Asian Journal of Biological Sciences, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.

A Morphological, Physiological and Biochemical Studies of Marine *Streptomyces rochei* (MTCC 10109) Showing Antagonistic Activity Against Selective Human Pathogenic Microorganisms

N.G. Reddy, D.P.N. Ramakrishna and S.V. Raja Gopal
Department of Biotechnology, GITAM Institute of Sciences, GITAM University, Visakhapatnam, India

Corresponding Author: N. Gopi Reddy, Department of Biotechnology, GITAM Institute of Sciences, GITAM University, Visakhapatnam-530045, Andhra Pradesh India Tel: +91-9298151592 Fax: +91-891-2790032

ABSTRACT

Marine antagonistic actinomycete strain *Streptomyces rochei* (MTCC 10109) was isolated from the sea water samples of Visakhapatnam coast of Bay of Bengal by serial dilution method. The aim of study was identification and characterization by its morphological, physiological and biochemical studies and deposited to Microbial Type Culture Collection and Gene bank, IMTECH, Chandigarh, India with accession number is MTCC 10109. It was a gram-positive, very long rods and filamentous organism, consisting of sporangia with spores observed by using cover-slip method and evaluated by phase contrast microscopes. It was capable to produce yellow pigments and possessing an earthy characteristic odour and exhibited optimum growth under aerobic conditions at temperature 30°C, pH at 7.0 and sodium chloride level at 2% (w/v). So, it was considered as a mesophillic, alkaliphillic and moderate salt tolerance in nature. The isolate showed difference in the carbon utilization, able to utilize all studied carbon sources, except xylose, adonitol, sorbitol, inositol and raffinose and showed positive results to methyl red test, nitrate reduction test, citrate utilization, urea hydrolysis, cytochrome oxidase, catalase test, gelatin hydrolysis, arginine dihydrolase, tween-60, tween-80 and esculin. It was exhibited broad antagonistic spectrum against all tested selective human pathogenic fungi and bacteria and its activity measured by zone of inhibition (mm), by agar well diffusion method. Among all the studied pathogens, *Candida tropicana, Staphylococcus aureus* and *Escherichia coli* were the most sensitive organisms from selective human test pathogens of fungi, gram-positive and gram-negative microorganisms respectively. Altogether, the results indicated that the natural marine environment is also good sources for isolation of novel varieties of antagonistic *Streptomyces*.

Key words: Marine actinomycete, morphology, optimum growth, carbon utilization and antagonistic spectrum

INTRODUCTION

Marine microbial biotechnology has opened up unexpected new horizons for finding novel organism for trapping their potential resources. Oceans account for more than 70% of the earth's surface and the microorganisms growing in marine environments are metabolically and physiologically diverse from terrestrial organisms (Takizawa *et al*., 1993). So, marine derived antibiotics are more efficient at fighting microbial infections than the terrestrial bacteria have not developed any resistance against them (Donia and Humann, 2003). Among, all the known

microbes, members of the actinomycetes genus especially *Streptomyces* sp. have long been recognized as prolific producer of useful bioactive metabolites with broad spectrum of activities, which has antibacterial, antifungal, antibiotic, antiparasitic, antitumor, antiviral, insecticide, herbicide, immunomodulators, antithrombotic agents (Atta and Ahmad, 2009; Baltz, 2008; Naeimpoor and Mavituna, 2000; Zheng *et al*., 2000; Desphande *et al*., 1988). They cover about 80% of total antibiotic products as compared to other actinomycetes genera (Keiser *et al*., 2000) and providing more than half of the naturally occurring antibiotics discovered to date and continuing to be a major source of many types of antibiotics and other class of biologically active secondary metabolites (Miyadoh, 1993; Okamoto Hosay *et al*., 2003). Of 9 maritime states in Indian peninsula only very few states have been extensively covered for the study of marine actinobacteria for antagonistic properties against different pathogens (Sivakumar *et al*., 2007) and especially, the east coast area of Bay of Bengal is major sources of actinomycetes (Sambamurthy and Ellaiah, 1974; Dhansasekaran *et al*., 2005; Vijaykumar *et al*., 2007).

Evolution of novel diseases, toxicity of currently used compounds and emergence of drug resistant pathogens which cause life threatening infections and risk undermining the viability of healthcare systems, especially in immunodeficient patients revealed the need for new and novel antibiotics (Hakvag *et al*., 2008; Talbot *et al*., 2006; Jensen *et al*., 2005; Jain and Jain, 2005). The prevalence of antimicrobial resistance among pathogens is increasing at an alarming rate worldwide (Singer *et al*., 2003).

It is perhaps not surprising that novel marine actinomycetes are proving to be such a valuable source of new bioactive compounds (Fiedler *et al*., 2005; Blunt *et al*., 2007) as actinomycete systematic is providing a taxonomic road map to genes hence products, including the discovery of first-in-class drug candidates (Kumar and Goodfellow, 2008; Blunt *et al*., 2007; Goodfellow *et al*., 2007; Ward and Goodfellow, 2004). Methods described by Shilling and Gottlieb (1966) have been used in the International Streptomyces Project (ISP) to characterizes the *Streptomyces* species, those characteristics were considered important and are now commonly used in the key for classification of *Streptomyces* species.

The aim of this present study was to isolation identification and characterization potent antagonistic alkaliphillic marine actinomycete isolate, *Streptomyces rochei* from sea water sample of Visakhapatnam coast of Bay of Bengal and to screen for their antagonistic activity against selective human pathogenic microorganisms. The strain was identified and deposited at Microbial Type Culture Collection and Gene bank, IMTECH, Chandigarh, India with accession number MTCC 10109.

MATERIALS AND METHODS

Sample collection: Sea water samples were collected in the year of 2008, from Visakhapatnam coast of the Bay of Bengal into the sterilized glass bottles and transported to the laboratory and stored in the refrigerator at 4°C until use.

Isolation of *Streptomyces rochei* from sea water: The medium used for the isolation and cultivation of marine actinomycetes was starch casein agar medium contains starch 1.0%; casein 0.1%; agar 2.0%; 30 days aged natural sea water 50 mL and distilled water 50 mL. After autoclaving, the medium was supplemented with 50 and 20 μg mL^{-1} of tetracycline and nystatin respectively as antibacterial and antifungal agents to inhibit the bacterial and fungal contamination. Sea water samples were serially diluted with filtered and sterilized 50% sea water

up to 10^{-2} dilutions (Sahu *et al*., 2004). From each suitable dilution, 0.1 mL was taken and spread evenly with sterile L-shaped glass rod over the surface of Starch-Casein Agar (SCA) and kept for incubation at 30°C. Streak Plate method was used to purify the marine actinomycetes colonies (Williams and Cross, 1971). The developed colonies that grow on petri plates can be individually purified by repeated streaking on SCA medium by using separate petri plates and then subcultured to ensure for their axencity. Pure culture was transferred on slants and preserved at 4°C for further analysis (Ellaih and Reddy, 1987; Kokare *et al*., 2004).

Morphological and physiological characterization: The potential isolate *Streptomyces rochei* was inoculated on Starch Casein Agar (SCA) and incubated at 30°C for 7 days. The culture was grown for 4 weeks and observation was made at weekly intervals. The micro-morphology of actinomycete strain was carried out for gram straining type, shape and size by under light microscope (Pathiranana *et al*., 1991; Duggiri, 1976; Jensen and Finical, 1994; Vimal *et al*., 2009) and cultural characteristics such as colony morphology like elevation, surface, density; aerial and substrate mycelium colour and pigment production was carried out (Kenneth, 1958). The spore chain morphology such as sporangia spore motility and spore surface ornamentation of the isolate was evaluated by phase contrast microscope magnifications 100x and 400x (Labomed Model: CXIII). This is done by using cover-slip method in which spore suspension culture of the actinomycete was inoculated at the intersection of the SCA medium and cover slip was buried in the solid SCA medium at an angle of 45° (Williams and Cross, 1971). Physiological characterization of *Streptomyces rochei* carried out by performing the growth at different temperatures range from 4 to 65°C, pH range from 5.0 to 10.5 and the growth under anaerobic condition.

Sodium chloride tolerance: Sodium chloride tolerance level (Tresner *et al*., 1968) of *Streptomyces rochei* was evaluated on yeast extract agar medium supplemented with graded doses of sodium chloride (2.0, 0.4, 0.6, 0.8 and 10.0% w/v). Maximum sodium chloride concentration in the medium allowing any growth was recorded.

Biochemical characterization: Various biochemical tests were performed for the identification of the potent isolate *Streptomyces rochei*. These tests includes growth on MacConkey agar, indole test, methyl red test, voges proskauer test, citrate utilization, gas production from glucose, casein hydrolysis, urea hydrolysis, nitrate reduction, H_2S production, cytochrome oxidase test, catalase test, gelatin hydrolysis, arginine dihydrolase, tween-40, tween-60, tween-80 and esculin tests. To determine the production of acids by utilizing the different sources of carbohydrates like adonitol, sorbitol, dextrose, fructose, inositol, lactose, maltose, raffinose, rhamnose, sucrose and xylose were tested (Pridham and Gottilieb, 1948; Nonomura, 1974) by inoculating the isolate in ISP1 broth supplemented respective sugars and incubated for 7 day at 30°C.

Test organisms: The selective human pathogenic microorganisms used for antagonistic activity were fungi-*Candida tropicana, Candida albicana* (MTCC 183), *Aspergillus niger* (MTCC 1344), *Saccharomyces cercvisiae* (MTCC 307), Gram positive bacteria - *Staphylococcus aureus* (MTCC 3160), *Micrococcus luteus* (MTCC 106) and Gram negative bacteria - *Vibrio alginolyticus, Escherichia coli* (MTCC 443), *Pseudomonas aerugenosa* (MTCC 424), *Pseudomonas fluorescence* (MTCC 103) and *Aeromonas hydrophylla*. The pathogenic microorganisms *Candida tropicana*,

Vibrio alginolyticus and *Aeromonas hydrophylla* were obtained from Department of Biotechnology, Andhra University, Visakhapatnam, India and remaining strains from IMTECH, Chandigarh, India. All the cultures were maintained on nutrient slants at 4°C.

Antagonistic activity: *Streptomyces rochei* was grow in 50 mL of starch casein broth by submerged culture containing in 250 mL flasks by incubating at 32°C for 7 days and centrifuged at 10,000 rpm for 15 min and the clear supernatant broth samples were tested for their antagonistic activity against the selected human pathogenic microorganisms by agar well diffusion method (Saadoun and Muhana, 2008). Wells of 6 mm diameter were prepared in the nutrient agar plates and the test pathogenic bacterial and fungal cultures were swabbed on to the nutrient agar surface (Mitra *et al*., 2008) and the wells were filled with the 50 µL of crude culture supernatant and the diameter of inhibition zones were measured after incubation for 24 h at 37°C for the bacterial species and 48 h at 28°C in the case of fungal species.

RESULTS

According to Bergey's manual of Determinative Bacteriology, Ninth edition by Holt (2000) and the Laboratory Manual for Identification of Actinomycetes (IMTECH, 1998), the organism was identified as *Streptomyces rochei* based on morphological, physiological and biochemical characteristics and deposited to Microbial Type Culture Collection and Gene bank, IMTECH, Chandigarh, India with accession number is MTCC 10109.

Morphological and physiological characterization: As shown in Table 1 *Streptomyces rochei* was isolated on starch casein agar medium is Gram-positive, very long, rod shaped, produces yellow pigments and possessing an earthy odour characteristic of actionomycetes. The colony was light brown to gray in colour. Mycelium was aerial and white in colour, where the substrate mycelium was light brown (Fig. 1a, b); colony elevation was raised with wrinkled surface and opaque density and tenaciously adhering to the medium and the fine structure were studied by under phase contrast microscopy (Fig. 2a, b); it was sporangia and spore forming in nature and spores were non-motile, smooth and hairy. *Streptomyces rochei* exhibited optimum growth under aerobic conditions at temperature 30°C and pH at 7.0 (Fig. 3, 4). It was a mesophillic and alkaliphillic in

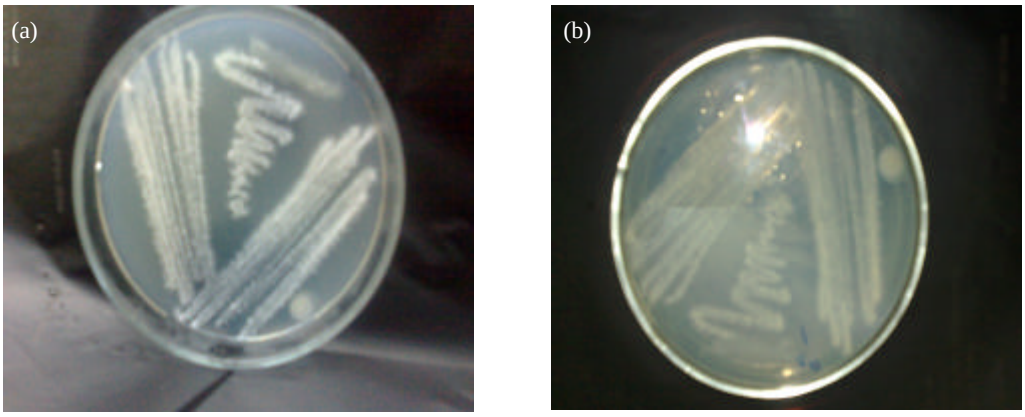

Fig. 1: Morphological view of *Streptomyces rochei* (MTCC 10109), (a) Aerial view and (b) substrate view

Table 1: Morphological and physiological characterization of *Streptomyces rochei*

Tests	Results
Colony	
Elevation	Raised
Surface	Wrinkled
Density	Opaque
Pigment	Yellow
Gram's Reaction	+ve
Shape	Rods
Size	Very long rods
Spore	+
Sporangia	+
Mycelium	Aerial
Spore Motility	Non-motile
Spore surface	Smooth and hairy
Growth at temperature	
4°C	–
10°C	–
25°C	+
30°C	+++ (Optimum)
37°C	++
42°C	+
55°C	–
65°C	–
Growth at pH	
pH5.0	–
pH6.0	+
pH7.0	+++ (Optimum)
pH8.0	++
pH9.0	+
pH10.5	+
Growth on NaCl (%)	
2.0	++ + (Optimum)
4.0	++
6.0	+
8.0	–
10.0	–
Growth under anaerobic condition	–

-: No growth; +: Normal growth; ++: Moderate growth; + ++: Optimum growth

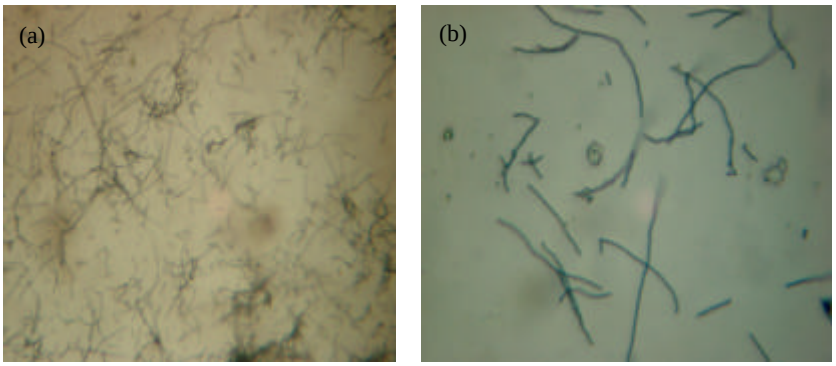

Fig. 2: Spore chain structure of *Streptomyces rochei* (MTCC 10109), (a) Phase contrast microscope ×100 and (b) Phase contrast microscope ×400

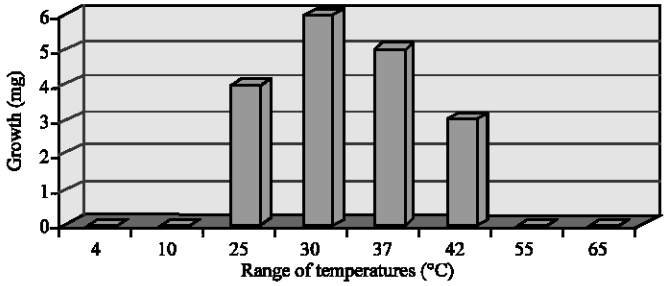

Fig. 3: Effect of different temperatures on growth of *Streptomyces rochei*

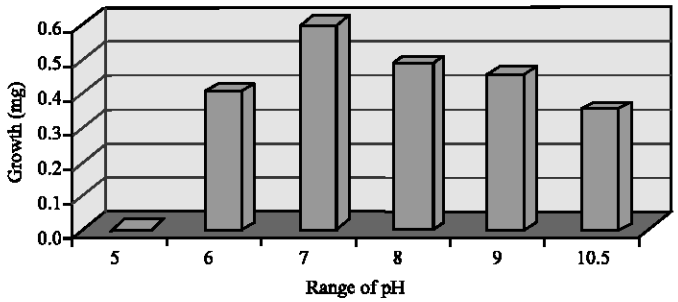

Fig. 4: Effect of different pH on growth of *Streptomyces rochei*

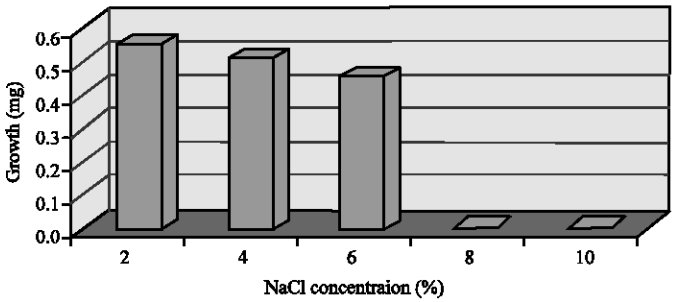

Fig. 5: Effect of different NaCl concentration on growth of *Streptomyces rochei*

nature which showed growth at temperature range from 25 to 42°C and pH 6 to 10.5, but growth was not observed at temperatures 4, 10, 15, 55 and 65°C and pH 5.0.

Sodium chloride tolerance: Optimum growth of *Streptomyces rochei* was observed (Fig. 5) at 2% (w/v) sodium chloride (NaCl), but maximum tolerance of sodium chloride concentration was exhibited growth upto to 6% (w/v), indicating it was indigenous to marine environment and moderate salt tolerance in nature.

Biochemical characterization: *Streptomyces rochei* could utilize dextrose, fructose, lactose, maltose, mannitol, rhamnose and sucrose as the carbon source along with acid production; however, xylose, adonitol, sorbitol, inositol and raffinose were not utilized by the organism (Table 2) and there is no growth on Mac conkey agar. The biochemical tests like methyl red test, nitrate reduction test, citrate utilization, urea hydrolysis, cytochrome oxidase, catalase test, gelatin hydrolysis,

Table 2: Biochemical identification of *Streptomyces rochei*

Tests	Results
Biochemical tests	
Growth on MacConkey Agar	NG
Indole test	–
Methyl red test	+
Voges proskaucer test	–
Citrate utilization	+
Gas production from glucose	–
Casein hydrolysis	–
Starch hydrolysis	–
Urea hydrolysis	+
Nitrate reduction	+
H_2S production	–
Cytochrome oxidase	+
Catalase test	+
Gelatin hydrolysis	+
Arginine dihydrolase	+
Tween-40	–
Tween-60	+
Tween-80	+
Esculin	+
Acid production from carbohydrates	
Adonitol	–
Sorbitol	–
Dextrose	+
Fructose	+
Inositol	–
Lactose	+
Maltose	+
Mannitol	+
Raffinose	–
Rhamnose	+
Sucrose	+
Xylose	–

NG: No Growth; +: Positive reaction; –: Negative reaction

arginine dihydrolase, tween-60, tween-80 and esculin were positive, but indole test, voges proskauer test, gas production from glucose, casein hydrolysis, starch hydrolysis, H_2S production and tween-40 were negative.

Antagonistic activity: The antagonistic principles secreted by the *Streptomyces rochei* exhibited broad antagonistic spectrum against all tested human pathogenic fungal and bacterial species studied (Table 3) and among all the tested pathogenic organisms, *Candida tropicana, Staphylococcus aureus* and *Escherichia coli* were the most sensitive organisms followed by *Candida albicana, Micrococcus luteus* and *Aeromonas hydrophylla* (Fig. 6, 7).

Table 3: Antagonistic activity of *Streptomyces rochei* against selective human pathogenic fungi, Gram-positive and Gram-negative bacteria

Test organisms	Inhibition zone (mm)
Fungi	
C. tropicana	20
C. albicana	18
Aspergillus niger	16
Sacharomyces cervisiae	15
Gram +ve bacteria	
S. aureus	19
Micrococcus luteus	18
Gram –ve bacteria	
E. coli	16
Vibrio alginolyticus	14
Ps. aeruginosa	12
Ps. fluroscence	12
A. hydrophylla	15

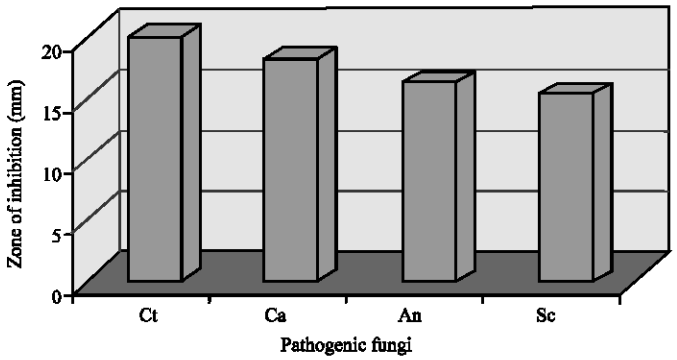

Fig. 6: Antagonistic activity against selective human pathogenic fungi. Ct-*Candida tropicana;* Ca-*Candida albicana;* An-*Aspergillus niger;* Sc-*Sacharomyces cervaceae*

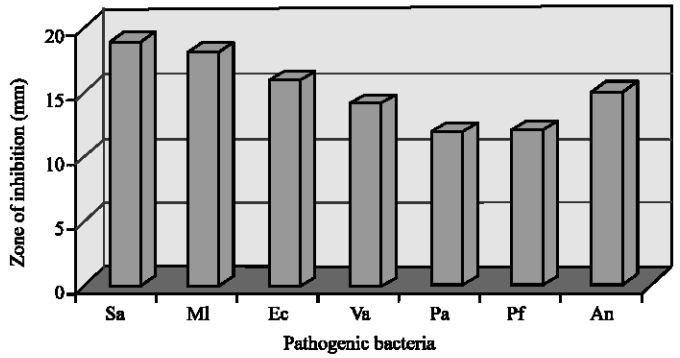

Fig. 7: Antagonistic activity against selective human pathogenic bacteria. Sa-*Staphylococcus aureus;* Ml-*Micrococcus luteus; Va-Vibrio alginolyticus; Ec-Escherichia coli; Pa-Pseudomonas aerugenos;* Pf-*Pseudomonas fluorescence;* Ah-*Aeromonas hydrophylla*

DISCUSSION

Many characteristics of actinomycetes have been employed for the purpose of classification and ideally, these should be constant under the same cultural conditions. Most of actinomycetes were

isolated from a marine environment which required seawater for growth and these strains were designated marine actinomycetes (Mincer *et al*., 2002) and *Streptomyces* have been reported to grow well on Starch Casein Agar (SCA) by earlier workers (Laidi *et al*., 2006), so, SCA media supplemented with 30 days aged 50% natural sea water was used for the isolation of present strain *Streptomyces rochei*.

In review literature reported growth is characterized by small compact, soft to leathery colonies tenaciously adhering to the medium, the surface being either flat or elevated (Sathi *et al*., 2001). The colony surface is usually dry with conical or elevated with mycelium. The colour of the mycelia can range from nearly colourless to white, chalky red or grey olive (Oskay *et al*., 2004). The colony morphology of the present studied isolate (10109), consisting of both aerial and substrate mycelium. Colony elevation was raised with wrinkled surface and opaque density, was similar to that of the others studied *Streptomyces rochei* species (Locci, 1989), This clearly indicates that present the isolate under investigation belonged to the genus *Streptomyces*. *Streptomyces rochei* (10109) showed white to light brown in and also capable of production of yellow pigment, the colour of the isolates is due to the pigment production and examples of pigments produced are phenazines, phenoxazinones and prodiginines reported by Rahman *et al*. (2000) and it was sporangia and spore forming in nature and spores were non-motile, smooth and hairy, regarding to sporulating culture surfaces in terms of morphological groups, described by Odakura *et al*. (1984) and Getha *et al*. (2004), these include shape of the spores, branching manner of the spore chains, nature of the spore chain, form or structure of sporophores (You *et al*., 2005).

The strain exhibited salt tolerance up to 6% and so that it may be placed in the moderate salt tolerance group. Larsen (1986), halophilic microorganisms can be conveniently grouped according to their requirements for NaCl concentrations, for the growth of slightly halophilic organisms in marine environments can grow in the presence of 2 to 3% NaCl. The moderate halophilic grow over a much wider NaCl concentration range (5 to 20%, w/v). The extreme holophililes, including the well-known halobacterial and halococci, are able to grow in saturated NaCl and unable to grow in the presence of NaCl concentrations lower than 12%. The present studied strain exhibited salt tolerance upto 6% and may be placed in the intermediate salt tolerance group as well as moderate halophilic in nature. *Actinomycetes* can use a variety of organic nutrients but special media are often preferable (Rahman *et al*., 2000; Sultan *et al*., 2002). For the carbon utilization tests, profuse growth of the *Streptomyces* colonies showed that, the particular carbon source was effectively utilized by the isolate as also reported by Oskay *et al*. (2004) slight or poor growth is an indication, that, the particular carbon source is not an adequate source of carbon or material may contain traces of other compounds (Sathi *et al*., 2001). These characteristics were useful to identify *Streptomyces rochei* (10109) isolate to the species level by based on its pigment production, morphological and physiological characteristics, additional feature such as studying microscopic micrographs of the spore surface and biochemical properties can provide more details, that can be used for identification purposes, was also reported by Oskay *et al*. (2004). Other advanced methods such as gene analysis of 16r RNA and Fourier transform infrared spectroscopy are more reliable, however these are more expensive and technically challenging (You *et al*., 2005).

According to review literatures, *Streptomyces strain* SLO-105 showed broad-spectrum of antibacterial activity against gram-positive bacteria (*Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA), *Micrococcus luteus*) and antifungal activity (against *Aspergillus niger*). However, no activity of the strain was observed against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) as well as on fungi *Candida albicana* (Morakchi *et al*., 2009;

Laidi *et al.*, 2008). Where as, certain *Streptomyces* strains showed strong antifungal activity (against *Aspergillus niger*, *Candida albicana*) and antibacterial activity against gram positive (*S. aureus*) and gram negative bacteria (*E. coli*) also (Laidi *et al.*, 2007; Oskay, 2009). *Streptomyces* sp. PM-32, showed good activity against both the Gram-positive and Gram-negative bacteria (*S. aureus* and *E. coli*) and weak antifungal activity (against *A. niger* and *C. albicana*) (Manivasagan *et al.*, 2009). According to Narayana *et al.* (2007), reported that *Streptomyces* sp. were highly susceptible to gram-negative (*Ps. aeruginosa* and *Ps. fluroscence*) and showed moderate activity against gram-positive bacteria (*S. aureus*) and less antifungal activity (against *C. albicana*). Different types novel *Streptomyces* strains identified by Sunanda *et al.* (2009), were *S. lydicus*, *S. lavendulae*, *S. albus*, *S. antibioticus*, *S. diastaticus* and *S. phaeochromogenes* reported that, has been showed various degrees of antibacterial activity against gram-positive (*S. aureus*) as well as gram-negative bacteria (*E. coli*) and antifungal activity (against *A. niger, C. albicana* and *S. cerevisiae*). In the present results, observed that our strain *Streptomyces rochei* (MTCC 10109) exhibited broad spectrum of antifungal and antibacterial activity against selective human test pathogenic fungi and gram positive bacteria, comparatively less active against studied gram negative bacteria, which was shows the similarities with the other researchers (Pandey *et al.*, 2004; Oskay *et al.*, 2004). By the above mentioned various reviewers literatures and present results, concluded that variety of *Streptomyces* sp. has been showed various degrees of activity against gram-positive, gram negative and fungi, according to nature of their produced antimicrobial secondary metabolites.

In the present study, the isolated alkaliphilic marine *Streptomyces rochei* have been most sensitive against *C. tropicana, S. aureus* and *E. coli* among the studied fungi, gram positive and negative bacteria respectively, followed by good activity against *Candida albicana* and *Micrococcus luteus* and *Aeromonas hydrophylla* and less effective against gram-negative pathogenic bacteria are *Ps. aeruginosa, Ps. fluroscence* and *Vibrio alginolyticus*, these present results supported by following reviewers reports within the species level (Kavitha and Vijayalakshmi, 2007). *S. rochei* inhibited a wide variety of gram positive (*S. aureus*), gram negative (*E. coli*) bacteria and fungi (*A. niger* and *C. albicana*); Kathresan *et al.* (2005) and Kokare *et al.* (2004), they observed good antibacterial activity against *S. aureus* and least activity against *Ps. aeruginosa* and fungi like *C. albicana*. As well as present results also, showed contradiction with reviews of Kotake *et al.* (1992), extracted antifungal substances from the *S. rochei* S785-16 reported that moderate activity on yeast, Ugur and Sahin (2002), reported that *S. rochei* (MU119) had no activity against the test organisms *B. subtilis, E. coli, S. auerus* and *C. albicana* and Augustine *et al.* (2005) informed that the metabolites of *S. rochei* AK 39 showed antifungal activity, while these metabolites had no effect on *C. albicana* and *A. niger*. So due to the above reviewer's contradiction, the produced secondary metabolite compounds of our *Streptomyces rochei* (MTCC10109) may be different from that of earlier studied *S. rochei* strains.

CONCLUSION

The predominance of *Streptomyces* in any actinomycete population is a well known fact (Alexander, 1961). This reflected in our present investigation of marine environment of Bay of Bengal of Visakhapatnam coast. Altogether, the results indicated that the natural marine environment is also good sources for isolation of novel varieties of antagonistic *Streptomyces*.

In the view of its broad antimicrobial spectrum, attempts are in progress to characterize the antimicrobial compounds produced by *S. rochei* MTCC10109.

ACKNOWLEDGMENT

The authors are grateful to GITAM University management for providing facilities required to carry out this work.

REFERENCES

Alexander, M., 1961. Introduction to Soil Microbiology. John Wiley and Sons, Inc., New York.

Atta, M.A. and M.S. Ahmad, 2009. Antimycin-A antibiotic biosynthesis produced by *Streptomyces* sp. AZ-AR-262: Taxonomy, fermentation, purification and biological activities. Aust. J. Basic Sci., 3: 126-135.

Augustine, S.K., S.P. Bhavsar and B.P. Kapadnis, 2005. Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK 39. Indian J. Med. Res., 121: 164-170.

Baltz, R.H., 2008. Renaissance in antibacterial discovery from actinomycetes. Curr. Opin. Pharmacol., 8: 557-563.

Blunt, J.W., B.R. Copp, W. Hu, M.H.G. Munro, P.T. Northcote and M.R. Prinsep, 2007. Marine natural products. Nat. Prod. Rep., 24: 31-86.

Desphande, B.S., S.S. Ambedkar and J.G. Shewale, 1988. Biologically active secondary metabolites from *Streptomyces*. Enzyme Microb. Technol., 10: 455-473.

Dhansasekaran, D., G. Rajakumar, P. Sivamani, S. Selvamani, A. Paneerselvam and N. Thanjuddin, 2005. Screening of salt pans actonomycetes for antibacterial agents. Int. J. Microbiol., 1: 1-8.

Donia, M. and M.T. Humann, 2003. Marine natural products and their potential applications as anti infective agents. Lancet Infect. Dis., 3: 338-348.

Duggiri, G.D., 1976. Drugs from sea. Science, 194: 491-497.

Ellaih, P. and A.P.C. Reddy, 1987. Isolation of actinomycetes from Marine sediments off Visakhapatnam, east coast of India. Indian J. Mar. Sci., 16: 134-135.

Fiedler, H.P., C. Bruntner, A.T. Bull, A.C. Ward and M. Goodfellow *et al.*, 2005. Marine actinomycetes as a source of novel secondary metabolites. Antonie van Leeuwenhoek, 87: 37-42.

Getha, K., S. Vikineswary, W.H. Wong, T. Seki, A. Ward and M. Goodfellow, 2004. Characterization of selected isolates of indigenous *Streptomyces* species and evaluation of their antifungal activity against selected plant pathogenic fungi. Malaysian J. Sci., 23: 37-47.

Goodfellow, M., Y. Kumar, D.P. Labeda and L. Sembiring, 2007. The *Streptomyces violaceusinger* clade: A home for *Streptomyces* with rugose ornamented spores. Antonie van Leeuwenhoek, 92: 173-199.

Hakvag, S., E. Fjarvik, K.D. Josefsen, E. Ian, T.E. Ellingsen and S.B. Zotchev, 2008. Characterization of *Streptomyces* spp. Isolated from the sea surface microlayer in the Trondheim Fjord, Norway. Mar. Drugs, 6: 620-635.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 2000. Bergeys Manual of Determinative Bacteriology. 9th Edn., Lipponcott Williams and Wilkins, Philadelphia, USA., pp: 189-255.

IMTECH, 1998. Laboratory Manual for Identification of Actinomycetes. Institute of Microbial Technology, Chandigarh, pp: 94.

Jain, P.K. and P.C. Jain, 2005. Production of heptaene antifungal antibiotic by *Streptomyces* purpoefuscus CM 1261. Ind. J. Exp. Biol., 43: 342-345.

Jensen, P.R. and W. Fenical, 1994. Strategies for the discovery of secondary metabolites from marine bacteria. Annu. Rev. Microbiol., 48: 559-584.

Jensen, P.R., T.J. Mincer, P.G. Williams and W. Fenical, 2005. Marine actinomycete diversity and natural product discovery. Antonie van Leeuwenhoek, 87: 43-48.

Kathresan, K., R. Balagurunathan and M. Selvam, 2005. Fungicidal acitivity of marine actinomycetes against photopathogenic fungi. Ind. J. Biotech., 4: 271-276.

Kavitha, M. and M. Vijayalakshmi, 2007. Studies on cultural, physiological and antimicrobial activities of *Streptomyces rochei*. J. Applied Sci. Res., 3: 2026-2029.

Keiser, T., M.J. Bibb, M.J. Buttner, K.F. Chater and D.A. Hopwood, 2000. General introduction to actinomycete biology. Proceedings of the Practical *Streptomyces* Genetics, 2000, The John Innes Foundation, Crowes, Norwich, England, pp: 1-21.

Kenneth, L.K., 1958. Prepared research paper RP 2911, central nations for the revised ISCC-NBS color name blocks. J. Res. NBS, 16: 427-427.

Kotake, C., T. Yamasaki, T. Moriyama, M. Shinoda and N. Komiyama *et al.*, 1992. Butyrolactols A and B, new antifungal antibiotics. Taxonomy, isolation, Physio-chemical properties, structure and biological activity. J. Antibiotics Tokyo, 45: 1442-1450.

Kokare, C.R., K.R. Mahadik and S.S. Kadam, 2004. Isolation of bioactive marine actinomycetes from sediments isolated from Goa and Maharashtra coast lines (West Coast of India). Ind. J. Marine Sci., 33: 248-256.

Kumar, Y. and M. Goodfellow, 2008. Five new members of the 16S rRNA gene clade: *Streptomyces violaceusniger* sp. nov., *Streptomyces himastatinicus* sp. nov., *Streptomyces mordarskii* sp. nov., *Streptomyces rapamycinicus* sp. nov. and *Streptomyces ruanii* sp. nov. Int. J. Syst. Evol. Microbiol., 58: 1369-1378.

Laidi, R.F., L.K. Amany, A.M. Elshafei and B. Cheikh, 2006. Taxonomy, identification and biological activities of a novel isolate of *Streptomyces tendae*. Arab. J. Biotech, 9: 427-436.

Laidi, R.F., A. Elshafei, M. Sanker, B. Cheick and H. Hocine, 2007. Screening, isolation and characterization of a novel antimicrobial producing actinomycetes, strain RAF10. Biotechnology, 6: 489-496.

Laidi, R.F., M. Sifour, M. Sanker and H. Hocine, 2008. A new actinomycete strain SK4-6 producing secondary métabolite effective against methicillin-resistant *Staphylococcus aureus*. World J. Microbiol. Biotechnol., 24: 2235-2241.

Larsen, H., 1986. Halophilic and halotolerant microorganism: an overview historical perspective. FEMS Microbial. Rev., 39: 3-7.

Locci, R., 1989. *Streptomyces* and Related Genera. In: Bergey's Manual of Systematic Bacteriology, Williams, S.T. and J.G. Holt (Ed.). Williams and Wilkins Company, Baltimore, USA., pp: 2451-2469.

Manivasagan, P., S. Gnanam, K. Sivakumar, T. Thangaradjou, S. Vijayalakshmi and T. Balasubramanian, 2009. Antimicrobial and cytotoxic activities of an actinobacteria (*Streptomyces* sp. PM-32) isolated from an offshore sediments of the bay of bengal in tamilnadu. Advances Biological Res., 3: 231-236.

Mincer, T.J., P.R. Jensen, C.A. Kauffman and W. Fenical, 2002. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. Applied Environ. Microbiol., 68: 5005-5011.

Mitra, A., S.C. Santra and J. Mukharjee, 2008. Distribution of actinomycetes, their antagonistic behavior and the Physio-chemical characteristic of the worlds largest tidal mangrove forest. Applied Microbial Biotchnol., 80: 685-695.

Miyadoh, S., 1993. Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach. Actinomycetologica, 9: 100-106.

Morakchi, H., A. Ayari1, M. Taok, D. Kirane1 and N. Cochet, 2009. Characterization of *Streptomyces* strain SLO-105 isolated from Lake Oubeira sediments in North-East of Algeria. Afr. J. Biotech., 8: 6332-6336.

Naeimpoor, F. and F. Mavituna, 2000. Metabolic flux analysis in *Streptomyces coelicolor* under various nutrient limitations. Metab. Eng., 2: 140-148.

Narayana Kolla, J.P., P. Peddikotla, V. Muwa, V. Yenamadra and S.J. Krihsna Palakodety, 2007. Biological activity of Phenylpropionic acid isolated from a terrestrial *Streptomyces*. Polish J. Microbiol., 56: 191-197.

Nonomura, H., 1974. Key for classification and identification of 458 species of the *Streptomyces* included in ISP. J. Ferment. Technol., 52: 78-92.

Odakura, Y., H. Kase, S. Itoh, S. Satoh and S. Takasawa *et al.*, 1984. Biosynthesis of astromicin and related antibiotics. ii biosynthetic studies with blocked mutant of *Micromonospora olivasterospora*. The J. Antibiot., 37: 1670-1680.

Okamoto Hosay, Y., S. Okamoto and K. Ochi, 2003. Development of antibiotic-overproducing strains by site-directed mutagenesis of the rps1. Gene *Streptomyces lividans*. J. Applied Environ. Microbiol., 69: 4256-4259.

Oskay, M., U.A. Tamer and C. Azer, 2004. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr. J. Biotechnol., 3: 441-446.

Oskay, M., 2009. Antifungal and antibacterial compounds from *Streptomyces* strains. Afr. J. Biotech., 8: 3007-3017.

Pandey, B., P Ghimire and V.P. Agarwal, 2004. Studies on the antibacterial activity of actinomycetes isolated the Khumbu region of Mt. Everest. Proceedings of the International Conference on the Great Himalayan: Climate, Health, Ecology, Management and Conservation, (ICGHCHEICGH'04), Kathmandu University and the Aquatic Ecosystem Health and Management Society, Canada, pp: 1-4.

Pathiranana, C., D. Tapiolas, P.R. Jensen, R. Dwight and W. Fenical, 1991. Structure determination of moduralide: A new 24-membered ring macrolide glycoside produced by a marine bacterium (actinomycetales). Tetrahedron Lett., 32: 2323-2326.

Pridham, T.G. and D. Gottlieb, 1948. The utilization of carbon compounds by some actinomycetales as an aid for species determination. J. Bacteriol., 56: 107-114.

Rahman, M.Z.A., A.T.M.Z. Azam and M.A. Gafur, 2000. *In vitro* antibacterial principles of two flavonoids and extracts from *Clerodendrum indicum* linn. Pak. J. Biol. Sci., 3: 1769-1771.

Saadoun, I. and A. Muhana, 2008. Optimal production condition, extraction, partial purification and characterization of inhibitory compound(s) produced by *Streptomyces* Ds-104 isolate against Multi-drug resistant *Candia albicana*. Curr. Trends. Biotechnol. Pharm., 2: 402-420.

Sahu, M.K., K.S. Kumar and L. Kanan, 2004. Isolation of actinamycetes from various samples of the vellar estuary, South east coast of India. Proc. Ecotech, 24: 45-48.

Sambamurthy, K. and P. Ellaiah, 1974. A new streptomycin producing Neomycin (B and C) comples, *S. marinensis* (part-1). Hind Antibiot Bull., 17: 24-28.

Sathi, Z.S., M.A.A. Rahman and M.A. Gafur, 2001. Identification and *in vitro* antimicrobial activity of compound isolated from *Streptomyces* speices. Pak. J. Biol. Sci., 4: 1523-1525.

Shilling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 313-340.

Singer, R.S., R. Finch, H.C. Wegener, R. Bywater, J. Walters and M. Lipsitch, 2003. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. The Lancet Infect. Dis., 3: 47-51.

Sivakumar, K., M.K. Sahu, T. Thangaradjou and L. Kannan, 2007. Research on marine actinobacteria in India. Indian J. Microbiol., 47: 186-196.

Sultan, M.Z., N.A. Khatune, Z.S. Sathi, S.A.M.D. Bhuiyan and G.M. Sadik *et al.*, 2002. *In vitro* antibacterial activity of an active metabolite isolated from *Streptomyces* species. Biotechnology, 1: 100-107.

Sunanda Kumari, K., K. Uma Devi and A. Apparao, 2009. Characterization of marine *Streptomyces* from Visakhapatnam coast. Drug Inven. Today, 1: 78-80.

Takizawa, M., R.R. Colwell and R.T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. Applied Environ. Microbiol., 59: 997-1002.

Talbot, G.H., J. Bradley, Jr. J.E. Edwards, D. Gilbert, M. Scheld and J.G. Bartlett, 2006. Bad Bugs Need Drugs: An update on the development pipeline from the antimicrobial availability task force of the infectious diseases society of America. Clin. Infect. Dis., 42: 657-668.

Tresner, H.D., J.A. Hayes and E.J. Backns, 1968. Differential tolerance of *Streptomyces* to sodium chloride as a taxonomic aid. Applied Microbiol., 16: 1134-1136.

Ugur, A. and N. Sahin, 2002. Accumulation of poly â-hydroxy butyrate in *Streptomyces* species during growth with different nitrogen sources. Turk. J. Biol., 26: 171-174.

Vijaykumar, R., C. Muthukumar, N. Thahuddin, A. Paneerselvam and R. Saravanamuthu, 2007. Studies on the diversity of actionomycetes in the Palk Strait region of bay of Bengal, India. Actinomycetol., 21: 59-65.

Vimal, V., R.B. Mercy and K. Kannabiran, 2009. Antimicrobial activity of marine actinomycete, *Nocardiopsis* sp. VITSVK 5 (FJ973467). Asian J. Med. Sci., 1: 57-63.

Ward, A.C. and M. Goodfellow, 2004. Phylogeny and Functionality: Taxonomy as a Roadmap to Genes. In: Microbial Diversity and Bioprospecting, Bull, A.T. (Ed.). ASM Press, Washington, DC, USA., pp: 288-313.

Williams, S.T. and T. Cross, 1971. Actinomycetes Isolation from Soil. In: Methods in Microbio, Booth, C. (Ed.). Vol. 4. Academic Press, London, New York, pp: 295-334.

You, J.L., L.X. Cao, G.F. Liu, S.N. Zhou, H. Tan and Y.C. Lin, 2005. Isolation and characterization of actinomycete antagonistic to pathogenic *Vibrio* spp. from near shore marine sediments. World J. Microbil. Biotechnol., 21: 679-682.

Zheng, Z., W. Zeng, Y. Huang, Z. Yang, J. Li, H. Cai and W. Su, 2000. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol. Lett., 188: 87-91.