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ANTIMICROBIAL ACTIVITY AND PHYLOGENETIC ANALYSIS OF *STREPTOMYCES PARVULUS* DOSMB-D105 ISOLATED FROM THE MANGROVE SEDIMENTS OF ANDAMAN ISLANDS

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Actinomycetes, especially species of *Streptomyces* are prolific producers of pharmacologically significant compounds accounting for about 70% of the naturally derived antibiotics that are presently in clinical use. In this study, we used five solvents to extract the secondary metabolites from marine *Streptomyces parvulus* DOSMB-D105, which was isolated from the mangrove sediments of the South Andaman Islands. Among them, ethyl acetate crude extract showed maximum activity against 11 pathogenic bacteria and six fungi. Presence of bioactive compounds in the ethyl acetate extract was determined using GC-MS and the compounds detected in the ethyl acetate extract were matched with the National Institute of Standards and Technology (NIST) library. Totally eight compounds were identified and the prevalent compounds were 2 steroids, 2 alkaloids, 2 plasticizers, 1 phenolic and 1 alkane. Present study revealed that *S. parvulus* DOSMB-D105 is a promising species for the isolation of valuable bioactive compounds to combat pathogenic microbes.

Keywords: antimicrobial activity, bioactive compound, actinobacteria, *Streptomyces parvulus,* Andaman Islands

Introduction

Status of marine sources for unearthing the novel natural products with pharmaceutical potential has been brought out during the last decade and high-

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lighted in various research articles [1–2]. Microorganisms of different marine environments have been found to produce pharmacologically significant secondary metabolites which are commercially explored for drug discovery. Among the microbes, actinobacteria, with an array of enzymes, yield a broad range of bioactive metabolites of industrial and medical importance. They are one of the most vital sources for new bioactive compounds such as antibiotics and enzymes [3, 4]. The isolated compounds from the marine actinobacteria have shown a broad spectrum of biological activities, viz. antibiotic, antifungal, toxic, cytotoxic, neurotoxic, antimitotic, antiviral and antineoplastic activities [5]. For many of the marine actinobacteria, taxonomy of the strain is poorly defined so that binomial identification is frequently uneasy to carry out [6].

Streptomyces is the principal genus of actinobacteria [7] and it has been now documented that over 55% of the antibiotics discovered from the genus *Streptomyces* represent a total of more than 5000 compounds [8]. However, this genus has been frequently isolated from marine samples such as decaying materials, sediments and animals, numerous attempts have been made to evaluate its antagonistic properties. *S. parvulus,* isolated from the terrestrial and marine environment, is having potential bioactive compounds, which are available in the market, e.g. Actinomycin D and Manumycin A [9]. Marine soils of India especially those of the Andaman and Nicobar Islands have rich microbial diversity, including actinobacteria. However, they have not been fully explored for microbial resources and their bioactive compounds.

Materials and Methods

Isolation of Streptomyces

Sediment samples were collected from the mangrove environment of Burmanallah, South Andamans and were subjected to pre-heat treatment and the pre-treated sediments were used for the isolation of Streptomycetes [10].

Taxonomy of strain DOSMB-D105

Colour of the matured aerial spore and substrate mycelium and melanin production were recorded in yeast malt extract agar (ISP2), oat meal agar (ISP3), inorganic salt starch casein agar (ISP4), glycerol asparagine agar (ISP5), tyrosine agar (ISP7) and Kuster's agar (KU) [11]. Spore bearing hyphae and spore chain were studied by the direct examination of the cultures under a microscope

(400 × magnifications). Spore morphology and mycelial structure were observed in 14 days old culture under scanning electron microscope [12]. Biochemical characterization of the isolate was also made by the methods of Shirling and Gottlieb [11]. Stability of growth in various pH, temperature, carbon and nitrogen sources were also studied. Further, analyses of cell wall amino acids [13] and whole cell sugars [14] were carried out.

Molecular characterization

Chromosomal DNA was isolated using the previously described method of Wilson and Brent [15]. The template DNA was amplified by using upstream primer (100 pmols) (5′-AGAGTTTGATCCTGGCTCAG 3′), 1 µL of downstream primer (100 pmols) (5′-CCGTACTCCCCAGGCGGGG 3′) in PCR thermal cycler. The amplification was carried out in the following manner of 35 cycles, denaturation for 60 sec at 92 °C, primer annealing for 60 seconds at 54 °C and polymerization for 90 sec at 72 °C. Ten µL of PCR products with 2 µL of loading dye was analysed by electrophoresis on agarose gel [16]. Retrieved gene sequences were compared with other bacterial sequences by using NCBI BLAST search for their pair wise identities. Multiple sequencing alignments and the phylogenetic tree construction were done with MEGA 4.1 software by using the neighbour joining (NJ) method with 1000 replicates as bootstrap value and NJ belongs to the distance-matrix method [17]. The 16S rRNA sequence was submitted to the GenBank. The 16S rRNA secondary structure prediction and restriction site of the isolate were analysed by Genebee and NEB cutter program version 2.0 online software (www.genebee.msu.su/services/rna2-reduced.htmlandwww.neb.com/NEBCutter2/index.php) respectively.

Extraction of bioactive compounds

S. parvulus DOSMB-D105 was cultivated in a 5 L-flask comprising 2.5 L of production media broth (dextrose – 20 g, soya bean – 20 g, soluble starch – 5 g, peptone – 5 g, $(NH_4)_2$ – SO_4 – 2.5 g, Mg SO_4 $7H_2O$ – 0.25 g, K2 HPO_4 – 0.02 g, NaCl – 4 g, $CaCo_3$ – 2g, seawater – 500 mL, dH_2O – 500 ml and pH – 7±0.2) with shaking at 28±2 °C and 250 rpm for seven days. The fermented broth was centrifuged at 10 000 rpm at 4 °C for 20 min and the supernatants were filtered using 0.45-µm-spore size membrane filter (Millipore). An equal volume (1:1) of ethyl acetate, methanol, chloroform, hexane, water and alcohol was added separately to the cell free culture filtrates and shaken for 12 h and the solvent

extracts were evaporated by rotary evaporator and the crude powder was collected and 20 µg was mixed with DMSO to check the activity against eleven bacteria (Muller Hinton agar) and six fungi (Sabouraud's dextrose agar) using agar well diffusion method [18].

GC-MS analysis

GC-MS analysis of ethyl acetate extract was performed using a Perkin-Elmer GC clauses 500 system and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Elite-5 ms, fused silica capillary column (30 m×0.25 mm ID×0.25 mdf), composed of 100% dimethyl poly siloxane. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 3 µL was employed split ratio of 10:1 injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C/min-No hold, then 50 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. The relative % amount of each component was calculated by comparing its average peak area to the total areas and the software used to handle the mass spectra and chromatograms was Turbomass. Interpretation of the mass spectrum GC-MS was made using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns. Spectrum of the compounds were compared with the spectrum of the known compounds stored in the NIST library. Name, molecular weight and structure of the compounds of the test material were also ascertained.

Results

Morphological and cultural characteristics

The isolate DOSMB-D105 was identified by morphological, cultural, cell wall chemical, biochemical, physiological and molecular characteristics, as per the ISP (International *Streptomyces* Project) description. Light and scanning electron microscopic (SEM) views of the isolate showed spiral and smooth spore surface (Fig. 1). Characteristics of the isolate's colony on various culture media

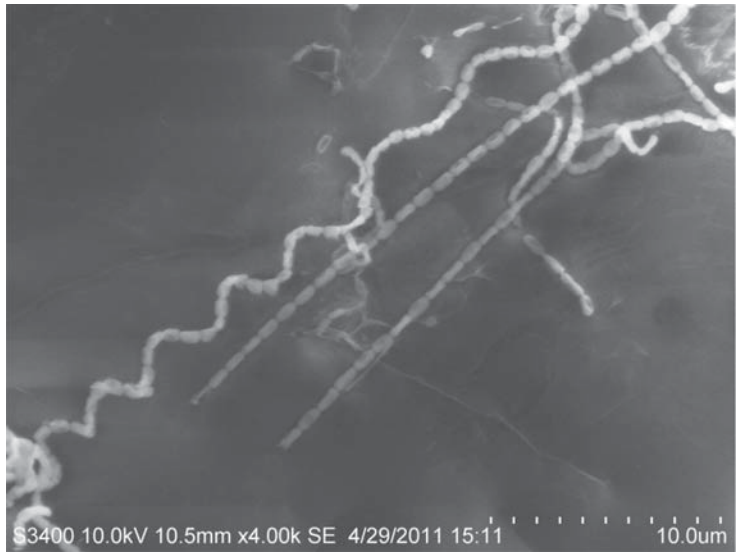

Figure 1. Morphology of *Streptomyces parvulus* DOSMB-D105

Table 1. Cultural characteristics of DOSMB-D105 on different media

Medium	Properties of *Streptomyces*			
	Arial mycelium	Substrate mycelium	Diffusible pigment	Melanin pigment
ISP2	Grey	Yellow	Nil	Nil
ISP3	Whitish grey	Dark yellow	Nil	Nil
ISP4	Whitish grey	Dark yellow	Yellow	Nil
ISP5	Grey	Yellow	Nil	Nil
ISP7	Grey	Yellow	Nil	Positive
KUA	Grey	Yellow	Yellow	Nil
AIA	Grey	Yellow	Yellow	Nil

and physiological and biochemical characteristics are given in Tables I, II and III. The isolate showed the presence of LL-diaminopimelic acid and, glycine and did not contain any diagnostic sugar in its cell wall, identifying that the isolate DOSMB-D105 had type-I cell walls.

Table II. Biochemical characteristics of DOSMB-D105

S. No.	Test	DOSMB-D105
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

+ ve (positive) – ve (negative)

Molecular characterization

Sequence of the strain DOSMB-D105 was compared with the reference sequences of the NCBI database for pair wise alignment. It revealed that 827 base sequence of the strain DOSMB-D105 matched with *Streptomyces parvulus* (JQ638518) (Fig. 2). The secondary structure of 16S rRNA of *S. parvulus* DOSMB-D105 showed 33 stems. Free energy structure of the 16S rRNA secondary structure of the strain showed 193.3 kkal/mol, as indicated by genebee software. The total restriction enzyme was 55, GC content was 59% and –78.6 kkal/mol, as detected by NEB Cutter Program V 2.0. Based on the colony morphology, cell chemistry, biochemical, physiological and molecular characteristics, the strain DOSMB-D105 was identified as *Streptomyces parvulus*.

Antimicrobial efficacy of Streptomyces parvulus *DOSMB-D105*

The fermented broth of *S. parvulus* DOSMB-D105 was extracted by using five different solvents. Antimicrobial activity of the solvent extracts was tested against eleven pathogenic bacteria and six fungal pathogens. Maximum zone of inhibition of the ethyl acetate solvent extract was obtained against *Pseudomonas* sp. (27.66 mm), followed by *Proteus* sp. (22.66 mm), *Bacillus* sp. (22.33 mm), *E. coli* (20.33 mm), *S. aureus* (22.33 mm), *L. lactis* (22.00 mm), *S. infantis* (16.66

Table III. Physiological characteristics of DOSMB-D105

S. No.	Test	D105
1.	*Temperature*	
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 (30 mcg)	R
b.	Clindamycin (2 mcg)	18
c.	Co-Trimoxazole (25 mcg)	R
d.	Erythromycin (15 mcg)	R
e.	Gentamycin (10 mcg)	21
f.	Oflaxin (1 mcg)	R
g.	Penicillin (10 unit)	R
h.	Vancomycin (mcg)	R
i.	Amikain	35
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	+

+ (positive) – (negative) R (drug resistance)

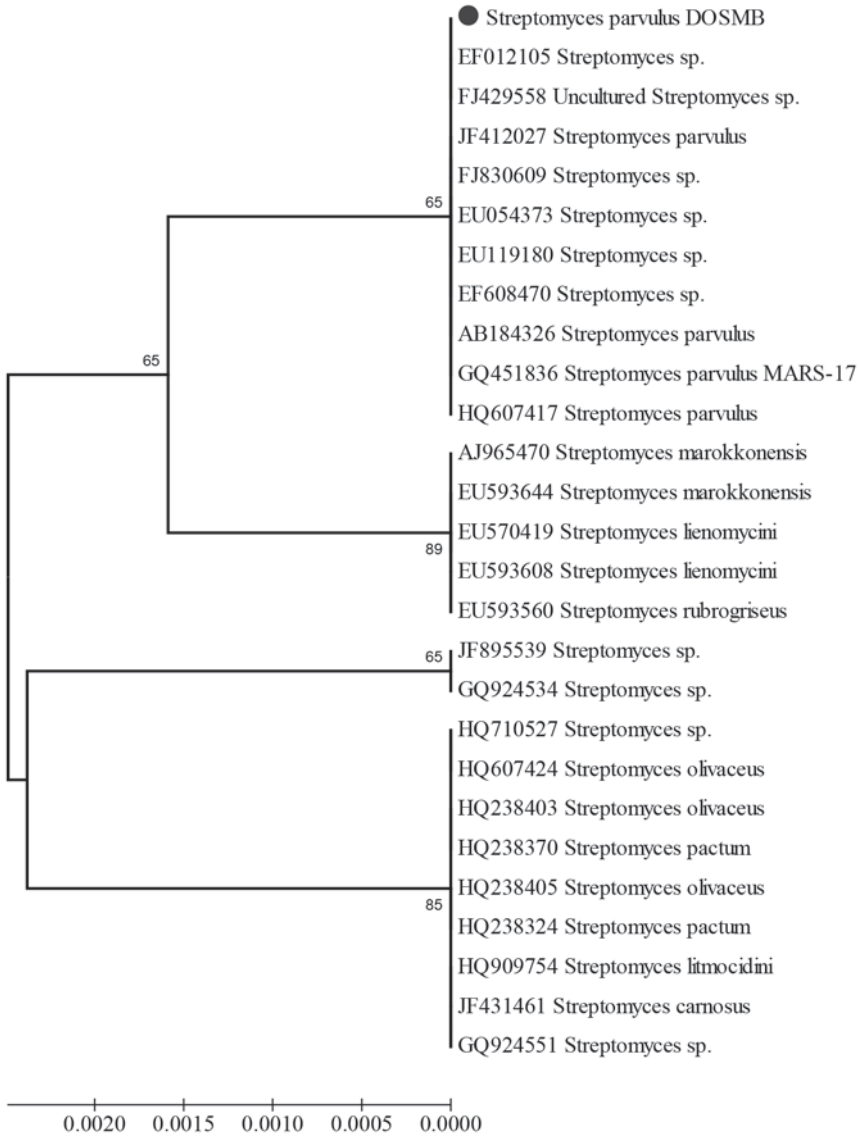

Figure 2. Phylogenetic tree of *Streptomyces parvulus* DOSMB-D105

mm), *K. pneumoniae* (15.66 mm), *V. cholarae* (15.33 mm), *C. diserus* (15.33 mm), *S. flexneri* (13.33 mm), *A. niger* (25.66 mm), *A. flavus* (20.33 mm), *A. fumigatus* (19.33 mm), *Penicillium* sp. (18.66 mm), *Fusarium* sp. (17.33 mm) and *C. magnolia* (20.66 mm) (Plate 1 and Table IV).

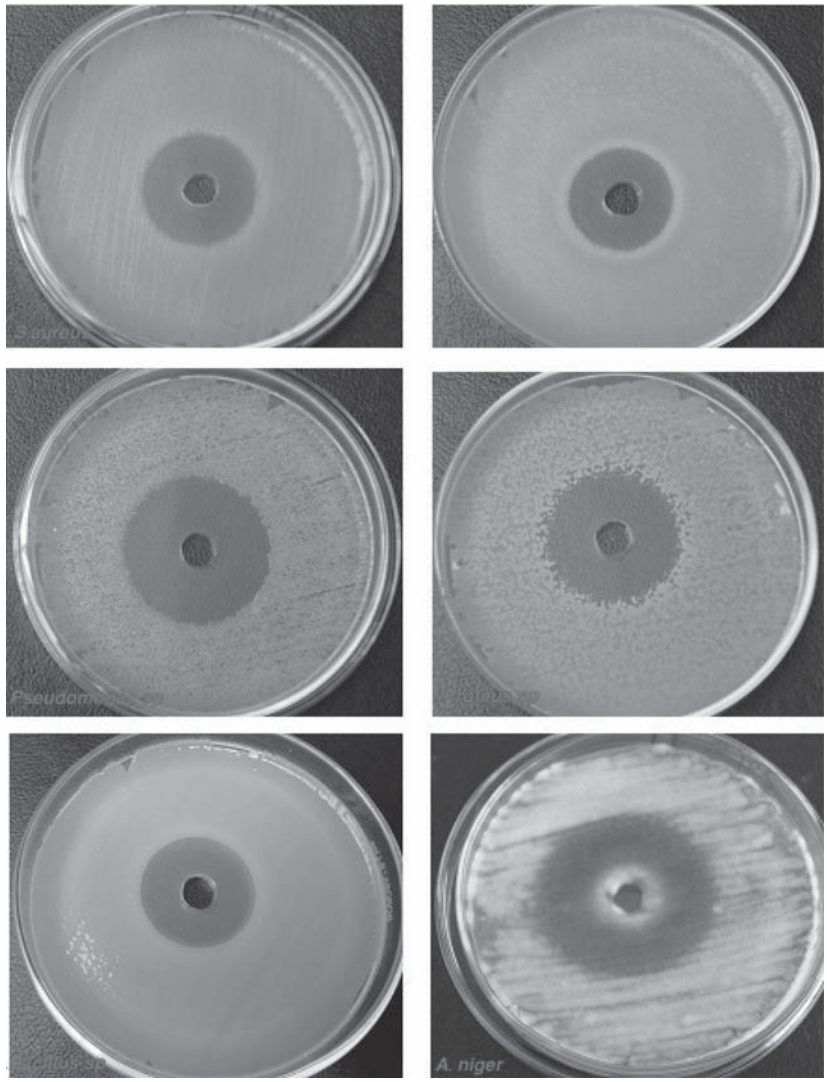

Plate 1. Antimicrobial activity of *Streptomyces* sp. DOSMB-D105

Maximum activity of chloroform extract was found against *S. aureus* (20.66 mm) followed by other tested pathogens and did not show any activity against *A. niger* and *A. fumigatus*. Methanol and alcohol extracts showed maximum activity against *Bacillus* sp. (17.66 mm and 16.33 mm, respectively) and moderate to minimum activity against other pathogens. Methanol and alcohol extract did not express any zone of inhibition against *K. pneumoniae, S. infantis,*

Table IV. Antimicrobial activity of *S. parvulus* DOSMB-D105 extracted by different solvents

S. No	Pathogens	Methanol	Chloroform	Ethanol	Ethyl acetate	Hexane	Control DMSO
1	*K. pneumoniae*	–	13.33±0.50	–	15.66±0.50	12.66±0.19	3.66±0.19
2	*S. infantis*	–	14.66±0.19	–	16.66±0.38	12.66±0.19	3.33±0.38
3	*S. aureus*	13.33±0.38	20.66±0.19	14.33±0.19	22.33±0.19	–	5.66±0.19
4	*L. lactis*	17.0±0.33	20.0±0.33	14.66±0.19	22.0±0.33	–	7.0±0.33
5	*E. coli*	12.66±0.19	18.66±0.19	13.33±0.19	20.33±0.19	–	5.33±0.19
6	*V. cholerae*	–	9.66±0.19	–	15.33±0.19	9.66±0.19	3.33±0.19
7	*S. flexheri*	–	8.33±0.19	–	13.33±0.38	–	3.33±0.19
8	*Pseudomonas* sp.	–	17.66±0.19	–	27.66±0.19	–	3.66±0.19
9	*Proteus* sp.	15.66±0.19	20.66±0.19	14.33±0.19	22.66±0.19	9.66±0.19	2.33±0.19
10	*C. diserus*	–	12.33±0.19	–	15.33±0.19	11.66±0.19	4.00±0.33
11	*Bacillus* sp.	17.66±0.19	20.66±0.19	16.33±0.19	22.33±0.19	15.33±0.19	3.66±0.19
12	*A. niger*	23±0.33	–	14.66±0.19	25.66±0.19	18.66±0.19	3.33±0.19
13	*A. flavus*	18.66±0.19	13.33±0.19	–	20.33±0.19	16±0.00	–
14	*A. fumigates*	16.33±0.38	–	10.66±0.19	19.33±0.19	14.66±0.19	3.66±0.19
15	*Penicillium* sp.	15.66±0.19	13.0±0.33	14.00±0.00	18.66±0.19	13.33±0.19	–
16	*Fusarium* sp.	14.33±0.38	11.0±0.00	11.66±0.19	17.33±0.19	–	3.00±0.00
17	*C. magnolia*	17.66±0.19	12.66±0.19	12.66±0.57	20.66±0.19	14.33±0.19	–

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

V. cholerae, S. flexneri and *C. diserus,* and alcohol extract had no activity against *Pseudomonas* sp. and *A. flavus*. Hexane extract showed highest activity against *A. niger* (18.66 mm) followed by other tested pathogens; however, no activity was found against *S. aureus, L. lactis, E. coli, S. flexneri, Pseudomonas* sp. and *Fusarium* sp. (Table IV).

Identification of compounds

GC-MS spectral results and comparison of results with NIST library search productively enabled the presence of eight compounds in *S. parvulus* DOSMB-D105: phenol, 2,4-bis(1,1-dimethylethyl); pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-metylpropyl); 7,8-epoxylanostan-11-ol, 3-acetoxy; dihydro-ergot-

Table V. Compounds identified from the *S. parvulus* DOSMB-D105

RT	Name of the compound	Molecular formula	MW	Peak area %	Nature	Activity (Ref. NIST)
7.94	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	3.39	Phenolic compound	Antioxidant Antimicrobial Anti-inflammatory Analgesic
11.57	Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210	10.32	Alkaloid compound	Antimicrobial Anti-inflammatory
12.87	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	502	24.81	Steroid	Antimicrobial Anti-inflammatory Anti-arthritic Antiasthma Anticancer
18.80	Dihydroergotamine Mesylate	$C_{33}H_{37}N_5O_5$	583	14.95	Alkaloid compound	Antimicrobial Anti-inflammatory
20.72	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	34.51	Plasticizer compound	Antimicrobial, Anti fouling
25.09	Phthalic acid, diisodecyl ester	$C_{28}H_{46}O_4$	446	5.08	Plasticizer compound	Antimicrobial Anti fouling
26.69	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	4.16	Alkane compound	No activity reported
34.48	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	2.77	Steroid	Same like 3rd compound

amin-emesylate; 1,2-benzenedicarboxylic acid, diisooctyl ester; phthalic acid, diisodecyl ester; octadecane, 3-ethyl-5-(2-ethylbutyl)and ethyl iso-allocholate. Among these, seven compounds were bioactive compounds except Octadecane, 3-ethyl-5-(2-ethylbutyl). Mass spectrum and structures of all the compounds (Fig. 3 and 4) and the details of the identified compounds are given in Table V.

Discussion

Identification of strain DSMB-A107

Smooth surfaced spores are characteristic of 75 to 80% of the *Streptomyces* [19–20]. When the strain DOSMB-D105 was examined under the SEM, it

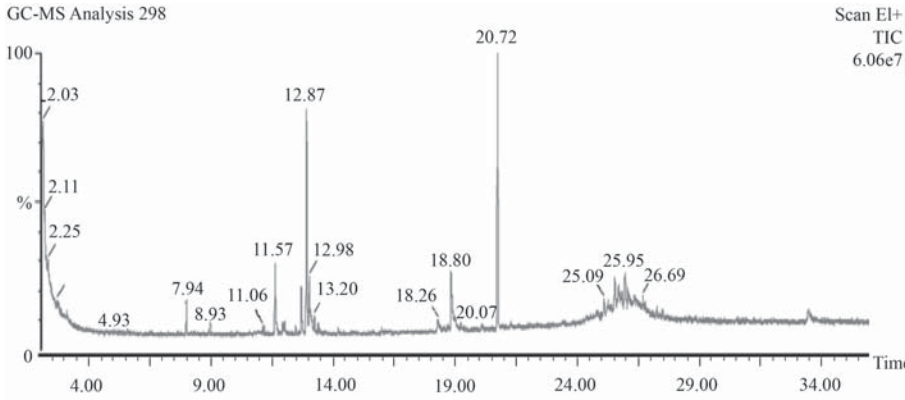

Figure 3. Chromatogram of *Streptomyces parvulus* DOSMB-D105 by GC-MS

showed spiral and smooth spore surface. Chemical composition of the medium has a bearing on the morphology of the organisms. The strain DOSMB-D105 was grown on various media, viz. ISP2, ISP3, ISP4, ISP5, ISP7, KUA and AIA. Of the different media used, Kuster's agar medium gave better result than the others as the strain DOSMB-D105 could easily utilize all the nutrients and sporulate in this medium. Similar types of observations have been made by many workers [21–22].

Cell wall chemistry

Simple quantitative analysis of cell wall amino acid is one of the most useful techniques for the primary identification of actinobacteria at the genus level. The most useful diagnostic marker is the diaminopimelic acid which occupies the anchor position in the tetrapeptide of the cell wall peptidoglycan, since many actinobacteria contain meso-diaminopimelic acid. Of the various sugars occurring in the cell hydrolysate of actinobacteria, four sugars, arabinose, galactose, madurose and xylose are of taxonomic importance. Members of the family *Streptomycetaceae* do not contain any of these sugars and in most cases they are devoid of any other carbohydrates. The cell wall chemistry of the *Streptomyces* spp. has also been reported by several researchers [23–24]. In the present study, *S. parvulus* DOSMB-D105 was investigated for the cell wall amino acids and whole cell sugars. It showed the presence of LL-diaminopimelic acid and glycine and did not contain any diagnostic sugars in the cell wall.

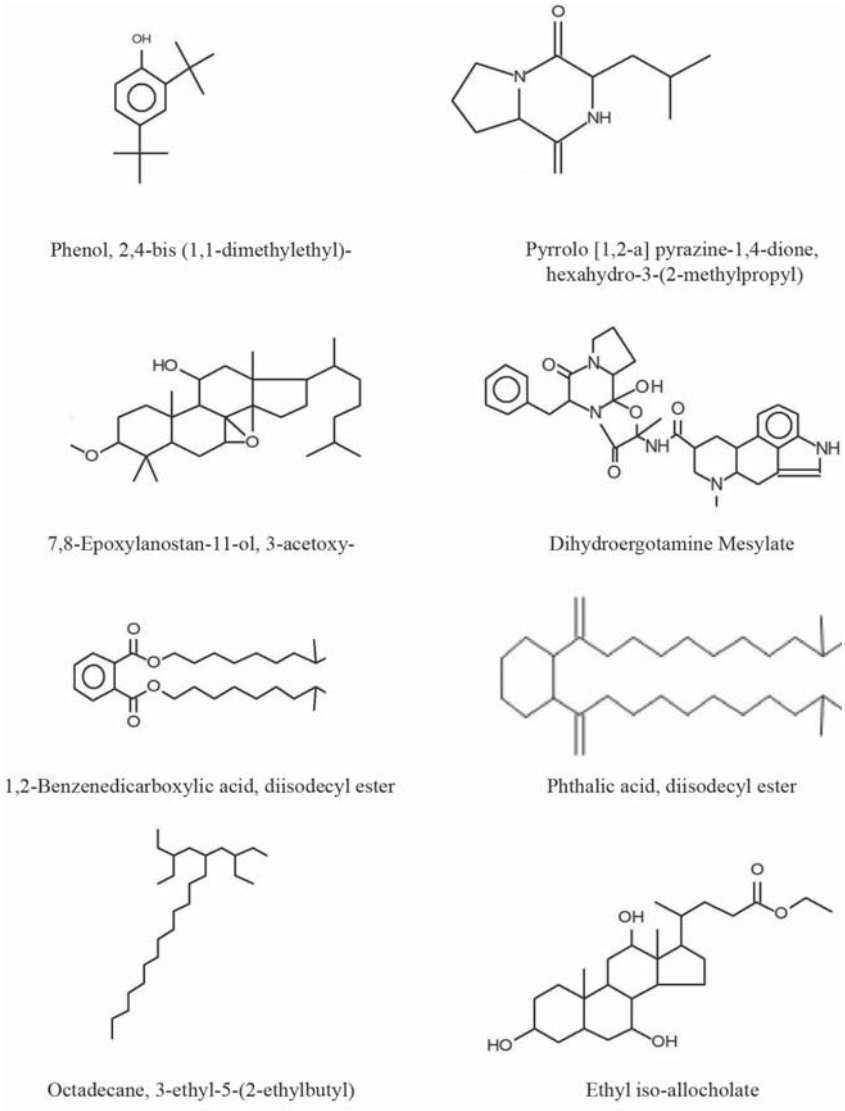

Figure 4. Structure of identified compounds of *Streptomyces parvulus* DSMB-D105

Biochemical characterization

Various biochemical characterizations of *Streptomyces* spp. were used for their identification [25]. In the present investigation, it was found that *S. parvulus*-DOSMB-D105 had nitrate reducing ability, as referred by others [26].

Production of urease, catalase, oxidase, β-lactamase and citrase are important for characterizing the *Streptomyces* species [27]. In the present study, it was found that *S. parvulus* DOSMB-D105 produced urease and citrase and did not produce catalase. Similarly, hydrolyse of starch, gelatin, casein, lecithin, xanthine and haemolysis as also used for characterizing the isolates. In the present study, DOSMB-D105 was found to hydrolyse starch, lecithin and casein and the isolate was not able to hydrolyse testosterone, lipid and gelatine.

Physiological characteristics of S. parvulus *DOSMB-D105.*

Physiological properties are very important for the identification of *Streptomyces;* however, they are not much of significance in the identification up to species level but they are used as markers. Strains can be recognized for various physico-chemical properties, influencing the growth of *Streptomyces* [28]. Present investigation revealed that the optimum pH was 6–9 and temperature was 25–42 °C, for the growth of *S. parvulus* DOSMB-D105. Growth response of the organism to the inhibitory compounds was studied and it was found that the isolate could tolerate crystal violet, sodium aside and sodium chloride. Likewise, other researchers have reported the tolerance of actinobacteria to inhibitory compounds [24, 29]. *S. parvulus* DOSMB-D105 was sensitive to gentamycin, clindamycin and amikacin and resistant to cepholothin, co-trimoxale, oflaxin and penicillin as observedin *Streptomyces* spp. by Gesheva and Gesheva [23]. Various carbon source utilization ability confirmed the identity of *Streptomyces* spp. [24]. The isolate DOSMB-D105 was able to utilize dextrose, cellulose, xylose, arabinose, raffinose, mannitol, fructose, sorbitol, maltose, lactose, rhamnose, adonital and starch. But, it did not utilize inositol and L-rhamose. Thus, the present investigation could find that the physiological characteristics of *S. parvulus* DOSMB-D105 varied depending on the nutritive and the physical conditions.

Molecular taxonomy of S. parvulus *DOSMB-D105*

DNA based molecular methods have been used for species differentiation and the identification of actinobacteria. The strain DOSMB-D105 was similar (100%) to that of *S. parvulus* (EF012105) isolated from the mangrove soil of China and *S. parvulus* (GQ451836), from the soil of Bangladesh, and K2P value of DOSMB-D105 was also similar to that of *S. parvulus* (EF012105). This is providing with the message for the geographical relationship of the microorganisms, their distribution and environmental tolerance.

Phylogenetic studies based on 16S rDNA sequences would enhance the knowledge on the systematics of actinobacteria [31]. Sequences of 16S rDNA have provided actinobacteriologists with phylogenetic trees that allow the investigation of evolution of actinobacteria and also provide the basis for identification. In the present study, secondary structure of 16S rDNA gene consisted of 33 stems and it showed energy threshold, cluster factor, conserved factor, compensated factor, conservativity, start position, end position, part of sequence greedy parameter and treated sequence. The free energy structure of the 16S rDNA secondary structure of the strain DOSMB-D105 (JQ638518) was 193.3 kal/mol. The total restriction enzymes 48% and the GC content was 59% and the free energy structure was –78.6 kal/mol. Distinct variation in the secondary structure, G+C composition and presence of restriction enzymes sites in 16S rDNA sequence of the strain DOSMB-D105 showed molecular level specificity of each and every individual isolates. Apparent phylogenetic relationships, secondary structure and restriction enzyme sites in 16S rDNA have been reported by many workers [32–34]. Based on the morphological, cell wall chemistry and molecular properties of the strain DOSMB-D105, it was identified as *Sparvulus*. DOSMB-D105 (JQ638518). The identity of the isolate was also confirmed with Bergey's Manual of Systematic Bacteriology [35], Bergey's Manual of Determinative Bacteriology [36] and phylogenetic analysis using sequence alignment program CLUSTAL W [37].

Antimicrobial efficacy of crude extract

Antimicrobial efficacy of *S.parvulus* DOSMB-D105 was tested against eleven pathogenic bacteria and six fungi with five different solvent extracts. The maximum antimicrobial efficacy of the ethyl acetate solvent extract of the isolate was found against the following pathogens: *Pseudomonas* sp., *A. niger, Proteus* sp., *Bacillus* sp. and *S. aureus*, *C. magnolia*, *E. coli* and *A. flavus*, *L. lactis*, *A. fumigatus*, *Penicillium* sp., *Fusarium* sp., *S. infantis*, *K. pneumoniae*, *V. cholerae*, *C. diserus* and *S. flexneri*.

Out of five different solvents used to extract the antimicrobial compounds of DOSMB-D105, ethyl acetate solvent extract only showed remarkable activity against all the pathogens tested. Whereas the methanol, chloroform, ethanol and hexane extracts showed moderate to minimum activity and they did not show activity against all the pathogens tested. This finding leads support to that of Vijayakumar et al. [38] who reported that the antimicrobial activity of different solvent extracts of the isolate *Streptomyces* sp. (VPTSA18) against 13 pathogens (11 species of bacteria and 2 species of fungi) was remarkable. The ethyl acetate-

treated compound (extract) was highly active against bacterial and fungal pathogens and the other solvent extracts had only moderate to minimum inhibitory effect. Also, Pugazhvendan et al. [39] reported that the ethyl acetate extract of *Streptomyces* showed the maximum inhibition range of 6–15 mm against bacterial pathogens. Likewise, many researchers have evaluated the antimicrobial efficacies of the actinobacteria using various solvents including n-butanol [40], chloroform [41], ethyl acetate and methanol [42], ethyl acetate, methanol, chloroform and alcohol [43], petroleum ether [44], *n*-butanol and ethyl acetate [45]. It was found that the ethyl acetate solvent extract had promising activity against most of the pathogens tested. Similar findings have also been reported by many researchers [46-47]. Thus, the present and previous studies have indicated that the ethyl acetate is a suitable solvent for the extraction of the antimicrobial compounds from the marine actinobacteria.

Identification of bioactive compounds

Antimicrobial compounds present in the cell free ethyl acetate extract of *S. parvulus* DOSMB-D105 were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethyl acetate extract of *S. parvulus* DOSMB-D105 were noted. Totally, eight compounds phenol, 2,4-bis(1,1-dimethylethyl); pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-metylpropyl); 7,8-epoxylanostan-11-ol, 3-acetoxy; dihydroergotamine mesylate; 1,2-benzenedicarboxylic acid, diisooctyl ester; phthalic acid, diisodecyl ester; octadecane, 3-ethyl-5-(2-ethylbutyl) and ethyl iso-allocholate were identified from the ethyl acetate extract of *S. parvulus* DOSMB-D105 as shown by NIST library. Previously, only a few bioactive compounds which are currently in use such as actinomycin D and manumycin A [48–50] have been reported from *S. parvulus*. Similarly, Livia and Avira [51] have reported different antimicrobial, antiviral, anticancer and antimalarial compounds from the marine *S. parvulus*.

Conclusion

As a conclusion of the present study we can state that the marine *S. parvulus* DOSMB-D105 is a good source for obtaining novel bioactive compounds for use as biomedicines. Identification of the bioactive compounds in *S. parvulus* DOSMB-D105 is a significant finding and it can pave way for the large scale production of antibiotics, when pursued in further.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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