Indian Journal of Geo-Marine Sciences
Vol. 44(1), November 2015, pp. ------

# Isolation and characterization of antagonistic *Streptomyces* spp. from marine sediments along the southwest coast of India

Rekha Devi Chakraborty[*†] , Kajal Chakraborty[#] & Bini Thilakan[#]

[*]Crustacean Fisheries Division, [#] Marine Biotechnology Division, Central Marine Fisheries Research Institute, Kochi - 682018, India.

[E.Mail:rekhadevi76@yahoo.com ]

Received ; revised

Antagonistic *Streptomyces* spp. were isolated from marine and mangrove sediment samples collected off Cochin, along the southwest coast of India. Sediment samples were pre-treated and following the soil dilution technique, samples were surface plated on starch casein agar and actinomycetes isolation agar. In the primary screening, 7.4% of presumptive actinomycetes (135 isolates) showed antibacterial activity against one or more bacterial fish pathogens and 3.7% of these cultures showed broad spectrum activity against the tested pathogens. Morphologically white powdery colonies with chalky white /grey appearance were selected as presumptive *Streptomyces* cultures. Isolates subjected to biochemical, physiological and 16S rDNA characters revealed the presence of three species of *Streptomyces* dominated by *Streptomyces tanashiensis* followed by *S. viridobrunneus* and *S. bacillaris*. Isolates characterized by 16S rDNA indicated the presence of 650 bp band in *Streptomyces* spp. Primary screening for activity against selected fish pathogens was done by a cross streak method using modified nutrient agar medium. Prominent isolates showing high zone of activity against the fish pathogens ranged 17-35 mm by the paper disc method. Enriched broth of selected isolates showing high antagonistic activity was screened for pharmacologically active agents revealed ethyl acetate fractions to be active against selected microbial pathogens.

**[Keywords:** *Actinomycetes, Streptomyces*, sediments, 16S rDNA, PCR ]

## Introduction

Disease control and management in the fish culture system has become one of the major problems as the fish bacterial pathogens are becoming more and more resistant to the conventional therapeutic drugs used in the industry causing heavy financial losses to the fish farmers. The need of new antimicrobial agents from marine origin is greater than ever because of the emergence of multidrug resistance in common pathogens[1]. Previous studies revealed that marine derived antibiotics are more efficient at fighting microbial infections because the terrestrial bacteria have not developed any resistance against them[2]. *Actinomycetes* spp. are generally considered as a good source of antibiotics and bioactive molecules and thus considered to be a rich biological resource [3-5].

*Streptomyces* is one of the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae[6] with over 500 species in it[7] characterized by a complex secondary metabolism[8]. They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g., neomycin, chloramphenicol, streptomycin, gentamicin, etc.)[9]. Nearly 9,500 antibiotics from actinomycetes have been reported by 2008, of which 85% are reported to belong to the genus *Streptomyces* producing prolific active secondary metabolites[10]. But members of the actinomycetes from marine and mangrove origin are poorly understood and only few reports are available[11-13]. Many reports from India stated that, the east-coast area is a major source of streptomycetes[14-18] but, only a few reports are available pertaining to streptomycetes diversity in west-coast of India[13,19].

Antibiotics and other chemotherapeutic agents are commonly used in fish farms either as feed additives or immersion baths to achieve either prophylaxis or therapy. Historically a large percentage of the world's natural antibiotics have been found in a group of micro-organisms known collectively known as *Streptomycetes*. So, there is a need for the search of novel bacterial strains or bio-active compounds with therapeutic potential which can be used to control the bacterial disease in an eco-friendly manner in aquaculture. In the present study, actinobacteria belonging to the *Streptomyces* genus were isolated from marine and mangrove sediments collected off Cochin along the southwest coast of India and characterized based on the morphological, biochemical, physiological, 16S rRNA partial gene sequencing and secondary metabolites were

tested for antagonistic activity against the bacterial fish pathogens.

**Materials and Methods**

Sediment samples were collected from Mangalavanam mangrove area and marine sediment from three different depths *viz*., near shore (1-2 m), 10 m, and 20 m located off Cochin waters along the southwest coast of India for a period of one year during 2011 by using sediment sampler grab (Van Veen grab). Sampling stations were located in the Arabian sea off Cochin at the following Latitude 9.96°67' N, and Longitude 76° 21' E. Approximately 100 g sample from the surface of each grab was collected from each site in a pre-labelled sterile containers and transported to the laboratory in aseptic conditions.

After sample collection, it was mixed properly and divided into four portions of 30 g each. One of the sample was treated with $CaCO_3$ (10:1w/w) and the other was processed without treatment and both these samples were given pre-heat treatment at 50 $^oC$ and 70 $^oC$ for a period of 15 min. After treatment, 10 g sample from each site was diluted with 90 ml 50% seawater and serial dilutions were made upto $10^{-6}$. About 0.5 ml of diluted sample was plated onto starch casein agar (SCA) and actinomycetes isolation agar (AIA) (Himedia, Mumbai, India) by the spread plate technique and incubated at 28 $^oC$ for the isolation of *Actinomycetes*. The SCA and AIA medium were supplemented with cycloheximide (CH: 80µg / L) and nalidixic acid (NA: 25µg / L) to prevent bacterial and fungal contaminants. Plates without CH and NA served as control to assess the effect of these chemicals. Petriplates were incubated at 28 ± 2 $^0C$ and were observed for bacterial colonies from 4$^{th}$ day onwards upto a period of 20 - 40 days. Presumptive *Streptomyces* colonies with powdery appearance were picked out and purified by repeated streaking on yeast malt extract agar medium (ISP No: 2). Purified cultures were maintained in ISP No: 2 agar slants for further investigation.

The potent *Actinomycetes* isolates giving broad spectrum activity were characterized according to the recommendations of International *Streptomyces* Project (ISP) [20] based on morphological, biochemical and physiological methods. Morphological method consists of macroscopic and microscopic characterization. Macroscopically the actinomycetes isolates were differentiated by their colony characteristics *viz*., size, shape, aerial mass colour, reverse side pigments and melanoid pigments in yeast extract and malt extract agar. For the microscopy, the isolates were grown by coverslip culture method [21]. They were then observed for their arieal, substrate mycelial structure and spore formation under light microscope (100X). The observed morphology of the isolates was compared with the *Actinomycetes* morphology provided in Bergey's Manual for the presumptive identification of the isolates.n

For physiological and biochemical characterization, their growth on media having four different concentrations of NaCl (2%, 5%, 7% and 10%), pH (5, 8, 9 and 10), temperature (15, 25, 37 and 42$^oC$), utilization of eleven different sugars and fourteen biochemical properties were studied following standard methods [20,22-23].

Antimicrobial activity was determined by cross streak plating as described by Lemos et al.[24] using the standard strains *Vibrio parahaemolyticus* ATCC 17802, *Vibrio vulnificus* MTCC 1145, *Vibrio alginolyticus* MTCC 4439 and few cultures confirmed as bacterial fish pathogens were collected from Central Institute of Brackishwater Aquaculture (*Aeromonas hydrophilla, V. anguillarum* A1, *V. anguillarum* O1, *Vibrio harveyi* 101). In brief, the test streptomycetes culture was streaked in the middle of the petridish poured with modified nutrient agar medium (glucose 0.5 g; peptone 0.5 g; beef extract 0.3 g; NaCl 0.5 g; agar 1.5 g; distilled water: 100 ml) and incubated at 28 ± 2 $^0C$ for 3-4 days. When thick growth of the culture was observed, young cultures of the test pathogens were streaked at right angles to the actinomycetes and all the plates were incubated at 28 ± 2$^oC$ for 24 h and inhibition zone (mm) was measured, simultaneously control plates were also streaked with the pathogens without the streptomycetes culture.

Isolates that showed activity against the test microorganisms were streaked on the nutrient agar plates supplemented with 1% NaCl (Himedia) and incubated for 6-7 days at 30 $^oC$. Spent agar was pooled from all the plates and extracted with different solvents (chloroform, methanol, alcohol, ethylacetate) 1:1 (v/v) and shaken vigorously for 2-3 h for complete extraction. The solvent was decanted and filtered and passed through sodium sulphate and filtrate was collected and concentrated in a pre-weighed vial. Solvent phase contains antibiotic substances separated from aqueous phase was used to determine antimicrobial activity. The crude extracts were resuspended in methanol at concentration of 1mg per ml for antimicrobial studies. Sterile filter paper discs 6 mm in diameter (Hi-Media, India) were impregnated with 50 µl (50 µg crude antibiotic) suspension, dried and placed onto the plates previously seeded with the test microorganism. Then the plates were kept at 4 °C for at least two hours to allow the diffusion of crude extracts. Then they were incubated for 24 hours at 37 °C and the diameter of inhibition zone were measured. Each test was repeated three

times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the secondary metabolite when compared to controls[14].

*Streptomycetes* cells were harvested by centrifugation, and cell pellets were suspended in TEG buffer (10 mM Tris HCl, 1 mM EDTA, glucose 0.1mM, pH 8.0) and cells were lysed with lysozyme. Samples were treated with sodium dodecyl sulfate (SDS: 10% (w/v)) and incubated at 30 °C for 10 min. DNA was purified from proteins and other cellular constituents using an equal volume of chloroform-isoamyl alcohol (24:1) followed by the addition of phenol: chloroform-isoamyl alcohol (25:24:1, by volume) & centrifugation (10,000xg) for 5 minutes after each step and DNA was precipitated by chilled absolute alcohol (2 volume). DNA pellets was washed with 70% (w/v) ethanol before drying under vaccum. The purified DNA was resuspended in TE buffer, quantified by absorption spectrophotometry at 260 nm and stored at -20$^{o}$C until further use.

PCR targeting for 16S rDNA partial gene sequence with specific primers (forward: 5'CGCGGCCTATCAGCTTGTTG3' and reverse 5'CCGTACTCCCCAGGCGGGG3') of actinobacteria was performed from the isolates as described previously[25] using a thermocycler (Bio-rad Mastercycler Germany). In order to find the correct annealing temperatures for this pair of primers, gradient PCR was carried out in a Bio-rad thermalcycler. PCR amplification was optimized in a total reaction volume of 25 µl consisting of sterile Milli Q water (18.7 µl), PCR buffer (2.5 µl), each primer (1.0 µl), dNTP mix (0.5 µl, 200 mM), template (1 µl), and Taq DNA polymerase (0.3 µl). The components were mixed well and the PCR amplification of the target sequence was arranged in a thermocycler (Bio-rad Mastercycler, Germany), and programmed for 35 cycles of amplification. Each cycle consisted of three step reactions i.e., initial denaturation (94$^{o}$ C, 5 min) followed by 35 cycles of denaturation (94 $^{o}$C, 1 min), annealing (62 $^{o}$C, 1 min) and extension (72 $^{o}$C, 1 min) followed by final extension (72 $^{o}$C, 5 min). PCR products were resolved on agarose (1.5% w/v) gel electrophoresis. The gel was stained with ethidium bromide (0.5 mg/ml) and visualized under a UV transilluminator (Gel Doc 2000 Gel Documentation System from Bio-Rad Laboratories, USA). GeneRuler$^{TM}$ 100 bp DNA Ladder (MBI Fermentas, USA) was used as a molecular size marker. PCR products were eluted as per the procedure given in Gel extraction kit (Sigma GenElute$^{TM}$ Gel extraction kit cat. No. NA IIII).

The PCR products after eluting were sequenced using an ABI 3730 Automated Sequencer (Applied Biosystems), and the sequences were edited using the program BioEdit[26] and submitted to the BLAST function of GenBank to check their homology with the published sequences. The 16S rRNA gene sequences were aligned using CLUSTAL-X software[27] in the program BioEdit[26]. A neighbor joining[28] phylogenetic tree was generated using MEGA 4.0 software[29], with a bootstrap analysis[30] of 1,000 replicates; a distance matrix was generated using Kimura's 2-parameter model[31]. Finally sequences were edited and submitted to NCBI (Sequin software) for their accession numbers.

**Results**

The sampling site off Cochin is located on the southwest coast of India (Latitude (N) 9.96º` and Longitude (E) 76º27`). A total of 135 strains were isolated from the mangrove and marine sediment samples at various depths (1-2 m, 10 m and 20 m) and designated as MSA1-MSA135 based on their colony morphology observed on the master plate. Presumptive colonies of streptomycetes started developing on the petriplates from the seventh day onwards and counting was done for a period of 28-40 days. Population density of streptomycetes varied with sample treatment and culture media used for isolation. Colony size varied from small to medium, powdery, and colour varied from chalky white and grey (Figure 1A – 1D).

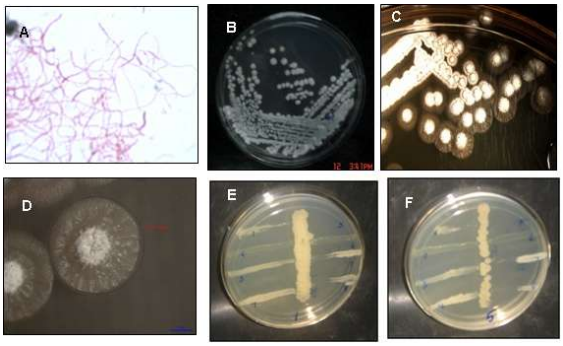


**Fig. 1.**Pure culture of *Streptomyces* spp. isolated from marine sediments. A: Filamentous Gram Positive colonies of *Streptomyces* observed under oil immersion light microscope (100X); B: streak plate showing pure culture of *Streptomyces* powdery white colonies; C &D: Streptomyces colonies observed under stereozoom light microscope

Samples which were given pre-heat treatment at 70 $^{o}$C for 15 min yielded more number of isolates, when compared to treatment

at 50 $^{o}$C for 15 min or air drying and treatment with $CaCO_3$. But the samples treated with $CaCO_3$ were found to give potent streptomycetes isolates. SCA yielded more isolates when compared to AIA along with the various pretreatment techniques. So, a combination of treatment was standardized *i.e.*, samples were treated with $CaCO_3$ and isolated from SCA were found to yield more potent isolates from the marine origin.

In the primary screening only ten isolates MSA4 (R1), MSA17 (R2), MSA36 (R3), MSA44 (R4), MSA59 (R5), MSA63 (R6), MSA84 (R7), MSA111 (R8), MSA123 (R9), MSA134 (R10) were found to give antimicrobial activity against one or two bacterial pathogens (Figure 1E, 1F). Of the ten isolates 6 active isolates (R4, R6, R8, R9, R10, and R1) were obtained from mangrove sediment and rest from marine sediment (R2, R3, R5, and R7). Among the ten isolates three isolates from mangrove sediment and two from marine sediment (MSA44 (R4), MSA59 (R5), MSA84 (R7), MSA123 (R9), MSA134 (R10) ) were found to give broad spectrum activity (zone of inhibition: 17- 35 mm) against all the selected aquaculture pathogens (Table. 1). These isolates were selected and characterized both by conventional biochemical and molecular methods. All experiments were done in triplicates. Maximum active cultures were obtained from 20 m depth followed by 10 m depth and none of the cultures obtained from nearshore region were found to have broad spectrum activity. Four cultures showing broad spectrum activity were obtained from 20 m depth and one such culture was from 10 m depth (Figure 2A-2D).

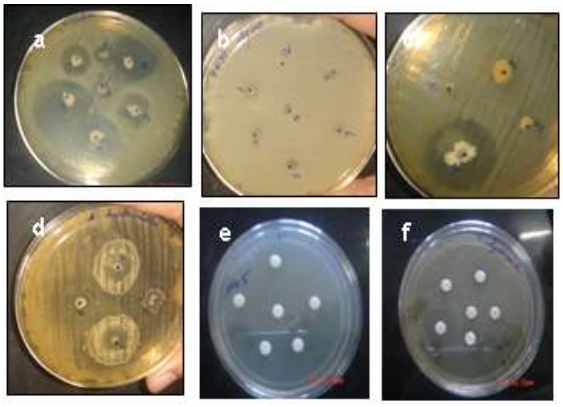


**Fig. 2.**Primary screening of presumptive *Streptomyces* cultures by cross streak method against test pathogens for antagonistic activity

These isolates exhibited a marked antagonistic activity against all the Gram negative bacterial pathogens causing diseases in aquaculture (Table 1). The ethyl acetate extract (1000 µg/ml) obtained from the isolate showed significant antimicrobial activity when compared with the standard, streptomycin (25 µg / disc). Antibacterial activity exhibited by the crude extract was equivalent to that of the activity of streptomycin (Figure 2E, 2F).

The observed morphology of the isolates was compared with the *Actinomycetes* morphology provided in Bergey's Manual for the presumptive identification of the isolates. The aerial mycelium is unbranched, white in color with sparse substrate mycelium. In the optimized yeast extract agar (ISP1) medium supplemented with sea water the isolate produces white spore mass. In brief, the isolated strains were Gram positive, non-motile exhibiting various biochemical characteristics (Table. 2) revealed the presence of *Streptomyces tanashiensis*, (4 strains), *S. viridobrunneus* (4 strains) and *S. bacillaris* (2 strains). Out of these ten active strains only 5 strains showed broad spectrum activity against the aquaculture pathogens and they belonged to *Streptomyces tanashiensis*, (3 strains), *S. viridobrunneus* (1 strains) and *S. bacillaris* (1 strain). Growth of these isolates was noticed at different temperatures (25, 30, 37, 42$^{o}$C); pH (8, 9, 10) and NaCl concentrations (2%, 5%, 7%, 10%).

Polymerase chain reaction assay using the purified DNA samples obtained from the 10 specific cultures with PCR conditions using 16S rRNA specific primers revealed the presence of product with a band size of 650 bp (Figure 3). The BLAST (Basic local alignment search tool) search of the 16S rDNA sequence of the isolates showed maximum (100%) similarity with *Streptomyces* spp. Sequences were submitted to GenBank of NCBI for their accession numbers as *viz*., JX155770 (R1), JX155771 (R2), JX155772 (R3), JX155773 (R4), JX155774 (R5), JX155775 (R6), JX155776 (R7), JX155777 (R8), JX155778 (R9), JX155779 (R10).

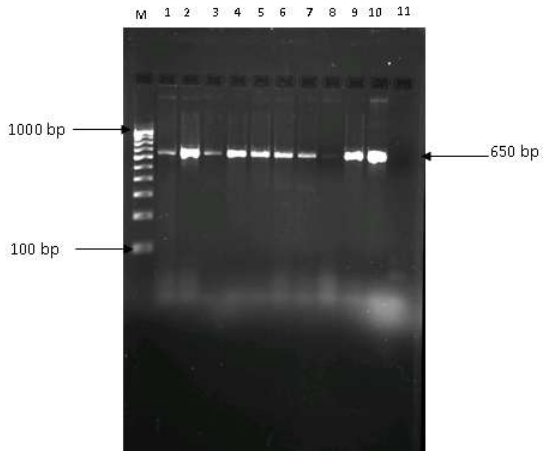


**Fig. 3**. PCR for the detection of Actinomycetes specific 16S rRNA gene (650 bp). Lane M: DNA ladder (100 bp); Lanes 1 to 10: Actinomycetes cultures MSA 4, 17, 36, 44, 59, 63, 84,111,123,134 ; Lanes 1–3: Actinomycetes cultures isolated from marbouth sediment; Lanes 4-6: cultures isolated from sediment collected at 10 m depth and Lanes 7-10: cultures isolated from sediment samples collected from 20 m depth; lane 11: PCR negative control.

Table 1 Antibacterial activity of the potent isolates and ethylacetate extract against the selected bacterial pathogens

| Aquaculture pathogens | Streptomycin (25 µg/disc) | Zone of inhibition (mm) at 30°C after 48 h | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MAS44 | | MAS59 | | MAS84 | | MAS123 | | MAS134 | |
| | | isolate | extract | isolate | extract | isolate | extract | isolate | extract | isolate | extract |
| *A. hydrophilla* | *34* | 32 | 32 | 14 | 15 | 25 | 34 | 24 | 35 | 40 | 32 |
| *V. anguillarum O1* | *25* | 17 | 25 | 15 | 20 | 30 | 25 | 20 | 18 | 25 | 28 |
| *V. parahaemolyticus* | *35* | 35 | 33 | 20 | 30 | 34 | 33 | 18 | 25 | 27 | 35 |
| *V. harveyi* | *30* | 30 | 30 | 30 | 23 | 34 | 30 | 17 | 19 | 23 | 29 |
| *V. anguillarum A1* | *18* | 20 | 23 | 30 | 20 | 22 | 15 | 25 | 15 | 24 | 15 |
| *V. alginolyticus 4439* | *29* | 25 | 25 | R | 25 | 17 | 32 | 20 | 26 | 25 | 23 |
| *V. vulnificus 1145* | *25* | 15 | 30 | 20 | 23 | R | 25 | 20 | 23 | 40 | 25 |

**R: resistant**

Table 2 Biochemical characteristics of the potent isolates with broad spectrum activity

| Tests | MAS44 | MAS59 | MAS84 | MAS123 | MAS134 |
|---|---|---|---|---|---|
| Gram staining | + | + | + | + | + |
| Catalase | + | + | + | + | + |
| Oxidase | + | + | + | + | - |
| MR | - | - | - | - | - |
| VP | - | - | - | - | - |
| Indole | - | - | - | - | - |
| H2S production | + | + | + | + | - |
| Nitrate reduction | - | - | - | - | - |
| Citrate utilization | - | - | - | - | - |
| Casein hydrolysis | - | - | - | - | - |
| Gelatin liquefaction | + | + | + | + | + |
| Urea | - | - | - | - | - |
| Triple sugar iron agar | alkaline | alkaline | alkaline | alkaline | Alkaline |
| Acid production from | | | | | |
| Arabinose | - | - | - | - | - |
| Galactose | - | - | - | + | + |
| Glucose | + | + | + | + | + |
| Mannitol | - | - | - | - | + |
| Raffinose | - | - | - | - | - |
| Salicin | - | - | - | - | - |
| Xylose | - | - | - | - | - |
| Sucrose | - | - | - | - | - |
| Rhamnose | - | - | - | - | - |
| Inositol | - | - | - | - | - |
| Fructose | - | - | - | - | + |
| Growth at 15°C | - | - | - | - | + |
| Growth at 25°C | + | + | + | + | + |
| Growth at 37°C | + | + | + | - | + |
| Growth at 42°C | + | + | + | - | + |
| Growth at pH 5.0 | - | - | - | - | - |
| Growth at pH 8.0 | + | + | + | + | + |
| Growth at pH 9.0 | + | + | + | + | + |
| Growth at pH 10.0 | + | + | + | + | + |
| Growth at 2% NaCl | + | + | + | + | + |
| Growth at 5% NaCl | + | + | + | + | + |
| Growth at 7% NaCl | - | - | - | - | - |
| Growth at 10% NaCl | - | - | - | - | - |

MAS44, MAS59, MAS84: *Streptomyces tanashiensis*; MAS123: *S. viridobrunneus*; MAS 134: *S. Bacillaris*

Phylogenetic tree was constructed with the ten strains (R1, R2, R3, R4, R5, R6, R7, R8, R9, R10) isolated from marine sediments and these strains having homology with the similar sequences were downloaded from NCBI, an outgroup (*Bacillus subtilis*) was added for bootstrap analysis using neighbour joining method (Mega 4.0 software). The cluster analysis revealed the presence of 2 major clusters under the root. The cluster showing 99% similarity (R1, R2, R3) formed a single group and rest of the isolates clustered into another group while *Bacillus subtilis* which was included as an outgroup formed a separate cluster (Figure 4).

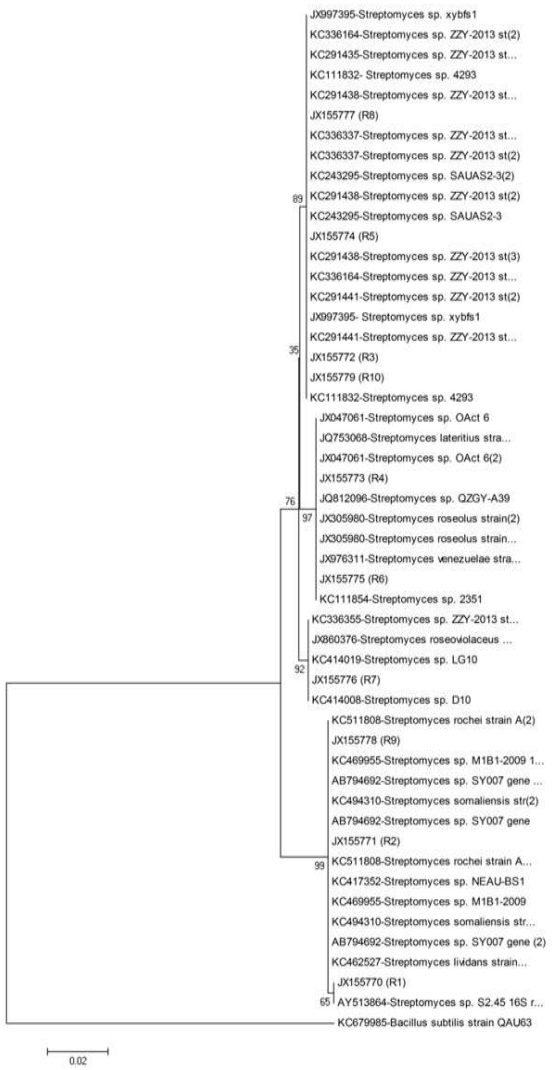


**Fig. 4**. Dendrogram illustrating the clustering of 16S rRNA profile of *Streptomyces spp*. from marine sediment samples. Bars are shown at each node, values at each node represents the replications of 100 corresponding to the standard deviation of values in that region of the similarity matrix. The average and the standard deviation of similarity values for the selected nodes are shown above the dendrogram. The similarity scale is shown below this dendrogram

## Discussion

In the present study, marine sediment samples were collected from mangrove area and from three different depths off Cochin along the southwest coast of India for a period of one year with an objective to isolate antagonistic *Streptomycetes*. Different media along with various pre-treatments was tried for the isolation of these Gram positive bacteria. The results of this study indicated that not only the population density of streptomycetes but also more number of isolates showing broad spectrum activity were obtained from the samples plated on starch casein agar treated with $CaCO_3$ collected from mangrove area and deeper waters (20 m). Isolates with broad spectrum activity were also obtained from sediments collected from 10 m and 20 m depth but none of the isolates obtained from nearshore region were found to give potent activity. Reasons attributed for this can be the dominance of pathogenic microflora in these areas. However, few authors have reported the incidence of antagonistic actinobacteria from surface seawaters[32] growing in various media[23]. Sahoo & Dhal[33] (2009) reported mangrove ecosystems as a source of predominant potential microbes having high specificity for various applications. Chakraborty et al.[34] reported the antibacterial properties of crude extracts obtained from *Ulva fasciata*. Identification of potent strains by both morphological and growth characteristics revealed the presence of white and grey isolates with powdery appearance having aerial hypae and substrate mycelium.

In the present study only 3.7% of the strains having broad spectrum antagonistic activity against all the tested aquaculture pathogens were found. While Alexander[35] found above 20% of the isolates exhibiting antibacterial activity, whereas Ellaiah and Reddy[36] reported little lesser percentage (18%) of the actinomycetes to have the antimicrobial activity. These isolates showing activity against the tested bacterial fish pathogens were characterized based on morphological characteristics[37, 20, 38]. Further based on the biochemical, physiological and 16S rRNA partial gene sequencing data all (100%) the isolates were assigned to the genus *Streptomyces* revealed the presence of *Streptomyces tanashiensis* as the dominant species followed by *S. viridobrunneus* and *S. bacillaris* having broad spectrum antimicrobial activity against the tested fish pathogens. In a similar study carried out by Remya and Viyayakumar[32] reported *Streptomyces* as the dominant genera (47%) isolated from Kerala coast having antibacterial and antifungal property but a very meager number of these isolates (two) showed broad spectrum activity. Frequency and dominance of *Streptomyces* among actinomycetes in various soil types were reported by several workers[15, 18, 39, 40]. Almost all the *Streptomyces* isolates studied here were found to exhibit similar morphological and biochemical characters with the published literature[32]. Methods for the isolation of genomic DNA and PCR was standardized using the primers specific for 16S rRNA and sequencing results showed 100% homology for *Streptomyces* spp confirming their identity to the genus level. All these five isolates were identified to species level based on their biochemical characteristics viz., *Streptomyces tanashiensis*, (3 strains), *S. viridobrunneus* (1 strains) and *S. bacillaris* (1 strain).

Cultures were deposited to MTCC, Chandigarh. Phylogenetic tree constructed by neighbour joining method using Mega 4.0 software indicated the presence of 2 major clusters under the root. The cluster showing 99% similarity belonged to the *Streptomyces*

*tanashiensis*, while 76% similarity was found in the clusters of *S. viridobrunneus* and *S. bacillaris*, respectively. In a similar study carried by Usha et al.[41], along the east coast isolated a strain belonging to *Streptomyces parvulus* KUAP106 having not only inhibitory effect against the prokaryotic and eukaryotic microorganisms but also antiangiogenisis activity. 16S rRNA gene sequence analysis of this study revealed that strain *S. tanashiensis* was most closely related to a similar strain, *Streptomyces tanashiensis* (Kala UC-5063) isolated by Johnson and Dietz[42] from which 'Kalafungin' a new antimicrobial agent having inhibitory activity against a variety of pathogenic fungi, yeasts, protozoa, gram-positive bacteria, and, to a lesser extent, gram-negative bacteria was isloated. Dastager et al.,[43] reported the isolation and identification of *S. gulbargensis* isolated from soil samples of Karnataka. This particular strain was able to produce alkaline protease and was further used to remove the blood stains from surgical instruments and cotton cloths[44]. 16S rRNA gene sequence data analysis in many other species of actinomycetes like *Micromonospora* revealed that they are active in the anti-tumor. Indeed, this also applies to the isolates assigned to some other taxa, such as the genera *Rhodococcus, Nomomuraea and Verrucosispora,* thereby indicating that marine habitats are likely to be a good source of rare actinomycetes. It is becoming increasingly evident that the taxonomic and metabolic diversity encompassed by *streptomycetes* is remarkable, as new and putatively novel *Streptomyces* species are being continuously isolated from under-researched habitats and shown to be valuable sources of new bioactive compounds[45-47]. The 16S rRNA gene sequence data acquired in the present study provided further evidence of this trend.

## Conclusion

Based on the screening result, it has been shown that the marine sediments off Cochin along the southwest coast of India has a potential for the isolation of antibiotic producing streptomycetes against the bacteria causing diseases in aquaculture thereby underpinning and extending results from previous studies which showed marine sediments at higher depths to be the source of bioactive *Streptomyces*. Further, these antibiotic producing *Streptomyces* may be tapped as one of the potential source for bioactive metabolites to provide high quality biological material for high throughput biochemical, anti-cancer and anti-infection screening programmes.

## Acknowledgements

Authors are grateful to Dr. G. Syda Rao, Director, Central Marine Fisheries Research Institute, Cochin for providing necessary facilities and encouragement for carrying out the study

### References

1. Spellberg B, Powers J H, Brass E P, Miller L G & Edwards J E, Trends in antimicrobial drug development: implications for the future, *Clin Infect Dis,* 38 (2004) 1279-1286.
2. Donia M & Hamann M T, Marine natural products and their potential applications as anti-infective agents, *Lancet Infect Dis*, 3 (2003) 338-348.
3. Chun J, Blackall L L, Kang S O, Hah Y C, & Goodfellow M, A proposal to reclassify *Nocardia pinensis* Blackall et al. as *Skermania piniformis* gen nov, comb Nov, *Int J Syst Bacteriol,* 47 (1997) 127-131.
4. Lechevalier H & Lechevalier A, Biology of actinomycetes, *Ann Rev Microbiol*, 21 (1967) 71-100.
5. Goodfellow M & Williams S T, Ecology of Actinomyces, *Ann Rev Microbiol*, 37 (1983) 189-216.
6. Kampfer P, *The Family Streptomycetaceae,* Part I: Taxonomy, The prokaryotes: a handbook on the biology of bacteria (Springer, Berlin) (2006) 538–604.
7. Euzeby J P, Genus *Streptomyces*, List of Prokaryotic names with Standing in Nomenclature, (2008). http://www.bacterio.cict.fr/s/streptomycesa.html.
8. Madigan M & Martinko J, *Brock Biology of Microorganisms* (Prentice Hall, New Jersey) 2005.
9. Kieser T, Bibb M J, Buttner M J, Chate K F & Hopwood D A, *Practical Streptomyces Genetics* (John Innes Foundation, Norwich, England) 2000.
10. Williams S T, *Streptomycetes in soil ecosystem* (Gustav fischer Verlag, Stuttgart) 1978, pp. 137-142.
11. Sivakumar K, *Actinomycetes of an Indain mangrove (Pichavaram) environment: An inventory* Ph.D. thesis, Annamalai University, India, 2001.
12. Vikineswary S, Nadaraj P, Wong H C & Balabaskaran S, Actinomycetes from a tropical mangrove ecosystem-Antifungal activity of selected strains, *Asian Pac J Mol Biol Biotechnol*, 5 (1997) 81-86.
13. Ratnakala R & Chandrika V, Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments, *Indian J Mar Sci*, 22 (1993) 297-299.
14. Sambamurthy K & Ellaiah P, A new Streptomycin producing Neomycin (B and C) Complex, S. marinensis (part-1), *Hind Antibiot Bull*, 17 (1974) 24-28.
15. Balagurunathan R, *Antagonistic actinomycetes from Indian shallow sea sediments with reference to α, β unsaturated δ-lactone, type of antibiotic from Streptomyces griseobrunneus.* Ph.D. thesis, Annamalai University, India, 1992.
16. Dhanasekaran D, RajaKumar G, Sivamani P, Selvmani S, Paneerselvam A & Thajuddin N, Screening of salt pans actinomycetes for antibacterial agents, *Intl J Microbiol*, 1 (2005) 1-8.
17. Vijayakumar R, Muthukumar C, Thajuddin N, Panneerselvam A & Saravanamuthu R, Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India, *Actinomycetologica,* 21 (2007) 59-65.
18. Gupta N, Mishra S & Basak UC, Diversity of *Streptomyces* in mangrove ecosystem of Bhitarkanika, *Iranian Journal of Microbiology*, 1(3) (2009) 37-42.

19. Baam R B, Gandhim N M & Freitas Y M, Antibiotic activity of marine microorganisms, Helgo-laender Wiss, *Meeresunters,* 13 (1966) 181-187.
20. Shirling E B & Gottlieb D, *Methods, classification, identification and description of genera and species* (The Williams and Wilkins Company, Baltimore) 1966, pp. 61-292.
21. Kawato M & Shinolue R, *A simple technique for the microscopical observation. In Memoirs of the Osaka university liberal arts and education* (Yamadaoka suita Osaka, Japan) 1959, p. 114.
22. Holding A J & Collee J G, *Routine Biochemical Tests* (Academic Press, New York) 1971, pp. 1-34.
23. Locci R, *Streptomycetes and Related Genera* (Williams and Wilkins, Baltimore) 1989, pp. 2451-2491.
24. Lemos M L, Toranzo A E & Barja J L, Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds, *Microb Ecol,* 11 (1985) 149-163.
25. Bredholt H, Fjaervik E, Johnsen G & Zotchev S B, Actinomycetes from sediments in the Trondheim Fjord, Norway: Diversity and biological activity, *Mar Drugs* 6 (2008) 12–24.
26. Hall U A, BioEdit, a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucl Acids Symp Ser,* 41 (1999) 95–98.
27. Thompson J D, Gibson T J, Plewniak F, Jeanmougin F & Higgins D G, The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res*, 25 (1997) 4876–4876.
28. Saitou N & Nei M, The neighbor-joining method: a new method for reconstructing phylogenetic trees, *Mol Biol Evol*, 4 (1987) 406–425.
29. Tamura K, Dudley J, Nei M & Kumar S, MEGA4, Molecular Evolutionary Genetics Analysis (MEGA software version 4.0), *Mol Biol Evol*, 24 (2007) 1596–1599.
30. Felsenstein J, PHYLIP (phylogenetic inference package,version 3.2), *Cladistics,* 5 (1989) 164–166.
31. Kimura M, A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences, *J Mol Evol,* 16 (1980) 111–120.
32. Remya M & Vijayakumar R, Isolation and characterization of marine antagonistic actinomycetes from west coast of India. Facta Universitatis series, *Medicine and Biology,* 15 (2008) 13-19.
33. Sahoo K, Dhal N K, Potential microbial diversity in mangrove ecosystems *Indian J Ma. Sci*, 38 (2009) 249-256.
34. Chakraborty K, Lipton A P, Paulraj R & Chakraborty RD, Antibacterial properties of green seaweed Ulva fasciata (Delile), *Eur J Med Chem,* 45 (2010) 2237-2244.
35. Alexander M, *Introduction to Soil Microbiology* (John Wiley and Sons Inc., New York) 1961.
36. Ellaiah P & Reddy A P C, Isolation of actinomycetes from marine sediments of Visakhapatnam East Coast of India, *Indian J Mar Sci*, 16 (1987) 134-135.
37. Waksman S A, The Actinomycetes resistant bacteria, *J Arid Environ*, 53 (1961) 365-371.
38. Nonomura H, Key for classification and identification of 458 species of the *Streptomyces* included in ISP, *J Ferment Technol*, 52 (1974) 78-92.
39. Saadoun I & AI-Momani F, Frequency and dominance of grey series *Streptomyces* in Jordan soils, *Actinomycetes,* 9 (1998) 61-65.
40. Peela S, Bapiraju K & Terli R, Studies on antagonistic marine actinomycetes from the Bay of Bengal, *World J Microbiol Biotechnol*, 21 (2005) 583-585.
41. Usha R, Ananthaselvi P, Venil C K & Palaniswamy M, Antimicrobial and Antiangiogenesis activity of Streptomyces Parvulus KUAP106 from Magrove soil, *European Journal of Biological Sciences,* 2 (2010) 77-83.
42. Johnson L E & Dietz A, Kalafungin a new antibiotic produced by *Streptomyces tanashiensis* strain Kala, *Appl Microbiol*, 16 (1968) 1815-1821.
43. Dastager S G, Wen-Jun Li, Dayanand A, Sulochana M B, Shu-Kun Tang, Xin Peng Tian, Xiao-Yang Zhi, Streptomyces gulbargensis sp. nov isolated from soil in Karnataka, India, *Antonie Van Leeuwenhoek,* 91 (2006) 99-104.
44. Vishalakshi N, Lingappa K, Amena S, Prabhakar M & Dayanand A, Production of alkaline protease from *Streptomyces gulbargensis* and its application in removal of blood stains, *Indian Journal of Biotechnology,* 8 (2009) 280-285.
45. Bull A T, Stach J E M, Ward A C & Goodfellow M, Marine actinobacteria: perspectives, challenges, future directions, *Antonie Van Leeuwenhoek,* 87 (2005) 259–276.
46. Okoro C K, Brown R, Jones A L, Andrews B A, Asenjo J A, Goodfellow M & Bull A T, Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile, *Antonie Van Leeuwenhoek*, 95 (2006) 121-133.
47. Fiedler H P, Bruntner C, Bull A T, Ward A C, Goodfellow M, Potterat O, Puder C. & Mihm G, Marine actinomycetes as a source of novel secondary metabolites, *Antonie Van Leeuwenhoek,* 87 (2005) 37–42.