Vol.57, n.3: pp. 349-356, May-June 2014
http://dx.doi.org/10.1590/S1516-89132014005000006
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

In vitro Antimicrobial Activity and Characterization of Mangrove Isolates of Streptomycetes Effective against Bacteria and Fungi of Nosocomial Origin

Arijit Das*, Sourav Bhattacharya, Abuelgasim Yegoup Hassan Mohammed and Subbaramiah Sundara Rajan
Department of Microbiology; Genohelix Biolabs; A Division of Centre for Advanced Studies in Biosciences; Jain University; Bangalore - Karnataka - India

ABSTRACT

The study aimed at determining the in vitro antimicrobial activity of alkaliphilic and halotolerant actinomycetes isolated from a mangrove ecosystem and identification of a potent strain. Twenty five isolates of actinomycetes were isolated from the sediment samples of Valapattanam mangrove swamp in Kerala, India. Antimicrobial activity of four selected actinomycete isolates was determined against bacterial and fungal pathogens of nosocomial origin by agar well diffusion method. Molecular characterization of the potent isolate was performed by 16S rDNA sequencing. Isolate no I-1 significantly inhibited Staphylococcus aureus *ATCC 25923 (12 mm),* S. aureus *(15±0.05 mm),* S. citreus *(20±0.5 mm),* Bacillus cereus *(17±0.2 mm) and* Serratia marcescens *(12 mm). It also demonstrated effective antifungal action against* Penicillium *sp. (12±0.2 mm),* Candida albicans *(20±0.5 mm),* C. parapsilosis *(12 mm) and* Cryptococcus neoformans *(12 mm). Morphological study revealed that all the isolated actinomycetes belonged to the genus* Streptomyces*. Based on 16S rDNA sequence data, the selected isolate I-1 was shown to be closely related to* Streptomyces xiamenensis*. The results revealed that the mangrove ecosystem of Valapattanam harboured a rich consortium of many potent actinomycetes, which could synthesize novel bioactive compounds of pharmacological significance.*

Key words: *Streptomyces xiamenensis*, Actinomycetes, Antimicrobial activity, Mangrove swamp

INTRODUCTION

Due to emergence of multidrug-resistant strains of bacteria and fungi, especially those causing nosocomial infections, there is an ever-increasing demand for novel antibiotics with broad antimicrobial spectra. Actinomycetes constitute a diverse group of microorganisms that are widely distributed in terrestrial, freshwater and marine habitats (Radhika et al. 2011). Compared to terrestrial actinomycetes, very few studies have been conducted on marine actinomycetes. Marine ecosystem constitutes oceans, the deep sea and the sea floor, estuaries and lagoons, salt marsh and intertidal zones, coral reefs and mangrove swamps.

Mangrove forests consist of woody trees and shrubs that plentifully thrive in the saline sediments of tropical and subtropical coastline. Due to the presence of high salinity, high temperature, extreme tides, high sedimentation and high evaporation the muddy anaerobic mangrove sediment differs from the terrestrial one in terms of the microbial diversity (Giri et al. 2011). The mangrove ecosystem remains largely unexplored, and therefore offers excellent

*Author for correspondence: jit2007das@gmail.com

opportunity for finding rare actinomycetes with unique properties, capable of producing many novel bioactive compounds such as antibiotics, enzymes and antitumor agents. Rare actinomycetes are considered as the strains whose isolation frequency by conventional methods is much lower than that of commonly occurring actinomycete strains (Naikpatil and Rathod 2011). Among the actinomycetes, species of *Streptomyces* constitute second highest percentage after *Nocardia* in terms of distribution in the mangrove ecosystem (George et al. 2012). They are prolific producers of secondary metabolites and about 80% of total antibiotics are produced from the species of *Streptomyces* (Sathiyaseelan and Stella 2011).

The coastal states of peninsular India are lined with numerous mangrove forests. These habitats offer unique ecological niche supporting the occurrence and interactions between many rare microbial forms. Therefore, the present study aimed at determining the *in vitro* antimicrobial activity of alkaliphilic and halotolerant actinomycetes isolated from a mangrove ecosystem and identification of a potent strain of actinomycete effective against the bacterial and fungal pathogens of nosocomial origin.

MATERIALS AND METHODS

Collection and processing of sediment samples

Seven samples of fresh sediments were collected from the Valapattanam mangrove ecosystem (11° 54′ 0″ N, 75° 22′ 0″ E) in Kannur district of Kerala, India during September 2011. Sediment samples (rich in plant debris) were collected with an auger (down to 10 cm depth) after removing approximately 5.0 cm litter of fallen leaves near the root system of *Avicennia marina.* Samples were stored in sterile zip-lock polythene packets, transported to the laboratory and stored at 4°C. After the determination of pH values, the sediment samples were air dried at room temperature, mixed thoroughly and sieved through a 2-mm pore size sieve (Retsch, Haan, Germany) to remove the large debris. The sieved samples were used for the isolation of actinomycetes.

Isolation of actinomycetes

Sieved sediment sample (1.0 g) was suspended in 100 mL sterile distilled water and incubated in an orbital shaker (Orbitek) at 28°C and 200 rpm for 1 h (Boroujeni et al. 2012). Particles were allowed to settle and then serial dilutions of the spore suspension were prepared up to 10^{-4}. From each dilution, 0.1 mL was spread evenly over the surface of starch casein nitrate (SCN) agar plates (supplemented with cycloheximide 50 μg mL^{-1}) with sterile L-shaped glass rod and incubated at 28°C for 10 days (Kuster and Williams 1964). Dilutions that yielded 30-300 colonies were chosen for further study. Actinomycete isolates were purified by streak-plate technique and the pure cultures were maintained on SCN agar slants at 4°C for further use.

Preliminary screening of actinomycetes for antagonistic activity

The test bacterial pathogens included *S. aureus* ATCC 25923, *S. aureus*, *S. citreus*, *B. cereus*, *Escherichia coli*, *S. marcescens*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. The test fungal pathogens included *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Fusarium* sp., *C. albicans*, *C. parapsilosis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans*. All these test bacterial and fungal pathogens were hospital isolates obtained from the Kempegowda Institute of Medical Sciences, Bangalore. For screening of antibacterial activity, pure isolates of actinomycetes were streaked at the centre of sterile SCN agar plates and incubated at 28°C for 5 days. Broth cultures of the test bacterial pathogens were inoculated into molten Mueller Hinton agar and overlaid on SCN agar plates. After solidification, the plates were incubated at 37°C for 24 h. Screening for the antifungal activity was performed by agar double layer method using Sabouraud dextrose agar medium and incubated at 27°C for 48 h. Antimicrobial activity was noted by observing the area of inhibition surrounding the streaked actinomycete isolate.

Production and extraction of antimicrobial metabolites

The selected actinomycete isolates were separately inoculated into sterile SCN broth and incubated at 28°C for 7 days in an orbital shaker at 150 rpm. The broth cultures were filtered using Whatman No. 1 filter paper and centrifuged at 5000 rpm for 30 min at 4°C (Hettich Universal 32R, Germany). The supernatants were aseptically transferred to sterile tubes and partially purified by adopting the method specified by Muiru et al.

(2007). The purified extracts were stored at 4°C for further assay.

Antimicrobial activity of actinomycete isolates

The antimicrobial activity of purified extracts derived from the actinomycetes was determined against all the test bacterial and fungal pathogens using agar well diffusion method. Test cultures of the bacterial pathogens were prepared in Mueller Hinton broth and incubated at 37°C. The 24 h old bacterial cultures were swabbed onto sterile Mueller Hinton agar plates. Wells were punched with a sterile cork borer (6 mm internal diameter) and 35 µL of the extract was added to each well. Ampicillin and streptomycin (50 mg/mL) were used as standard antibiotics for Gram positive and Gram negative bacteria, respectively. Similarly, spore suspensions of the fungal pathogens were prepared in Sabouraud dextrose broth. After swabbing the sterile Sabouraud dextrose agar plates with the suspensions, 35 µL of the extract was added to each well. Fluconazole (20 mg/mL) was used as the standard antifungal. The inoculated plates containing the extracts were incubated first under refrigeration for 6 h for diffusion of the extracts. The bacterial plates were then incubated at 37°C for 24 h. The fungal plates were incubated at 27°C for 48 h. Following incubation, diameters of the inhibitory zones were measured to the nearest millimetre and recorded.

Characterization of actinomycete isolates

The actinomycete isolates that exhibited effective antimicrobial activities were characterized morphologically by observing the spore chains of the Gram stained smears of 10 days old colonies. The organisms were identified by observing the structures and comparing with Bergey's Manual of Determinative Bacteriology (Bergey and Holt 2000). Colour of aerial mycelia was determined from the mature, sporulating aerial mycelia of the actinomycete colonies on SCN agar (Hamedani et al. 2012). Colour of the substrate mycelia (reverse of the plate) was also observed along with diffusible pigments, if any (Padmadhas and Ragunathan 2010). Physiological characterization such as the effect of pH (3.3-9.3), temperature tolerance (4-42°C), salt tolerance (0.1-10% w/v NaCl) and melanin production were analyzed.

Extraction of genomic DNA from the actinomycete isolate

The most potent actinomycete isolate was inoculated in SCN broth and incubated at 28°C for 7 days in an orbital shaker at 130 rpm. Genomic DNA was extracted using the Fungal Genomic DNA Isolation Kit RKT 41/42 (Chromous Biotech Pvt. Ltd., Bangalore, India) according to the manufacturer instructions and visualized using 0.8% agarose gel (with ethidium bromide) electrophoresis (Hamedani et al. 2012).

PCR amplification

DNA amplification by polymerase chain reaction (PCR) was performed in a total volume of 25 µL. Each reaction mixture contained the following solutions: 1.5 µL genomic DNA, 1.0 µL 10 pmol forward universal 16S rDNA primer (5'-AGAGTTTGATCCTGGCTCA-3') (Sigma, USA); 1.0 µL of 10 pmol reverse universal 16S rDNA primer (5'-ACGGCTACCTTGTTACGACT-3') (Sigma, USA); 1.0 µL of 30 mm deoxyribonucleoside 5'-triphosphate (N= A,T,G,C) (dNTP's); 2.5 µL of 10X PCR buffer and 1 µL Taq polymerase (1 U) (Chromous Biotech Pvt. Ltd., Bangalore, India) and water was added up to 25 µL. The thermal cycler (MJ Research PTC 200, USA) was programmed as follows: 2 min initial denaturation at 94°C, followed by 30 cycles that consisted of denaturation for 1 min at 94°C, annealing for 30 s at 57°C and extension at 74°C for 1 min and a final extension of 5 min at 74°C. The PCR-amplified product was detected by 1.2% agarose gel (with ethidium bromide) electrophoresis and the results were visualized under UV light using gel documentation system (Herolabs, Germany) (Hamedani et al. 2012).

Partial 16S rDNA sequencing and analysis of sequenced data

The partial 16S rDNA sequencing of the PCR-amplified product was performed at Chromous Biotech Pvt. Ltd., Bangalore, India. The 16S rDNA sequence data was aligned manually with the available nucleotide sequences retrieved from NCBI database by using BLASTn (Prapagdee et al. 2008). This nucleotide sequence was also submitted to GenBank database under an accession number.

RESULTS AND DISCUSSION

The rapid emergence and widespread occurrence of multidrug-resistant strains of bacteria and fungi, especially those causing nosocomial infections, has threatened the treatment regimen with conventional antibiotics. Several such cases are being reported worldwide (Ruscher et al. 2010). A recent study reported that nosocomial infections at the surgical sites were responsible for 42.9% of the mortality cases in paediatric living donor liver transplantation (Nafady-Hego et al. 2011).

Actinomycetes are ubiquitously prevalent diverse groups of prokaryotic microorganisms, which are known to synthesize a wide range of bioactive metabolites such as enzymes, antibiotics, pigments, antitumor compounds and immunosuppressive agents (Valli et al. 2012). They are specially recognized for their ability to synthesize various secondary metabolites, which possess antibacterial, antifungal and antiprotozoal activities (Ravikumar et al. 2011). Actinomycetes are present in terrestrial, marine as well as fresh water habitats (Boroujeni et al. 2012). They are also found in brackish water and estuarine ecosystems.

Mangrove ecosystem consists of pneumatophores bearing woody plants, which grow in waterlogged saline soil of intertidal coasts in the tropical and subtropical zones. They mainly occur along the coastline at the confluence of rivers and sea. This ecosystem produces large amounts of detritus (organic matter) due to the autolysis and microbial decomposition of fallen leaves, twigs, flowers and fruits. Abundance of these organic matters, salinity and high degree of moisture content favour the prevalence of actinobacterial population and other life forms in the mangrove ecosystem (Nag et al. 2012). These alkaliphilic actinomycetes are naturally capable of producing different plant fibre hydrolyzing enzymes and secondary metabolites (Tsujibo et al. 2003).

Isolation of actinomycetes from mangrove sediment

All the sediment samples were collected post monsoon in order to obtain a diverse population of actinomycetes. The sediment samples showed pH values ranging from 6.5 to 8.3. Twenty five isolates of actinomycetes were recovered from the sediment samples collected from the rhizosphere of *A. marina.* Some previous studies have reported the isolation of novel species of actinomycetes from the mangrove habitats. In a study investigating the actinobacterial population from the Pitchavaram mangrove forests in Tamil Nadu, India, maximum number of actinomycetes were recovered from the rhizosphere of *A. marina.* About 50% of the isolates revealed activity against *S. aureus, Bacillus subtilis, E. coli, Vibrio cholerae, K. pneumoniae, Proteus vulgaris* and *S. typhi* (Balagurunathan et al. 2010). It could be deciphered from these findings that a diverse group of pharmacologically potent actinomycetes remained in close association with the roots of these plants, protecting them from the adverse environmental conditions and successfully competing with various soil borne pathogens (Kumaresan and Suryanarayanan 2001). Ravikumar et al. (2011) also studied the biodiversity of actinomycetes in the Manakkudi mangrove ecosystem located in Tamil Nadu, India and found maximum population in the rhizospere soil.

Antibacterial activity of actinomycete isolates

Preliminary screening of all the actinomycetes for antagonistic activity against the pathogenic bacteria and fungi revealed four isolates, which showed better antimicrobial spectra. These four isolates were designated as I-1, I-2, I-3 and I-4. The secondary metabolites produced by these four isolates were extracted and partially purified. The antimicrobial activity of these four isolates is presented in Table 1. Among the test bacteria, *S. citreus* was maximally inhibited by I-1 (20±0.5 mm) and I-2 (14±0.05 mm), whereas, *S. aureus* was inhibited by I-3 *(*16±0.5 mm) and I-4 (16±0.2 mm), respectively. Interestingly, among the four actinomycete isolates, I-1 inhibited most of the Gram positive and Gram negative test bacteria, which indicated its broad antibacterial spectra. This was in agreement with that of another recent study wherein only five actinomycetes among a total of 107 marine isolates inhibited *B. subtilis, S. aureus, Proteus vulgaris, E. coli, Klebsiella aerogenes* and *P. aeruginosa* (Gulve and Deshmukh 2012). An earlier study revealed that *Enterococcus* sp., multidrug-resistant *P. aeruginosa* and methicillin-resistant *S. aureus* were the predominant bacterial pathogens causing nosocomial infections in liver transplantation patients (Nafady-Hego et al. 2011). In the present study, the streptomycete isolate I-1 inhibited *S. aureus* ATCC 25923 with a zone of 12 mm, even

though no inhibition was recorded with ampicillin. Overall, the antagonistic effect of I-1 against the Gram positive bacteria was significantly higher than that of Gram negative bacteria. On the other hand, extracts from the I-2, I-3 and I-4 showed higher inhibition against the Gram positive bacteria than the Gram negative pathogens, with zone diameters ranging between 8-20 mm. The greater degree of resistance exhibited by the Gram negative bacteria might be due to the presence of an outer membrane consisting of lipopolysaccharide (Parunago et al. 2007).

Table 1 - Antibacterial activity of selected actinomycete isolates against bacterial pathogens showing diameters of inhibitory zones (in mm) by agar well diffusion method.

Bacterial Pathogens	I-1	I-2	I-3	I-4	Antibiotic
S. aureus ATCC 25923	12±0.0*	8±0.0	10±0.0	11±0.5	-[a]
S. aureus	15±0.05	10±0.1	16±0.5	16±0.2	43±0.5[a]
S. citreus	20±0.5	14±0.05	12±0.05	10±0.0	43±0.5[a]
B. cereus	17±0.2	8±0.0	15±0.4	10±0.0	10±0.1[a]
E. coli	10±0.0	8±0.0	8±0.0	8±0.0	13±0.3[s]
S. marcescens	12±0.0	10±0.1	10±0.0	8±0.0	13±0.0[s]
P. mirabilis	10±0.0	10±0.0	10±0.0	10±0.1	13±0.0[s]
P. aeruginosa	11±0.01	10±0.5	8±0.0	8±0.0	16±0.2[s]
S. typhi	10±0.05	8±0.0	8±0.0	8±0.0	15±0.2[s]
K. pneumoniae	10±0.01	8±0.0	8±0.0	8±0.0	18±0.2[s]

*: values are mean ± standard deviation (n=3); [a]: ampicillin; [s]: streptomycin; -: no zone.

A total of 55 actinomycetes comprising of *Actinomyces*, *Nocardia*, *Streptomyces* and *Micromonospora* were isolated from the soil sample of Karanjal region in Sundarbans (Arifuzzaman et al. 2010). Among these, 20 actinomycete isolates showed antibacterial activity against the Gram negative bacteria such as *Shigella boydii*, *S. flexneri*, *S. sonnei*, *Pseudomonas* sp., *S.* dysenteriae type-1, *Vibrio cholerae*, *S. typhi*, *Plesiomonas*, *Hafnia* sp. and *E. coli*. Their study revealed that three isolates exhibited broad spectrum antibacterial activity. A previous study reported the isolation of 42 actinomycetes from the mangrove sediments of Andaman and Nicobar Islands, India, among which 22 species showed antagonistic activities against *S. aureus*, *B. subtilis*, *S. typhi* and *K. pneumoniae* (Baskaran et al. 2011). Another investigation revealed the isolation of an actinomycete from the mangrove forest soil of Guangxi Beihai, China, with designated strain no. BH0954, which was 99% related to *Streptomyces sindenensis*. This isolate inhibited *S. aureus*, *S. epidermidis*, *E. coli* and *P. vulgaris*, with no effect against *P. aeruginosa* and *C. albicans* (Dalin et al. 2010). A recent study also reported the isolation of *Streptomyces* sp. from the Coringa mangrove forest, Andhra Pradesh, India, whose metabolite was inhibitory to *S. aureus*, *P. fluorescens*, *P. aeruginosa*, *Lactobacillus acidophilus*, *L. casei*, *C. albicans*, *Streptococcus mutans*, *B. subtilis*, *B. megaterium* and *Xanthomonas* sp. (Deepthi et al. 2012).

Antifungal activity of actinomycete isolates

Extract from the isolate I-1 also exhibited antifungal activity against all the test fungal pathogens as illustrated in Table 2. Molds such as *A. niger*, *A. flavus*, *Penicillium* sp. and *Fusarium* sp. were inhibited with zones ranging between 8-12 mm. Some important systemic fungal pathogens affecting the humans are *C. albicans*, *C. neoformans* and *A. fumigatus*, some of these causing nosocomial infections in the patients during the postoperative months (Oskay 2009; Nafady-Hego et al. 2011). Among the pathogenic yeasts, *C. albicans* was inhibited by I-1 (20±0.5 mm). The extract of I-1 was equally effective against *C. parapsilosis* and *C. neoformans*. Isolates I-2 and I-4 also demonstrated maximum activity against *C. albicans* but I-3 revealed highest inhibition against *C. neoformans*. Extracts of the isolates I-2, I-3 and I-4 either demonstrated moderate to weak, or no antagonistic activity against the other fungi.

The antifungal activity of a rare actinomycete has been reported recently (Mangamuri et al. 2012). This actinomycete strain, isolated from the mangrove sediments of Nizampatnam, India, was found closely related to *Pseudonocardia endophytica* and inhibited *A. niger*, *F. oxysporum* and *C. albicans*. The present results were in accordance with those of a previous study, which reported the antifungal activity of selected strains of *Streptomyces* sp. against *C. albicans* with inhibitory zones ranging between 10-20 mm (Oskay 2009).

Table 2 - Antifungal activity of selected actinomycete isolates against fungal pathogens showing diameters of inhibitory zones (in mm) by agar well diffusion method.

Fungal Pathogens	I-1	I-2	I-3	I-4	Fluconazole
A. niger	10±0.0*	9±0.0	9±0.05	7±0.0	17±0.0
A. flavus	8±0.0	7±0.0	7±0.0	-	18±0.0
Penicillium sp.	12±0.2	10±0.0	7±0.0	8±0.0	21±0.5
Fusarium sp.	10±0.0	8±0.0	9±0.0	7±0.0	-
C. albicans	20±0.5	12±0.05	9±0.0	12±0.05	10±0.05
C. parapsilosis	12±0.0	11±0.02	8±0.0	9±0.0	37±1.0
T. rubrum	8±0.0	8±0.0	7±0.05	-	19±0.03
T. mentagrophytes	9±0.1	-	-	7±0.0	18±0.05
C. neoformans	12±0.05	8±0.0	11±0.0	7±0.0	22±0.0

*: values are mean ± standard deviation (n=3); -: no zone.

Characterization of actinomycete isolates

The morphological characterization of the actinomycete isolates was performed based on the study of colony characters and observation of Gram stained smears. On SCN agar, all the actinomycetes produced dry, compact, chalk-like colonies, similar to those of genus *Streptomyces*. The colonies on SCN agar showed hues of white, cream, grey, pale brown, dark brown, yellow, tan, pink and purple shades. The four selected strains had white to grey aerial mycelia, whereas the colour of their substrate mycelia showed distinct difference. The dark brown hue of the substrate mycelium as exhibited by the actinomycete isolate I-1 indicated its ability to produce unique pigment and/or any secondary metabolite. This observation could be correlated to the fact that among all the mangrove isolates, the isolate I-1 revealed the highest antagonistic activity against the nosocomial pathogens. Gram stained smears revealed the presence of filamentous structures bearing the spores in verticillate and spiral arrangements. The morphological characters of the actinomycete isolates are shown in Table 3.

Table 3 - Morphological characterization of selected actinomycete isolates.

Characteristics	I-1	I-2	I-3	I-4
Colour of aerial mycelium	Grey	Pale yellow	Light grey	Dark grey
Colour of substrate mycelium	Dark brown	Yellow	Dark purple	Dark Grey
Gram's reaction	Positive	Positive	Positive	Positive
Spore chain	Spirales	Spirales	Spirales	Spirales

The selected actinomycete isolates exhibited optimum growth within the mesophilic range of 25-37°C. Interestingly, all the selected isolates grew well at pH ranging from 5.3 to 9.3 but optimum growth was observed between pH 7.3-9.3. Furthermore, they were also tolerant to 0.1-5% NaCl but failed to grow at 10% salt concentration. This could probably be due to the hypertonic medium wherein the isolates would have suffered from severe osmotic shock. On par with the present findings, a marine strain of *S. rochei*, isolated from Visakhapatnam coast in India, was reported to be tolerant to pH 10.5 and 6% NaCl (Reddy et al. 2011). From these observations, it was clearly evident that mangrove actinomycetes were generally alkaliphilic and halotolerant. Furthermore, the isolates I-1, I-3 and I-4 also demonstrated melanin production. The physiological characters of four selected isolates are presented in Table 4.

Table 4 - Physiological characterization of selected actinomycete isolates.

Characteristics		I-1	I-2	I-3	I-4
Growth at	4°C	-	-	-	-
	25°C	+	+	+	+
	30°C	+	+	+	+
	37°C	+	+	+	+
	42°C	-	-	-	-
Growth at pH	3.3	-	-	-	-
	4.3	+	-	+	+
	5.3	+	+	+	+
	6.3	+	+	+	+
	7.3	+	+	+	+
	8.3	+	+	+	+
	9.3	+	+	+	+
Growth at NaCl (%)	0.1	+	+	+	+
	0.5	+	+	+	+
	1	+	+	+	+
	3	+	+	+	+
	5	+	+	+	+
	10	-	-	-	-
Melanin production		+	-	+	+

+: positive; -: negative.

Recently, molecular methods have been extensively used for the identification of bacterial species. They are preferred over the conventional methods because they yield faster and accurate results. 16S rDNA sequencing is one of the molecular methods that has revolutionized the bacterial systemics and classification. Based on the results of antimicrobial assay, the most potent alkaliphilic and halotolerant actinomycete isolate I-1 was subjected to molecular characterization. PCR amplification of its 16S rDNA gene sequence showed an amplicon of around 1500 bps. The automated sequencing of this PCR-amplified product was carried out. The sequence data when manually aligned in NCBI database using BLASTn revealed that the isolate I-1 was closely related to *S. xiamenensis* MCCC 1A01550 with 96% homology. Previously, many antimicrobial metabolite-producing actinomycetes isolated from marine sediments were identified as *S. roseoverticillatus, S. roseorubens* and *S. septatus* (Valan Arasu et al. 2012). The nucleotide sequence of the isolate I-1, designated as *S. xiamenensis* GHBA11, was provided a GenBank accession number JX827497. Most of the morphological, cultural and physiological characters of the isolate I-1 were in agreement with that of a rare actinomycete, *S. xiamenensis* sp. nov., isolated from the sediment of national mangrove reserve in Fujian Province, China (Xu et al. 2009). They reported its uniqueness in having a very high DNA G+C content of 71.6 mol%. To the best of our knowledge, this is the first report illustrating the antibacterial and antifungal activity of *S. xiamenensis.*

CONCLUSIONS

Mangrove ecosystem harbours many rare actinomycetes with interesting physiological properties. It is suggested that these unique strains of mangrove actinomycetes be further studied in search for some broad-spectrum, novel antibiotics, which could be effective in the treatment of nosocomial infections.

ACKNOWLEDGMENTS

We wish to extend our sincere gratitude to Dr. R. Chenraj Jain, Chairman, Jain Group of Institutions, Bangalore; Dr. N. Sundararajan, Vice-Chancellor of Jain University, Bangalore; Prof. K. S. Shantamani, Chief Mentor, Jain Group of Institutions and Dr. S. Sundara Rajan, Director of Genohelix Biolabs, A Division of Centre for Advanced Studies in Biosciences, Jain University, for their encouraging support. We express our heartfelt thanks to Mrs. K. Prashanthi for her assistance in molecular characterization.

REFERENCES

Arifuzzaman M, Khatun MR, Rahman H. Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *Afr J Biotechnol*. 2010; 9(29): 4615-4619.

Balagurunathan R, Masilamani Selvam M, Kathiresan K. Bioprospecting of mangrove rhizosphere actinomycetes from Pitchavaram with special reference to antibacterial activity. *J Pharm Res.* 2010; 3(5): 909-911.

Baskaran R, Vijayakumar R, Mohan PM. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. *Mal J Microbiol.* 2011; 7(1): 26-32.

Bergey DH, Holt JG. Bergey's Manual of Determinative Bacteriology. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2000.

Boroujeni ME, Das A, Prashanthi K, Suryan S, Bhattacharya S. Enzymatic screening and random amplified polymorphic DNA fingerprinting of soil streptomycetes isolated from Wayanad district in Kerala, India. *J Biol Sci.* 2012; 12(1): 43-50.

Dalin H, Guifeng Y, Yajuan X, Naixiang L, Jianhong C, Jiang lianxiu L, Senzhou C. Isolation and identification of *Actinomycetes* sp. BH0954 from the mangrove forest soil of Guangxi Beihai. *Chin Agric Sci Bull.* 2010; 26(18): 406-409.

Deepthi MK, Sudhakar MS, Devamma MN. Isolation and screening of *Streptomyces* sp. from Coringa mangrove soils for enzyme production and antimicrobial activity. *Int J* Pharmaceut *Chem Biol Sci*. 2012; 2(1): 110-116.

George M, Anjumol A, George G, Hatha AAM. Distribution and bioactive potential of soil actinomycetes from different ecological habitats. *Afr J Microbiol Res.* 2012; 6(10): 2265-2271.

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J, Duke N. Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecol Biogeogr.* 2011; 20(1):154-159.

Gulve RM, Deshmukh AM. Antimicrobial activity of the marine actinomycetes. *IMRJ*. 2012; 2(3):16-22.

Hamedani K, Soudbakhsh MN, Das A, Prashanthi K, Bhattacharya S, Suryan S. Enzymatic screening, antibacterial potential and molecular characterization of streptomycetes isolated from Wayanad district in Kerala, India. *Int J Pharm Bio Sci.* 2012; 2(1): 201-210.

Kumaresan V, Suryanarayanan TS. Occurrence and distribution of endophytic fungi in a mangrove community. *Mycol Res.* 2001; 105:1388-1391.

Kuster E, Williams ST. Selection of media for isolation of streptomycetes. *Nature.* 1964; 202: 928-929.

Mangamuri UK, Muvva V, Poda S, Kamma S. Isolation, identification and molecular characterization of rare actinomycetes from mangrove ecosystem of Nizampatnam. *Mal J Microbiol.* 2012; 8(2): 83-91.

Muiru WM, Mutitu EW, Mukunya DM. Characterization of antibiotic metabolites from actinomycete isolates. *African Crop Science Conference Proceedings.* 2007; 8: 2103-2107.

Nafady-Hego H, Elgendy H, El Moghazy W, Fukuda K, Uemoto S. Pattern of bacterial and fungal infections in the first 3 months after pediatric living donor liver transplantation: an 11-year single-center experience. *Liver Transplant*. 2011; 17(8): 976-984.

Nag C, Bhattacharya S, Das A. Evaluation of antagonistic activities of microbes from Vallapattanam and Pappinishery mangrove ecosystems of Kannur district in Kerala, India. *Int J Pharm Life Sci.* 2012; 3(5):1650-1659.

Naikpatil SV, Rathod JL. Selective isolation and antimicrobial activity of rare actinomycetes from mangrove sediment of Karwar. *J Ecobiotechnol*. 2011; 3(10): 48-53.

Oskay M. Antifungal and antibacterial compounds from *Streptomyces* strains. *Afr J Biotechnol*. 2009; 8(13): 3007-3017.

Padmadhas R, Ragunathan R. Isolation, characterisation and identification of novel actinomycetes collected from Western Ghats region of India. *J Pharm Biomed Sci.* 2010; 1(7): 1-7.

Parunago MM, Maceda EBG, Villano MAF. Screening of antibiotic-producing actinomycetes from marine, brackish and terrestrial sediments of Samal Island, Philippines. *J Res Sci Comp Engg.* 2007; 4(3): 29-38.

Prapagdee B, Kuekulvong C, Mongkolsuk S. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. *Int J Biol Sci*. 2008; 4(5): 330-337.

Radhika S, Bharathi S, Radhakrishnan M, Balagurunathan R. Bioprospecting of fresh water actinobacteria: isolation, antagonistic potential and characterization of selected isolates. *J Pharm Res*. 2011; 4(8): 2584-2586.

Ravikumar S, Fredimoses M, Gokulakrishnan R. Biodiversity of actinomycetes in Manakkudi mangrove ecosystem, southwest coast of India. *Annals Biol Res.* 2011; 2(1): 76-82.

Ravikumar S, Inbaneson SJ, Uthiraselvam M, Priya SR, Ramu A, Banerjee MB. Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. *J Pharm Res.* 2011; 4(1): 294-296.

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 J Biol Sci.* 2011; 4(1): 1-14.

Ruscher C, Lübke-Becker A, Semmler T, Wleklinski CG, Paasch A, Soba A, et al. Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. *Vet Microbiol*. 2010; 144(3-4): 340-346.

Sathiyaseelan K, Stella D. Isolation, identification and antimicrobial activity of marine actinomycetes isolated from Parangipettai. *Rec Res Sci Tech.* 2011; 3(9): 74-77.

Tsujibo H, Kubota T, Yamamoto M, Miyamoto K, Inamori Y. Characteristics of chitinase genes from an alkaliphilic actinomycete, *Nocardiopsis prasina* OPC-131. *Appl Environ Microbiol.* 2003; 69(2): 894-900.

Valan Arasu M, Asha KRT, Duraipandiyan V, Ignacimuthu S, Agastian P. Characterization and phylogenetic analysis of novel polyene type antimicrobial metabolite producing actinomycetes from marine sediments: Bay of Bengal India. *Asian Pac J Trop Biomed.* 2012; 2012: 803-810.

Valli S, Suvathi Sugasini S, Aysha OS, Nirmala P, Vinoth Kumar P, Reena A. Antimicrobial potential of actinomycetes species isolated from marine environment. *Asian Pac J Trop Biomed.* 2012; 2012: 469-473.

Xu J, Wang Y, Xie S, Xu J, Xiao J, Ruan J. *Streptomyces xiamenensis* sp. nov., isolated from mangrove sediment. *Int J Syst Evol Micr.* 2009; 59(Pt 3): 472-476.

Received: March 21, 2013;
Accepted: November 04, 2013.