	++	+++	+
SMRO 2	-	+++	-	SMRO 24	++	+	-
SMRO 3	+	+++	-	SMRO 25	-	++	-
SMRO 4	-	+++	+	SMRO 26	-	+++	-
SMRO 5	+	++	-	SMRO 27	-	++	-
SMRO 6	+	++	-	SMRO 28	+	+++	+
SMRO 7	+	+++	++	SMRO 29	++	+++	-
SMRO 8	-	-	-	SMRO 30	-	+++	-
SMRO 9	-	+++	++	SMRO 31	++	++	-
SMRO 10	++	+++	-	SMRO 32	+	+++	-

SMRO 11	+	++	+	SMRO 33	+	+++	-
SMRO 12	++	-	+	SMRO 34	+	-	-
SMRO 13	++	++	-	SMRO 35	+	++	-
SMRO 14	-	-	-	SMRO 36	+++	++	+
SMRO 15	-	-	-	SMRO 37	+	++	-
SMRO 16	-	+++	-	SMRO 38	+	+++	-
SMRO 17	+	++	-	SMRO 39	-	++	-
SMRO 18	-	-	-	SMRO 40	-	-	-
SMRO 19	++	-	++	SMRO 41	-	-	-
SMRO 20	-	++	-	SMRO 42	++	-	-
SMRO 21	++	++	-	SMRO 43	++	+++	-
SMRO 22	+	+++	-				

('-' Negative, '+' Positive, '++' Good, '+++' Very good)

DISCUSSION

The Uttara Kannada district is unique in the Western Ghats with regards to the distribution of Biodiversity. The estuaries are one among many unique habitats. Major population of marine actinomycetes resides in ocean water and sediments and requires sea water for growth and adaptation. Actinomycetes are Gram positive eubacteria with high G+C content, found in terrestrial, fresh water and marine water and are responsible for the production of geosmin responsible for characteristic earthy odour. The actinomycetes found in marine environment are found to offer a reliable source of new natural products as these organisms have not been tapped for their exhaustive resources[29, 30]. Marine actinomycetes are of peculiar importance because they have high potential for bioactive compounds to be effective in the marine environment.

The present study was aimed to isolate the actinomycetes from the estuarine environment of Uttara Kannada district and screen them for the production of secondary metabolites. The sediment and water samples were collected from different sites of Kali, Sharavathi and Aghanashini estuaries. Serial dilution and plating was done for isolation of Actinomycetes. A total of 43 isolates were obtained from these three estuaries. Similar studies were carried out by[31, 32] showing presence of actinomycetes in soil samples of sea shores and estuary, mangrove forests[33], saline waters[34, 35], sediments[6, 36, 37], marine soils[4, 38, 39, 9].

The spore arrangement studies and biochemical characterization studies assists classification of majority of actinomycetes isolates as members belonging to the genus *Streptomyces*[40, 41, 42]. The results of morphological and biochemical characterization are tabulated in Table no 1, 2 and 3. The results obtained are in agreement with earlier studies of[13, 37, 43], who have isolated actinomycetes from marine environment and the genus *Streptomyces* alone accounts for a remarkable 80% of actinomycetes.

Cross streak method is one of the important technique used for preliminary screening of antimicrobial activities for actinomycetes. This method determines the inhibition potential of any microbe by producing antimicrobial compounds. This method was selected in our study for preliminary screening of marine actinomycetes for their antimicrobial potential against a panel of bacteria and fungi. It has become one of the routinely used methods and has been employed by several Researchers to study antimicrobial potential of actinomycete isolates[8, 31, 36, 44].

It has been reported that actinomycetes isolated from marine environment exhibit antimicrobial activity. The marine actinomycetes species from West coast of India[31], Mahabalipuram sea shores and Adyar estuary[32], shore area of Vishakhapatnam[36], Baten West Coast[37], from Puducherry Coastal Region[4], from Antarctica[45], from Hypersaline soils of Kolhapur district[46] have exhibited antibacterial activity. The present study was successful in isolating marine actinomycetes species with broad antibacterial and antifungal potential. The isolates have shown inhibition of both Gram positive bacteria and Gram negative bacteria. Maximum inhibition of *Candida albicans* and *Cryptococcus neoformans* was recorded. The inhibition of *C. neoformans* was higher than that of *C. albicans.* Similar results were observed in earlier studies of [31, 47]. *Trichosporon* was least inhibited by all the 43 isolates in our study.

It is anticipated that isolation, characterization and study of Actinomycetes can be useful in the discovery of novel species of Actinomycetes. These microorganisms are the most important sources of secondary metabolites. The results of the present investigation reveal that the marine actinomycetes from the estuarine environment are the potent source of novel antibiotics and can be explored for antibiotic production.

CONCLUSION

The marine environments are increasingly appreciated as a reservoir for bioactive natural products. The present study was successful in determining the diversity and antimicrobial activity of the actinomycetes species from the Estuaries of Uttara Kannada District. The Actinomycetes isolates have shown potential antimicrobial activity against the pathogenic bacteria and fungi. Further studies are under progress to characterize these organisms at molecular level and to study cytotoxic activity of the isolates on cancer cell lines.

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REFERENCES

1. Maskey RP, Kock I, Helmke E, Laatch H, "Isolation and Structure Determination of Phenazostatin D, a New Phenazine from Marine actinomycete isolate Pseudonocardia sp. B6273" Z. Naturforsch, 2003, 58 , 692-694.
2. Fenical W, Jensen PR, "Developing a new resource for drug discovery marine actinomycete bacteria" Nature Chemical Biology, 2006, 2: 666-673.
3. Solanki R, Khanna M, RupLal R, "Bioactive compounds from Marine actinomycetes" Indian Journal of Microbiology, 2008, 48, 410-431.
4. Priya AJ, Sagadevan E, Dhanalakshmi P, Satish Kumar S, Karthikeyan V, Armugam P, "Detection of Antioxidant activities in marine actinomycetes from Puducherry Coastal Region" Journal of Modern Biotechnology, 2012, 1(2), 63-69.
5. Donia M, Humann MT, "Marine natural products and their applications as anti-infective agents" Lancet Infect Dis, 2003, 3(6), 338-348.
6. Magarvey NA, Keller JM, Bernan V, Dworkin M, Sherman DH, "Isolation and Characterization of Novel Marine Derived Actinomycete Taxa Rich in Bioactive Metabolites" Applied and Environmental Microbiology, 2004, 70(12), 7520-7529.
7. Singh P, Trivedi B, Soma, "Antimicrobial potential of Actinomycetes against microbes isolated from Ayurvedic Drugs" International Journal of Pharmaceutical Research and Development, 2012, 3(12), 132-135.
8. Oskay M, Tamer UA, Azeri C, "Antibacterial activity of some actinomycetes isolated from farming soils of Turkey" African Journal of Biotechnology, 2004, 3(9), 441-446.
9. Kalyani ALT, Sravani RKM, Annapurna J, "Isolation and Characterization of antibiotic producing Actinomycetes from marine soil samples" International Journal of Current Pharmaceutical Research, 2012, 4(2), 109-112.
10. Dhinakaran DI, Manohari V, Atchya B, Tamilselvi K, Lipton AP, "Antifungal and Cytotoxic activities of some Marine Sponges Collected from the South East Coast of India" Journal of Applied Pharmaceutical Science, 2012, 2(1), 52-55.
11. Jayaprakashvel M, "Therapeutically Active Biomolecules from Marine Actinomycetes" Journal of Modern Biotechnology, 2012, 1(1), 1-7.
12. Siva Kumar K, "Actinomycetes of an Indian Mangrove (Pichavaram) environment: An Inventory" Ph.D. thesis, Annamalai University, India, 2001, pp-91.
13. Jensen PR, Mincer TJ, Williams PG, Fenical W, "Marine actinomycete diversity and natural product discovery" Antonie van Leeuwenhoek, 2005, 87, 43-48.
14. Bibb MJ, "Regulation of secondary metabolism in Streptomycetes" Current Opinion in Microbiology, 2005, 8, 208-215.
15. Ward AC, Bora N, "Diversity and biogeography of marine actinobacteria" Current Opinion in Microbiology, 2006, 9, 279-286.
16. Ramachandra TV, "Vegetation status in Uttara Kannada District" MJS, 2007, 6(1),1-26.
17. Naik AR, Technical Report of Uttara Kannada, 2009. P. 2-4.
18. Basha NS, Rekha R, Komala M, Ruby S, "Production of extracellular anti-leukemic enzyme L-asparaginase from marine actinomycetes by solid state and submerged fermentation: Purification and characterization" Tropical Journal of Pharmaceutical Research, 2009, 8(4), 353 – 360.
19. Vimal V, Rajan BM, Kannabiran K, "Antimicrobial activity of marine actinomycete, *Nocardiopsis* sp. VITSVK 5 (FJ973467)" Asian Journal of Medical Sciences, 2009, 1(2), 57-63.
20. Reddy NG, Ramakrishna DPN, Raja Gopal SV, "A morphological, physiological and biochemical studies of marine *Streptomyces rochei* (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms" Asian Journal of Biological Sciences, 2011, 1-14.
21. Imada C, Koseki N, Kamata M, Kobayashi T, Sato NH, "Isolation and Characterization of antibacterial substances produced by marine actinomycetes in the presence of seawater" Actinomycetologica, 2007, 21(1), 27-31.
22. Shrilling EB, Gottlieb D, "Methods of characterization of *Streptomyces* species" International Journal of Bacteriology, 1966, 61, 313-340.
23. Kannan N. Hand book of laboratory culture media, reagents, stains and buffers. Panima Publishing Corporation, New Delhi; 2003. P. 152-161.
24. Holt JG, Krieg RN, Sneath PHA, Staley JT, Williams ST, Bergeys manual of determinative Bacteriology, Lippincot Williams and Wilkins, Baltimore, Maryland. 9th edition; 2000.
25. Aneja KR. Experiments in Microbiology, Plant pathology, Tissue culture and Mushroom cultivation. 2nd Edition. Wishwa Prakashan, New Delhi; 1996.
26. Cappuccino and Sherman, Microbiology a Laboratory Manual. 4th edition. The Benjamin/ Cummings Publishing Company Inc; 1999.
27. Sahin N, Ugur A, "Investigation of the Antimicrobial activity of some Streptomyces isolates" Turkish Journal of Biology, 2003, 27, 79-84.
28. Haritha R, Siva Kumar K, Jagan Mohan YSYV, Ramana T, "Amylolytic and Proteolytic actinobacteria isolated from Marine Sediments of Bay of Bengal" International Journal of Microbiological Research, 2010, 1(2), 37-44.
29. Devi GY, Kumar LB, Lokeshwari N, Arjun P, Ramulu HS, "Isolation of the marine actinomycetes from the Bay of Bengal sediments" International Research Journal of Pharmaceutical and Applied Sciences, 2013, 3(2), 121-123.
30. Sirisha B, Haritha R, Mohan YSYVJ, Sivakumar K, Ramana T, 2013. Bioactive compounds from Marine actinomycetes isolated from the sediments of Bay of Bengal. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2013, 3(2), 256-264.
31. Remya M, Vijayakumar R, "Isolation and Characterization of Marine Antagonistic Actinomycetes from West Coast of India" Medicine and Biology, 2008, 15(1), 13-19.
32. Valli S, Sugasini SS, Aysha OS, Nirmala P, Kumar VP, Reena A, "Antimicrobial potential of Actinomycetes species isolated from marine environment" Asian Pacific Journal of Tropical Biomedicine, 2012, 469-473.
33. Haque U, Rahman A, Haque A, Sarker AS, Islam A, "Antimicrobial and Anticancer activities of Ethyl acetate extract of co culture of *Streptomyces* sp ANAM-5 and AIAH-10 isolated from Mangrove forest of Sundarbans, Bangladesh" Journal of Applied Pharmaceutical Science, 2012, 6(2): 51-55.
34. Sharma C, Mehtani P, Bhatnagar P, Mathur N, "Saline water: A potential source of Actinomycetes possessing antibacterial activity" International Journal of Pharmacy and Biological Sciences, 2013, 3(3): 242-251.
35. Sagar S, Esau L, Hikmawan T, Antunes A, Holtermann K, Stingl U, Bajic VB, Kaur M, "Cytotoxic and apoptotic evaluations of marine bacteria isolated from brine seawater interface of the Red sea" BMC complementary and Alternative medicine**,** 2013, 13-29.
36. Dasari VRRK, Nikku MY, Donthireddy SRR, "Screening of Antagonistic Marine Actinomycetes: Optimization of Process Parameters for the Production of Novel Antibiotic by Amycolatopsis Alba var. nov. DVR D4" Journal of Microbial and Biochemical Technology, 2011, 3(5), 92-98.
37. Sunaryanto R, Marwoto B, "Marine Actinomycetes Screening of Banten West Coast and their antibiotics purification" Biodiversitas, 2010, 11(4), 176-181.
38. Rajan CP, Priya MA, Jayapradha D, Devi SS, "Isolation and Characterization of Marine Actinomycetes from West Coast of India for Its Antioxidant Activity and Cytotoxicity" International Journal of Pharmaceutical and Biological Archives, 2012, 3(3), 641-645.
39. Bais YG, Nimbekar TP, Wanjari BE, Timande SP, "Isolation of Antibacterial compounds from marine soil actinomycetes" International Journal of Biomedical and Advance Research, 2012, 3(3), 193-196.
40. Gautham SA, Shobha KS, Onkarappa R, Kekuda TRP, "Isolation, characterization and antimicrobial potential of *Streptomyces* species from Western Ghats of Karnataka,

India" Research Journal of Pharmacy and Technology, 2012, 5(2), 233-238.
41. Kekuda PTR, Shobha KS, Onkarappa R, Gautham SA, Raghavendra HL, "Screening Biological Activities of a *Streptomyces* Species Isolated from soil of Agumbe, Karnataka, India" International Journal of Drug Development and Research, 2012, 4(3), 104-114.
42. Kekuda PTR, Onkarappa R, Gautham SA, Mesta SC, Raghavendra HL, "Antimicrobial, Antioxidant and Cytotoxic activity of *Streptomyces* species from Western Ghat Soils of Karnataka, India" Science, Technology and Arts Research Journal, 2015, 4(2), 164-180.
43. Haggag WM, Sehemy RIA, "Screening of Marine Actinomycetes for their Antifungal activities in Egypt" International Journal of Pharma and Bio Sciences, 2014, 5(4), 527-536.
44. Kekuda PTR, Dileep N, Junaid S, Rakesh KN, Mesta SC, Onkarappa R, "Biological activities of Streptomyces species SRDP-07 isolated from soil of Thirthahalli, Karnataka, India" International Journal of Drug Development and Research, 2013, 5(3), 268-285.
45. Nedialkova D, Naidenova M, "Screening The Antimicrobial Activity Of Actinomycetes Strains Isolated From Antarctica" Journal of Culture Collections, 2004, 4, 29-35.
46. Nakade DB, "Biodiversity of Actinomycetes in Hypersaline soils of Kolhapur district and their Screening as potential antibiotic producer, India" Research Journal of Recent Sciences, 2012, 1, 317-319.
47. Shobha KS, Onkarappa R, "*In vitro* susceptibility of *C. albicans* and *C. neoformans* to potential metabolites from Streptomycetes*"* Indian Journal of Microbiology, 2011, 51(4), 445-449.