Indian Journal of Geo-Marine Sciences
Vol. 44(5), May,2015 pp. 714-723

Characterization and antimicrobial activity of *Streptomyces* sp. DOSMB-A107 isolated from mangrove sediments of Andaman Island, India

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Received 26 April 2013; revised 25 February 2014

In the present study, focused the antimicrobial compound production by the marine actinobacteria isolated from the mangrove sediments of Andaman Island. *Streptomyces* sp. DOSMB-A107 isolated from mangrove sediments of Andaman Island was identified by morphological, chemistry of cell wall and molecular characterization. Bioactive compounds were extracted with five different solvents and were identified using GC-MS analysis. Among the five different solvents ethyl acetate extract showed maximum activity against eleven bacteria and six fungal pathogens than other solvents. Twelve compounds were identified, off the following eight compounds such as 2-Butanol, 2-nitroso-, acetate(ester), Phenylethyl Alcohol, Phenol, 2,4-bis(1,1- dimethylethyl)-, 1-Hexadecanol, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3- (2-methylpropyl)-, 1Hentetracontanol, 1-Eicosanol, Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5' (phenylmethyl)-, (5'à)- were identified as anti-infective agent. Ethyl acetate would be a suitable solvent for the extraction of antimicrobial agents from *Streptomyces*.

[**Keywords:** Andaman, Antimicrobial activity, bioactive compounds, *Streptomyces*]

Introduction

Biologically active compounds produced by mangrove microorganisms may be a source for producing new bioactive compounds[1]. The importance of marine sources of novel natural products with pharmaceutical potential has been proved during the last decade and was high-lightened in various review articles[2,3]. Microorganisms present in mangrove environment produce naturally and traditionally active compounds against pathogenic microorganisms [4]. These natural organic compounds produced by microorganisms are an important screening target for a variety of bioactive substances. The actinobacteria, including members of the genera *Streptomyces*, *Sacchropolyspora* and *Amycolatopsis* are adapted to survive in a highly erratic and competitive soil environment. They are not only equipped with a wide array of enzymes for exploiting nutrients but also produce a broad range of bioactive metabolites of industrial and medical importance, e.g. compounds with antibiotic activity against fungus and bacteria. Actinobacteria are recognized as a source of novel antibiotic and anticancer agents with unusual structure and properties[5] and also they are known to produce chemically diverse compounds with a wide range of biological activities[6]. Therefore, actinobacteria hold a prominent position due to their diversity and proven ability to produce new compounds. Members of the marine actinobacteria are poorly understood and few reports only available pertaining to actinobacteria from mangroves[7]. Search for novel actinobacteria constitute an essential component in natural product based drug discovery.

Experimental Section

Isolation of actinobacteria

Mangrove sediment samples were collected from Guptapara, south Andaman. The sediments were air dried aseptically for five days then it was ground with a mortar and pestle, mixed thoroughly and passed through 2mm sieve filter to remove gravel and debris. The samples were kept at 70°C for 15 min. in separate glass container for pre-treatment. Ten fold serial dilutions of the sediment samples were made using sterile 50% sea water. About 0.1ml of the serially diluted sample was spread over the Kuster's agar medium pH 7±0.1 which was prepared using 50% sea water and supplemented with 80µg/ml of cycloheximide and 75µg/ml of nalidixic acid (Himedia, Mumbai) to minimize other bacterial and fungal growth[8]. The plates were incubated at

28±2°C for 28 days. After growth appeared, the actinobacterial colonies were sub-cultured and maintained in ISP No. 2 agar slants for further investigation.

Primary screening for antagonistic activity

The strain DOSMB-A107 was screened by cross streak method for antagonistic activities against *Klebsiella pneumoniae., Salmonella infantis., Staphylococcus aureus, Lactococcus lactis, Escherichia coli, Vibrio cholerae, Shigella flexneri, Pseudomonas* sp. *Proteus* sp., *Citrobactor diserus., Bacillus* sp., *Aspergillus niger, A. flavus., A. fumigates., Pencillium* sp., *Fusarium* sp., *Candida magnolia*. Modified nutrient agar and potato dextrose agar medium were used for bacteria and fungi respectively. Initially, these agar plates were inoculated with actinobacteria and incubated at 28±2°C. After observing a good ribbon like growth of the *Streptomyces* on the petriplates, the bacterial and fungal pathogens were streaked at right angles to the original streak of *Streptomyces* and incubated at 37°C for bacteria and 28±2°C for fungi. The inhibition zones were measured after 24 and 48 h[8].

Fermentation and preparation of crude extract

The isolate was inoculated into production media (dextrose- 20g, soya bean- 20g, soluble starch - 5g, peptone - 5g, $(NH_4)_2$-SO_4 - 2.5g, $MgSO_4.7H_2O$ - 0.25g, K_2HPO_4 - 0.02g, NaCl - 4g, $CaCo_3$ - 2g, seawater - 500ml, water - 500ml and pH 7±7.2), and incubated at 28±2°C on a rotary shaker at 250 rpm for seven days. The fermented broth was centrifuged at 10,000 rpm at 4°C for 20min. and the supernatant was filtered using 0.45µm filter (Millipore). An equal volume (1:1) of the solvent such as ethyl acetate, methanol, chloroform, hexane and ethanol was added separately to the cell free culture filtrates and mixed on a shaker for 12h, the solvent was separated and the extract was evaporated using a rotary evaporator and the crude powder was collected as an antimicrobial compound. The crude powder (20µg) was mixed with DMSO and used to test the activity against bacterial and fungal pathogens [9].

GC-MS analysis for compound identification

GC/MS analysis of ethyl acetate extract was performed using a Perkin-Elmer GC clauses 500 system and G C interfaced to a M S equipped with a Elite-5ms fused silica capillary column (30m x 0.25 mm ID x 0.25 m df), composed of 100% Dimethyl poly siloxane. An electron ionization system with ionizing energy of 70eV was applied for GC/MS detection. Helium gas (99.999%) was used as carrier at a constant flow rate of 1 ml/min and an injection volume of 3µl was employed split ratio of 10:1 injector at the temperature of 250ºC while ion-source temperature was 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min.) with an increase of 10ºC / min.-No hold, then 50ºC/min. to 280ºC, ending with a 9 min. isothermal at 280ºC. Mass spectra were taken at 70eV at a scan interval of 0.5 sec. and a fragment from 45 to 450 Da. Total GC running time was 36 min. The relative amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatogram was Turbomass. Interpretation of GC-MS data was carried out using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Phenotypic characterization of strain DOSMB-A107

The well matured aerial spore colour, reverse side pigments and melanin production was recorded in yeast malt extract agar (ISP2), oat meal agar (ISP3), inorganic salt starch casein agar (ISP4), glycerol asparagine agar (ISP5), tyrosine agar (ISP7), Kuster's agar (KU)[10]. The spore bearing hyphae and spore chain was determined by direct examination of culture under microscope (400 x mag.) by cover slip method using a well grown sporulated culture. The spore morphology and mycelia structure were observed in 14 days old culture under scanning electron microscope[11]. Biochemical tests such as indole test, methyl red test, Voges-Proskauer test, citrate utilization test, hydrogen sulphide production test, nitrate reduction test, urease test, catalase test, oxidase test, hydrolyses of starch, casein, gelatin and lipid and haemolysin assay were analyzed by the methods of Shirling and Gottlieb[10]. The ability of the streptomycetes grow in various pH (4, 6, 7, 8, 9 and 10), temperature, carbon and nitrogen sources were carried out. Further, analyses of cell wall amino acids [12] and whole cell sugars [13,14] were also carried out to differentiate the streptomycetes

Molecular characterization

The isolate was grown up to the late exponential phase in inorganic salt starch casein broth at 28±2ºC then the culture was harvested and washed twice with Tris EDTA buffer [15]. The chromosomal DNA was isolated by re-suspending of cells (0.5-1.0 g) in 5 ml of lysis buffer (25 mM Tris; 25 mM EDTA, pH 8.0; 10-15 µg lysozyme and 50 µg/ml Rnase) and incubated for 30-80 min at 37ºC, followed by the addition of 500 µl of 5 M NaCl solution. The suspension was agitated on a vortex mixer until it became translucent. To the sample 1.2 ml of 10% SDS was added and was incubated for 15-30 min at 65ºC. After the addition of 2.4 ml of 5 M potassium acetate, the solution was mixed and kept in ice for 20 min. The precipitate was removed by centrifugation at 6,000 rpm for 30 min., and the volume of the supernatant was adjusted to 8 ml. The DNA was recovered by precipitation with two volumes of isopropanol. The precipitate was dissolved in 700 µl/g 50 mM Tris, 10 mM EDTA (pH 8.0). Any insoluble substances were spun off and the aqueous phase was transferred to a 1.5 ml microfuge tube. Subsequently, 75 µl of 3 M sodium acetate and 500 µl isopropanol were added and the solution was centrifuged for 30 sec to 2 min. The precipitate was washed with cold 70% ethanol, dried and dissolved in 100µl TE (10mM Tris/1 EDTA, pH 8.0).

PCR amplification of 16S rDNA

A mixture of 39µl of water, 1µl of upstream primer (100 Pmols) (5'AGAGTTTGATCCTGGCTCAG 3'), 1µl of downstream primer (100 Pmols) (5'-CCGTACTCCCCAGGCGGGG 3'), and 5µl of 10x PCR buffer, 1 µl of 25 mM $MgCl_2$, 1µl of dNTP mix (10 mM), 1µl of template DNA (50ng) and 1 µl of *Taq* DNA polymerase (3U/µl), in a 0.5 ml microcentrifuge tube was taken. The total 50 µl mixture in the tube was gently spun for 10 sec. and the contents were allowed to settle. The amplification was carried out in Eppendrof PCR thermal cycler in the following steps of 35 cycles, denaturation for 60 sec. at 92ºC, primer annealing for 60 sec. at 54ºC and polymerization for 90 sec. at 72ºC. Finally, the tubes were ensured complete polymerization at 72ºC for 15 min. The samples were analysed by electrophoresis on a 1.2% agarose gel and was observed on UV trans-illuminator and compared with the DNA standard[16].

Phylogenetic analysis

The DNA sequences obtained were compared with other existing actinobacterial sequences by using NCBI BLAST search for their pair wise identities. Multiple sequence alignments and the phylogenetic tree was constructed with MEGA 4.1 software using the neighbour joining (NJ) tree method with 1000 replicates as bootstrap value and NJ belongs to the distance-matrix method. The 16S rRNA gene sequence was submitted to the GenBank and received accession number. The 16S rRNA gene secondary structure prediction and restriction site analysis of the isolate were analyzed by using Genebee and NEB cutter program version 2.0 online software (www.genebee.msu.su/services/rna2-reduced.html and www.neb.com/NEBCutter2/index.php) respectively.

Results and Discussion

Antimicrobial efficacy

The antimicrobial efficacy of five different solvent extracts of the *Streptomyces* sp. DOSMB-A107 was tested against eleven bacteria and six fungi. Notably, the ethyl acetate extract showed maximum efficacy against *Pseudomonas* sp. (24.66mm), followed by *A. niger* (24.33mm), *L. lactis* (23.33mm), *K. pneumoniae* (21.66mm), *Fusarium* sp. (20.33mm), *S. flexneri* (19mm), *C. magnolia* (19mm). *Bacillus* sp. (18.33mm), *A. flavus* (17.66mm), *S. infantis* (17mm), *S. aureus* (16.66mm) *A. fumigatus* (16.33mm), *E. coli* (15.66mm), *Penicillium* sp. (15.33mm), *V. cholerae* (15.33mm), *Proteus* sp. (14mm) and *Citrobacter diserus* (13.66mm) (Table 1). However, chloroform, hexane, ethanol, and methanol showed moderate to least activity against 15, 15, 12 and 8 pathogens respectively. Even though, these solvents did not show any activity against 2, 2, 5 and 9 pathogens respectively (Table 1). Similarly, Vijayakumar et al. have been studied the antimicrobial activity of different solvent extracts of *Streptomyces afghaniensis* VPTS3-1 against 13 pathogens (11 species of bacteria, 2 species of fungi). Of the various solvents used, ethyl acetate-treated extract was highly active against *Vibrio cholerae* (26 mm), followed by *Salmonella typhi* (24 mm), *Proteus vulgaris* (23 mm), *Staphylococcus epidermidis* (18 mm), *Bacillus subtilis* (17 mm), *Candida albicans* (17 mm), *Klebsiella pneumoniae* (16 mm), *Proteus mirabilis* (15 mm), *Staphylococcus aureus* (15 mm) and *Escherichia coli* (14 mm). However, other solvent extracts had a moderate to minimum

inhibitory effect against all the pathogens tested[17]. Likewise, many researchers have been evaluated the antimicrobial efficacies of the actinobacteria using various solvents including ethyl acetate, methanol, chloroform and alcohol[18]; n-butanol and ethyl acetate[19] and they were reported that, the ethyl acetate solvent extract had promising activity against most of the pathogens tested. In this view, the present and previous studies cleared recommended that, the selection of suitable solvents could be an essential step for the extraction of the antimicrobial compounds from microorganisms.

Table 1. Antimicrobial activity of *Streptomyces* sp. extracted by different solvents.

Pathogens	Methanol	Chloroform	Ethanol	Ethyl acetate	Hexane	Control DMSO
K. pneumoniae	-	16	-	21.66	11.66	3.66
S. infantis	-	15.33	-	17	14.33	3.33
S. aureus	12	11.66	9.66	16.66	10.66	5.66
L. lactis	13.66	13.33	12.33	23.33	13.66	7
E. coli	11.33	14.66	9.33	15.66	11	5.33
V. cholerae	-	11.66	-	13	12	3.33
S. flexneri	-	10.66	-	16	-	3.33
Pseudomonas sp.	15	21.66	13.66	24.66	19.66	3.66
Proteus sp.	12.66	14.33	9.33	14	12.33	2.33
C. diserus	-	11.66	-	13.66	13.33	4
Bacillus sp.	11.66	14	8.33	18.33	11.66	3.66
A. niger	12.66	-	19.66	24.33	14	3.33
A. flavus	-	14.66	13.33	17.66	11	-
A. fumigates	-	-	12.33	16.33	12.33	3.66
Penicillium sp.	12.33	10.33	9.33	15.33	14.66	-
Fusarium sp.	-	-	14.66	20.33	-	3
C. magnolia	-	-	17	19	12.33	-

Mean values are present in the table (zone of inhibition mm)

Identification of bioactive compounds of Streptomyces sp. DOSMB-A107

The results of GC/MS data were compared and interpreted with NIST Library to find out their nature. This enabled the identification of twelve compounds from the *Streptomyces* sp. DOSMB-A107. The mass spectrum, structure and nature of the compounds were given in figure 1, 2 and table 2. The present work has been reported about four alkane compounds, three alcoholic compounds, two alkaloids, one phenolic compound, one aromatic alcohol and one ester compound from the isolate *Streptomyces* sp. DOSMB-D107. Out of the twelve compounds, eight compounds were recorded as antibiotics and four recorded as non-antibiotic compounds. Of the eight antimicrobial compounds, three were identified as anti-inflammatory, one as an antioxidant and another one as an analgesic compound. Likewise, many researchers have been reported about their identified compounds from different actinobacteria. The compounds such as 2-butanol, 2-nitroso-, acetate (ester) have been reported from *Avicennia marina* (mangrove) but was not mentioned any antimicrobial activity[20]. However, NIST library reports showed with bioactivity. Phenylethyl alcohol was isolated from *Streptomyces globisporus* along with antifungal activity[21]. Phenol 2, 4-bis (1,1-dimethylethyl) has been reported from *Streptomyces* sp. TN272[22]. Similarly, the molecule has been reported from *Plumbago zeylanica*, Linn. and showed an antioxidant property[23]. 1-hexadecanol was reported with antimicrobial activity from the plant namely *Dacryodes edulis*p[24&25]. Pyrrolo[1,2-a] pyrazine - 1,4-dione, hexahydro - 3 - (2-methylpropyl) has been recorded with antibacterial activity from *Winogradskyella poriferorum* a marine sponge associated bacteria[26]. The NIST library data shows the pyrrolo[1,2-a] pyrazine -1,4-dione, hexahydro - 3 - (2-methylpropyl) is having anti-inflammatory

activity. 1-hentetracontanol was isolated from *Calluna vulgaris* with antimicrobial activity[27]. The 1-hentetracontanol was mostly reported from plants but, the present study reported the same compound from marine *Streptomyces* sp. DOSMB-A107. 1-Eicosanol has been documented with antimicrobial activity from *Geranium columbinum*[28]. Ergotaman-3', 6', 18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'à) was isolated with antifungal activity from *Streptomyces erumpens*[29]. Four non bioactive compounds reported by the present study such as cyclotetradecane, 1-nonadecene, 1-docosene, 17-pentatriacontene have already been reported from *Aspergillus versicolor*[30], *Rosa damascene* (plant)[31], *Penicillium chrysogenum*[30] and *Naringi crenulata* (plant)[32], respectively and also the present study has also proved that the four compounds did not possessed any antimicrobial activity.

Most of the compounds identified from the present study were already reported by many workers and showed that, the compounds retrieved from plant species have potential antimicrobial activity. The present study has been suggested that, the *Streptomyces* sp. DOSMB-A107 would be a suitable candidate for mass production of these bioactive compounds in pharmaceutical industries if the compounds are properly evaluated for their course of action in particular to those antimicrobial compounds. Further, these compounds could be used as an alternative drug for the multi-drug resistant pathogens.

Characterization of the isolate DOSMB-A107

Phenotypic characterization

The isolate DOSMB-A107 was examined under Scanning Electron Microscope (SEM), observed spiral shaped smooth spore surface (Fig. 3) which is the characteristic features of 75 to 80% of *Streptomyces* species [33]. The isolate showed variation in their colony morphology like colour of the aerial and substrate mycelia, such as black, brown and grey, whereas it does not produce any pigment on the culture media (Table 3). Similar type of observation has been reported earlier by Shinbu *et al.* in *Streptomyces*[34].

Various biochemical and physiological characterizations of the *Streptomyces* were also used for their identification (Table 4)[35&36]. In the present investigation, it was found that *Streptomyces* sp. A107 was not showed close relation with any available report according to their biochemical and physiological tests. Previously, it was reported that physiological properties are very significant for the identification of *Streptomyces*, however, it have no much significance in the identification up to species

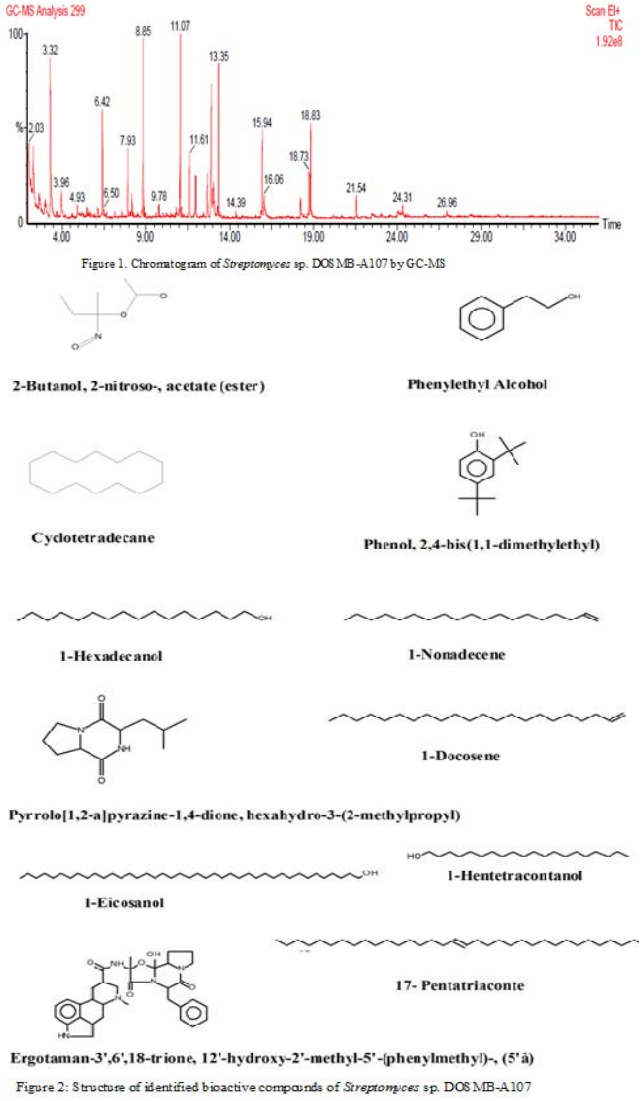

Figure 1. Chromatogram of *Streptomyces* sp. DOSMB-A107 by GC-MS

Figure 2: Structure of identified bioactive compounds of *Streptomyces* sp. DOSMB-A107

level. But, they used as a marker by which individual strains can be recognized for various physico-chemical properties which are influencing the growth rate of the *Streptomyces*[37&38]. The nitrate reducing ability of *Streptomyces* has also reported by many researchers[35&39], and production of H_2S has been considered as another useful character for the identification of streptomycetes[10]. Production of enzymes was considered for characterizing the

reported that the physiological characteristics of

Table 2. Major compounds identified by MS from ethyl acetate extract of *Streptomyces* sp.

RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
2.29	2-Butanol, 2-nitroso-, acetate(ester)	$C_6H_{11}NO_3$	145	4.38	Ester compound	Antimicrobial
3.32	Phenylethyl Alcohol	$C_8H_{10}O$	122	18.78	Aromatic alcohol	Antimicrobial
6.42	Cyclotetradecane	$C_{14}H_{28}$	196	7.50	Alkane compound	No activity
7.93	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	3.93	Phenolic compound	Antioxidant, Antimicrobial Anti-inflammatory Analgesic
8.85	1-Hexadecanol	$C_{16}H_{34}O$	242	9.6	Alcoholic compound	Antimicrobial
11.07	1-Nonadecene	$C_{19}H_{38}$	266	9.01	Alkene compound	No activity
12.92	Pyrrolo[1,2a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210	15.18	Alkaloid	Antimicrobial, Anti-inflammatory
13.35	1-Docosene	$C_{22}H_{44}$	308	9.79	Alkene compound	No activity
15.94	1-Hentetracontanol	$C_{41}H_{84}O$	592	6.46	Alcoholic compound	Antimicrobial
18.73	1-Eicosanol	$C_{20}H_{42}O$	298	3.40	Alcoholic compound	Antimicrobial
18.83	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'à)-	$C_{33}H_{35}N_5O_5$	581	10.25	Alkaloid	Antimicrobial Anti-inflammatory
24.31	17-Pentatriacontene	$C_{35}H_{70}$	490	1.70	Alkene compound	No activity

Streptomyces sp.[40&41]. In the present study, it was found that *Streptomyces* sp. DOSMB-A107 was able to produce urease and citrase. Thus, the ability to produce enzymes was also an important characteristic feature of *Streptomyces*, which are responsible for their adaptability and survivability of the isolates in the different soil environment. The ability to degrade many of such compounds by *Streptomyces* has been reported[42&43]. The present investigation revealed that the pH 6-9 and temperature 25-42°C were optimum for the growth of *Streptomyces* sp.DOSMB-A107 (Table 5). The growth response of the actinobacteria to the inhibitory compounds was studied by many researchers [44&45]. Thus, the different species/strains vary in their response to different inhibitory compounds depending on their genetic makeup. Hence, the *Streptomyces* sp. DOSMB-A107 was distinctly different from prior report.

The antibiotic sensitivities of the *Streptomyces* spp. have been reported by Gesheva and Gesheva[46]. Various amino acid and carbon source utilization statuses were used to confirm the identity of *Streptomyces* sp.[47&43]. Thus, the present investigation *Streptomyces* spp. varied depending on the nutritive and the physical conditions of the culture media. It is evident that the growth of the *Streptomyces* spp. was influenced by the environmental factors such as pH, temperature, inhibitory compounds and the availability of nutrients.

Cell wall chemistry of the isolate DOSMB-A107

The cell wall composition such as cell wall amino acid and whole cell sugars of the isolate DOSMB-A107 was analysed. The isolate showed the

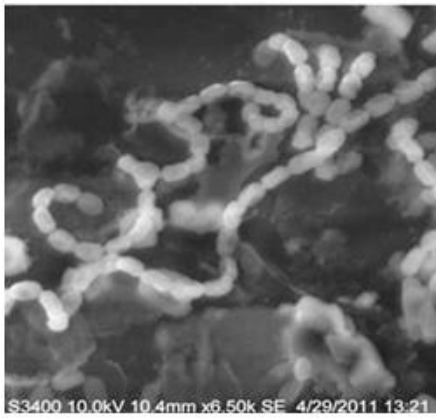

Figure 3. Spore surfece views of DOSMB-A107

Table 3. Cultural characteristics of DOSMB-A107 on different media

Media	Arial mycelium	Substrate mycelium	Diffusible pigment	Melanin pigment
Yeast malt extract agar (ISP2)	Gray	Brown	Nil	Nil
Oat meal agar (ISP3)	Whitish gray	Black	Nil	Nil
Inorganic salt starch casein agar (ISP4)	Gray	Brown	Nil	Nil
Glycerol asparagines agar (ISP5)	Gray	Black	Nil	Nil
Tyrosine agar (ISP7)	Gray	Black	Nil	Nil
Kuster's agar (KU)	Gray	Black	Nil	Nil
Actinomycetes isolation agar (AIA)	Gray	Brown	Nil	Nil

Table 4. Biochemical characteristics of *Streptomyces* sp. A107

S.No.	Biochemical Test	DOSMB-A107
1.	Indole production	-ve
2.	Methyl red	-ve
3.	Vogues-Proskauer	-ve
4.	Citrate utilization	+ve
5.	H_2S production	+ve
6.	Nitrate utilization	+ve
7.	Urease	+ve
8.	Catalase	+ve
9.	Oxidase	+ve
10.	Starch hydrolysis	+ve
11.	Gelatin hydrolysis	-ve
12.	Lipid hydrolysis	-ve
13.	Casein hydrolysis	+ve
14.	Lecithin hydrolysis	+ve
15.	Xanthine	+ve
16.	Testosterone	-ve
17.	Haemolysis	-ve
Carbon source utilization		
1	Inositol	-
2	Dextrose	+
3	Sucrose	-
4	Cellulose	+
5	Xylose	+
6	Arabinose	+
7	Raffinose	+
8	Mannital	+
9	Fructose	+
10	Sorbitol	+
11	Maltose	+
12	Lactose	+
13	Rhamnose	+
14	Adonital	+
15	Starch	+
16	L-rhamnose	-

LL-diaminopimelic acid and glycine. It does not contain any diagnostic sugar in their cell. Thus, the present investigation has concluded that the isolate DOSMB-A107 had a type-I cell wall. The similar cell wall chemistry has been reported in *Streptomyces* spp.[48].

Table 5. Physiological characteristics of *Streptomyces* sp. DOSMB-A107

S. No.	Test	Observation
1.	*Temperature* (°C)	
a.	4	-
b.	15	-
c.	25	+
d.	28	+
e.	35	+
f.	42	+
g.	55	-
2	*pH*	
a.	4	-
b.	6	+
c.	7	+
d.	8	+
e.	9	+
	10	-
3.	*Antibiotic sensitivity* (mm)	
a.	Cephalothin (30mcg)	R
b.	Clindamycin (2mcg)	R
c.	Co-trimoxazole (25mcg)	R
d.	Erythromycin (15mcg)	12
e.	Gentamycin (10mcg)	22
f.	Oflaxin (1mcg)	R
g.	Penicillin (10unit)	R
h.	Vancomycin (mcg)	25
i.	Amikacin	34
4	*Inhibitory compound* (%w/v)	
a.	Crystal violet (0.0001)	+
b.	Potassium tellurite (0.001)	-
c.	Sodium aside (0.01)	+
d.	Sodium chloride	
	1%	-
	3%	+
	5%	+
	7%	+
	10%	+
5	*Utilization of amino acids*	
a.	L-tyrosine	+
b.	D-tryptophan	+
c.	L-proline	-
d.	L-methionine	+
e.	L-lysine	+
f.	L-arginice	+

Phylogenetic analysis of Streptomyces sp. A107

Significance of phylogenetic studies based on gene sequences enhanced the knowledge on the systematics of actinobacteria[49]. Sequences of 16S rRNA gene have provided actinobacteriologists with

highly reproducible data and phylogenetic trees that allows the investigation on evolution of actinobacteria and also provided the basis for identification. The 16s rRNA sequencing and phylogenetic analysis reveals that the isolate DOSMB A107 correspond the *Streptomyces* sp. (Gen Bank Acc. No. JQ638519.1) (Figure 4). The secondary structure of 16S rRNA gene of the *Streptomyces* sp. DOSMB-A107 (JQ638519) showed 16 stems in their structure as well as the free energy structure of the 16S rRNA secondary structure showed -92.1kkal/mol as indicated by genebee software www.genebee.msu.ru. The total restriction enzymes were 48 and the GC content was 58%. The free energy structure was -92.1 kkal/mol determined by NEB Cutter Program V 2.0 in www.neb.com/nebcutter2/inde.php.

In the present study, a distinct variation in the secondary structure, GC composition, presence of restriction enzyme sites in 16S rRNA gene sequence of the isolate DOSMB-A107 showed molecular level specificity of each individual isolates. The apparent phylogenetic relationships, secondary structure and restriction enzyme sites in 16S rRNA has been reported by Cook and Mayer[50]. Based on

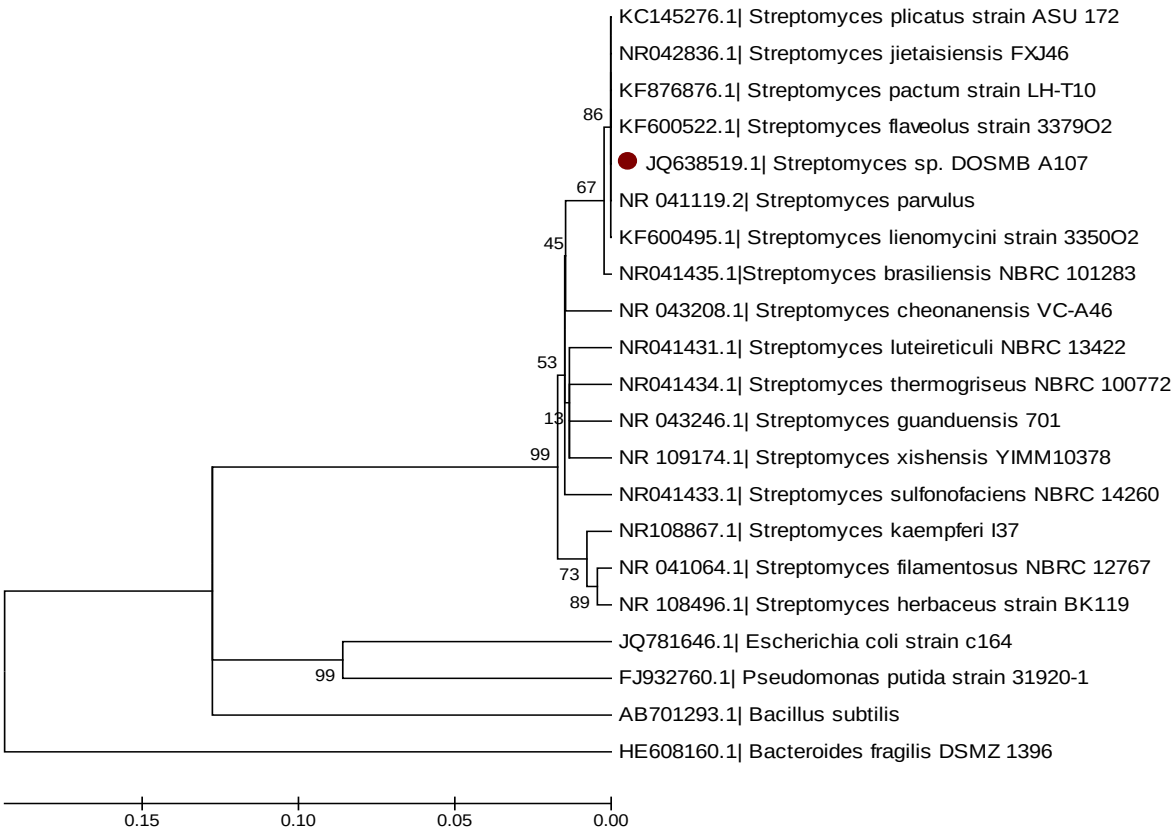

Figure 4. The inferred phylogenetic tree of marine isolate DOSMB A107 (●). Evolutionary distances were determined with pairwise dissimilarities of the 16S rRNA gene sequences, and the dendrogram was generated using the neighbor-joining algorithm (Mega 5). The isolate DOSMB A107 correspond to the *Streptomyces* spp.

the morphological, cultural, chemistry of cell wall and molecular properties of the isolate DOSMB-A107 was identified as *Streptomyces* sp. DOSMB-A107 (JQ638519). The identity of the isolate was also confirmed by Bergey's Manual of Systematic Bacteriology, Bergey's Manual of Determinative Bacteriology and phylogenetic analysis using sequence alignment program CLUSTAL W. However, the complete 16S rRNA gene of the isolate has to be sequenced for species level identification.

Acknowledgements

The authors thank to University Grants Commission (UGC) for providing Research Fellowship in Science to Meritorious Student (RFSMS) to the first author. The authors also obliged to Central Instrumentation Facility Section (CIF), Pondicherry University, Puducherry for SEM experiment.The authors also obliged to Central

Instrumentation Facility Section (CIF), Pondicherry University, Puducherry for SEM experiment.

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