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Research Article
Antimicrobial Efficiency of a Marine *Streptomyces* sp. VPTSA1-4 Isolated from Coromandel Coast of Bay of Bengal, India

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Abstract

Background and Objective: The need and search for therapeutic agents in the treatment of existing and emerging microbial diseases have intensified. The ever-reliable marine streptomycetes have been less explored for such antimicrobial agents and the present study is an attempt in that direction, wherein actinobacteria from marine sources are assessed for their antimicrobial activity. **Materials and Methods:** The actinobacteria isolated from coastal soils were screened for antimicrobial activity against microbial pathogens. Streptomycetes with the best activity were identified by 16S rRNA gene sequencing. The bioactive compound was extracted and subjected to spectral studies. Mean values of the data derived in antimicrobial efficacy were statistically analyzed. **Results:** Out of 19 isolates, *Streptomyces* sp., VPTSA1-4 exhibited the best antimicrobial activity and hence, 16S rRNA genome was sequenced to ascertain the identity of the isolate produced maximum antimicrobial compound at 30°C on the 9th day with a pH of 8 and salinity of 4%, on a starch-casein medium. Further, the potential isolate was identified as *Streptomyces tuirus* VPTSA1. **Conclusion:** The study suggested that antimicrobial compounds from *Streptomyces tuirus* could be used as alternative antimicrobial drugs to drug-resistant pathogens.

Key words: Marine actinobacteria, 16S rRNA gene sequencing, *Streptomyces tuirus* VPTSA1-4, screening, antimicrobial activity, extraction, purification, spectral studies

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Actinobacteria are widely distributed in all environments and have an immeasurable array of secondary metabolites. They are bio-factory for commercially valuable enzymes, vitamins and therapeutically beneficial bioactive molecules. The time-tested, ever-reliable *Streptomyces* species has accounted for 80% of antibiotics, while other rare genera of actinobacteria are credited for the remaining 20% of antibiotics[1]. *Streptomyces* species have been extensively exploited in the terrestrial environment worldwide[2] to such an extent that these terrestrial environments have almost been exhausted of new antimicrobial compounds[3]. As such the extensive screening program of the past several decades is yet to yield significant bioactive compounds, adding up to existing ones. But, the marine ecological niches harbour diverse microbial populations which are yet to be harvested to find their potential. Marine actinobacteria gain more attention for valuable bioactive compounds, produced as a consequence of challenges to culture on conventional culture media[3,4]. Moreover, the impending threat in the form of the alarming increase in drug-resistant pathogens and the inadequacy of multi-dimensional research strategies in elaborating new drugs presents more significant bottlenecks in devising effective antimicrobials[5,6]. Enhanced metagenomic and culture-dependent products are genomically assessed and clonally propagated to detect new actinobacteria from environmental samples[7]. Extensive research has been conducted on the isolation and cultivation of actinobacteria in various coastal areas of India[8,9]. Gulf of Mannar remains remarkably unexplored and even less is the isolation of salt-tolerant actinobacteria from the ecologically demanding saltpan. Therefore, the current study was planned to isolate actinobacteria from saltpan soil on the East Coast of India and discover novel bioactive compounds to overcome drug-resistant bacterial and fungal organisms.

MATERIALS AND METHODS

Study area: The study was conducted between March 2020 and January, 2022. The soil samples were collected along the Coromandel Coast (Bay of Bengal) at Vedaranyam and Thondi, India. The *in vitro* antagonistic and spectral assessments were made in the Department of Microbiology, Government Arts and Science College, Perambalur, India.

Isolation of actinobacteria: Soil samples were obtained from different locations in the salt pan environment at Vedaranyam (latitude 10°22'30.36"N and longitude 79°50'53.88"E) and Thondi (9°44'37.68"N and 79°1'6.6"E), Tamil Nadu, East Coast of India. Accurately measured 0.1 mL of serially diluted sample was spread over starch casein agar plates composed of amphotericin B (50 µg mL^{-1}) and tetracycline (20 µg mL^{-1}) (Himedia, Mumbai). The temperature of incubation was 28±2°C for 7-10 days. The colonies that appeared on culture plates were purified and stored in SCA slants for further study.

Test organisms: The test pathogens (MTCC cultures) were obtained from IMTECH, Chandigarh, viz. *Proteus mirabilis*, *Vibrio cholerae*, *Salmonella typhimurium*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella paratyphi* B, *Escherichia coli*, *Candida albicans* and *Cryptococcus neoformans*.

Evaluation of the antimicrobial activity of actinobacteria

Primary screening: The purified actinobacterial colonies were primarily screened for antimicrobial activity by cross streak method[8]. A single centre streak was made on the modified nutrient agar plates and incubated at 28±2°C. Over the appreciable growth of the actinobacteria, the log phase culture of test pathogens such as *E. coli* (MTCC: 43), *B. subtilis* (MTCC: 121) and *C. albicans* (MTCC: 183) was inoculated by a single streak at right angles to the original streak of actinobacteria. The inoculated plates were incubated at 37 and 27°C for bacteria and fungus, respectively. After incubation, the growth inhibition zone was observed and measured. Following the production of the growth inhibition, the actinobacteria with antagonistic activity against bacteria and fungi were selected.

Secondary screening: The selected potential actinobacterial isolate was inoculated in 10% of starch casein broth and kept in a shaker (250 rpm) at 28±2°C for 10 days. The mycelial mat of actinobacteria was filtered using Whatman No. 1 paper and Seitz filter (G5, 0.23 µm), respectively. Then, the crude extract was extracted by adding an equivalent (1:1) amount of various solvents, such as chloroform, ethyl acetate, distilled water, methanol and alcohol and shaken well for 2 hrs. The culture filtrate was used for antimicrobial efficacy by well diffusion method[9]. Eleven different pathogenic bacteria and two fungal pathogens mentioned in the test pathogens were spread over Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA), respectively. Accurately measured 25 µL of every solvent extract were poured in a sample well made using a well cutter and incubated at the appropriate temperature. After incubation, the antimicrobial activities of the actinobacterial isolate were evaluated by measuring the diameter of the zone of inhibition.

Phenotypic characterization and optimization of cultural condition: The sporophore nature of the selected potential isolate was observed in the Scanning Electron Microscope (SEM). The optimum culture conditions for maximum production of active compound was determined by using various ranges of temperature (4-50°C), pH (4-10), incubation period and salinity (1-32%) on different culture media, including ISP (1-7) media recommended by the International *Streptomyces* Project (ISP) and other standard procedures[10].

Purification and structure prediction of antimicrobial compound: The pure active compound was obtained using column chromatography by eluting with ethyl acetate and purified using thin-layer chromatography with n-butanol:acetic acid:water (2:1:1) techniques. The melting point, solubility nature, pH and thermostability of the active compounds were screened by the standard methods[11]. Further, the active compound was analyzed with various spectral studies such as UV spectra at 200-400 nm, FT-IR spectra in the range of 4000-400 cm^{-1} and mass spectrum at a current of 100 MA and 90°C. The ^{1}H NMR spectra (JEOL, Tokyo, Japan) of the active compound was also measured at 400 MHZ. Further, the structure and molecular weight of the active compound were determined using spectral data[12].

Molecular characterization of potential actinobacteria: The genomic DNA of the phenotypically identified potential actinobacterial isolate was isolated as per the procedure of Chen *et al.*[13]. The isolated and purified 16S rDNA was amplified in a thermal cycler with 1 mL of upstream primer (100 Pmols) (5'-AGAGTTTGATCCTGGCTCAG 3'), 1 mL of downstream primer (100 Pmols) (5'-AGGGCTACCTTGTTACGACTT 3'). The procedure adopted for amplification was the same as that of Monciardini *et al.*[14]. The PCR centrifugal filter extracted 16S rDNA was partially sequenced as per the method of Chen *et al.*[13]. The sequence data were aligned manually with the available actinobacterial sequence in the GenBank nucleotide database and analyzed by using the BLAST program. The aligned sequence was directly used to build a phylogenetic tree by the neighbour-joining (NJ) method[15]. Moreover, the Genebee structure prediction software was used to predict the secondary structure of *Streptomyces* sp., VPTSA1-4. The restriction sites in 16S rDNA were also studied using the NEB cutter program.

Statistical analysis: Three replicates were maintained for antimicrobial efficacy experiments evaluated against thirteen different bacterial and fungal pathogens and the mean values of the standard deviation of the data (n = 3) were analyzed.

RESULTS

Isolation and genetic identification of actinobacteria: About 10 salt pan soil samples were collected from two different sites of the Coromandel Coast of the Bay of Bengal, India. Altogether, 37 white, ash and grey-coloured colonies were isolated. The 19 morphologically distinct isolates (of 37 actinobacterial colonies), belonging to five genera are depicted in Table 1. The *Streptomyces* (13) were dominant flora, followed by *Actinopolyspora* (3) and one each from *Saccharopolyspora*, *Nocardiopsis* and *Actinoplanes*.

Antimicrobial activity of actinobacteria: All 19 morphologically different actinobacteria were primarily screened against pathogenic bacteria and fungi by cross streak plate technique. Ten isolates among 19 exhibited antagonistic properties against selected microbial pathogens. Isolates with greater than 5 mm of inhibition zone were considered as antagonistic activity. Of these, nine isolates had antibacterial activity, eight isolates with antifungal activity and seven exhibited both antibacterial and antifungal activity. The antagonistically superior streptomycete isolate, *Streptomyces* sp., VPTSA1-4 showed a higher degree of activity against *B. subtilis* (13 mm), followed by *C. albicans* (10 mm) and *E. coli* (7 mm) in preliminary screening (Table 1). The antimicrobial efficacy results are tabulated in Table 2. Notably, the ethyl acetate solvent extract of the strain VPTSA1-4 showed a higher degree of activity against *Proteus mirabilis* and *Vibrio cholerae* (23 mm), followed by *Salmonella typhimurium* (20 mm), *Salmonella typhi* and

Table 1: Screening of antimicrobial activity of actinobacterial isolates

	Zone of inhibition (mm)		
Actinobacterial isolates	*B. subtilis*	*E. coli*	*C. albicans*
Streptoverticillium sp., VPTS1-6	8	7	6
Streptomyces sp., VPTS1-7	2	-	3
Actinomadura sp., VPTS2-4	7	8	7
Actinopolyspora sp., VPTS2-7	3	-	4
Streptomyces sp., VPTS3-6	-	3	4
Streptomyces sp., VPTM1-7	5	10	-
Saccharopolyspora sp., VPTM2-2	4	-	-
Streptomyces sp., VPTM2-3	10	-	9
Actinopolyspora sp., VPTM2-5	-	3	-
Streptomyces sp., VPTM2-9	2	-	4
Streptomyces sp., VPTM2-12	9	8	7
Streptomyces sp., VPTM3-9	6	10	-
Streptomyces sp., VPTSA1-4	13	7	10
Actinopolyspora sp., VPTSA2-3	8	-	13
Streptomyces sp., VPTSA2-5	4	-	-
Streptomyces sp., VPTSA2-7	-	3	-
Nocardiopsis sp., VPTSA2-9	-	3	-
Streptomyces sp., VPTSA2-10	-	-	13
Streptomyces sp., VPTSA2-11	-	7	14

- : No antimicrobial activity

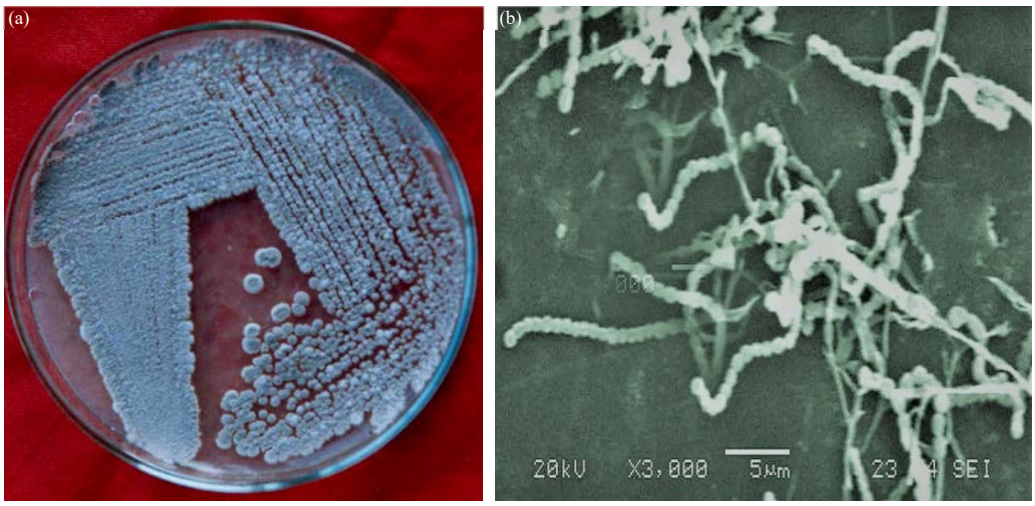

Fig. 1(a-b): (a) Colony morphology and (b) Microscopy of *Streptomyces* sp., VPTSA1-4

Table 2: Antimicrobial efficacy of crude extract of active isolate VPTSA1-4

	Zone of inhibition (mm)				
Name of the pathogens (MTCC No.)	Alcohol	Chloroform	Distilled water	Ethyl acetate	Methanol
Bacillus subtilis (121)	9.5±0.28	8.2±0.60	8.1±0.46	15.4±0.35	8.2±0.64
Escherichia coli (43)	11.0±0.78	9.4±0.13	6.2±0.40	14.4±0.48	8.3±0.62
Klebsiella pneumoniae (39)	10.4±0.13	7.5±0.31	12.5±0.33	16.4±0.62	7.4±0.84
Proteus mirabilis (425)	8.3±0.15	7.4±0.53	9.5±0.44	23.5±0.82	10.3±0.66
Proteus vulgaris (426)	12.4±0.88	6.5±0.488	7.2±0.42	15.3±0.77	9.2±0.73
Salmonella typhi (733)	9.2±0.20	5.3±0.37	10.1±0.55	19.4±0.68	11.4±0.64
Salmonella typhimurium (98)	10.5±0.15	9.2±0.42	11.5±0.51	20.4±0.62	9.3±0.75
Salmonella paratyphi B (735)	9.4 ±0.13	10.1±0.37	12.5±0.46	15.2±0.71	8.4±0.68
Staphylococcus aureus (87)	6.2±0.22	7.5±0.42	14.3±0.37	19.3±0.82	12.5±0.82
Staphylococcus epidermidis (2639)	8.4±0.26	6.3±0.44	9.4±0.48	16.3±0.75	10.3±0.71
Vibrio cholerae (3904)	8.3±0.26	11.4±0.55	9.3±0.42	23.4±0.71	11.1±0.75
Candida albicans (183)	8.2±0.57	8.0±0.46	12.2±0.44	17.3±0.55	7.3±0.66
Cryptococcus neoformans (4410)	11.3±0.42	6.2±0.64	13.2±0.37	16.2±0.82	8.2±0.48

MTCC: Microbial type culture collection and GenBank (Chandigarh, India)

Staphylococcus aureus (19 mm), *C. albicans* (17 mm), *Klebsiella pneumoniae*, *S. epidermidis* and *Cryptococcus neoformans* (16 mm), *Bacillus subtilis*, *S. paratyphi* B and *P. vulgaris* (15 mm) and *E. coli* (14 mm). The remaining solvent extracts of the isolate exhibited modest activity against all other tested organisms (Table 2).

Phenotypic characterization: This potential isolate produced different colony colours such as ash in the SCA medium (Fig. 1a) and yellow, brown and white series when grown in other culture media, including ISP media. The spore morphology of the isolate forms a smooth spore surface and looped (retinaculiaperti) spore chains (Fig. 1b). The isolate VPTSA1-4 was optimized with various cultural conditions for the observation of growth and maximum compound production. Growth of the potential isolate was optimal at temperature 30°C, pH 7-8 and NaCl 4-6%, whereas, no growth was observed at 10°C and poor growth was observed at temperature 50°C, pH at 5.0 and 1 and 32% of NaCl.

Purification and structure elucidation of antimicrobial compound: The antimicrobial compound was separated and observed as a single band in TLC with an Rf value of 0.41. The melting point of the light brown-coloured active compound was identified at 170°C. Maximum stability of the compound was observed at pH 6-9 and temperature 20-50°C. The antimicrobial compound dissolved completely in ethyl acetate and the maximum absorption was observed between 200-300 nm in the UV spectrum (Fig. 2a). The absorption peak at 1133.3 cm^{-1} in the IR spectrum concerning VPTSA1-4 indicates the presence of a compound with R-O-R- (aliphatic) group. The single absorption peak at 1644.4 cm^{-1}

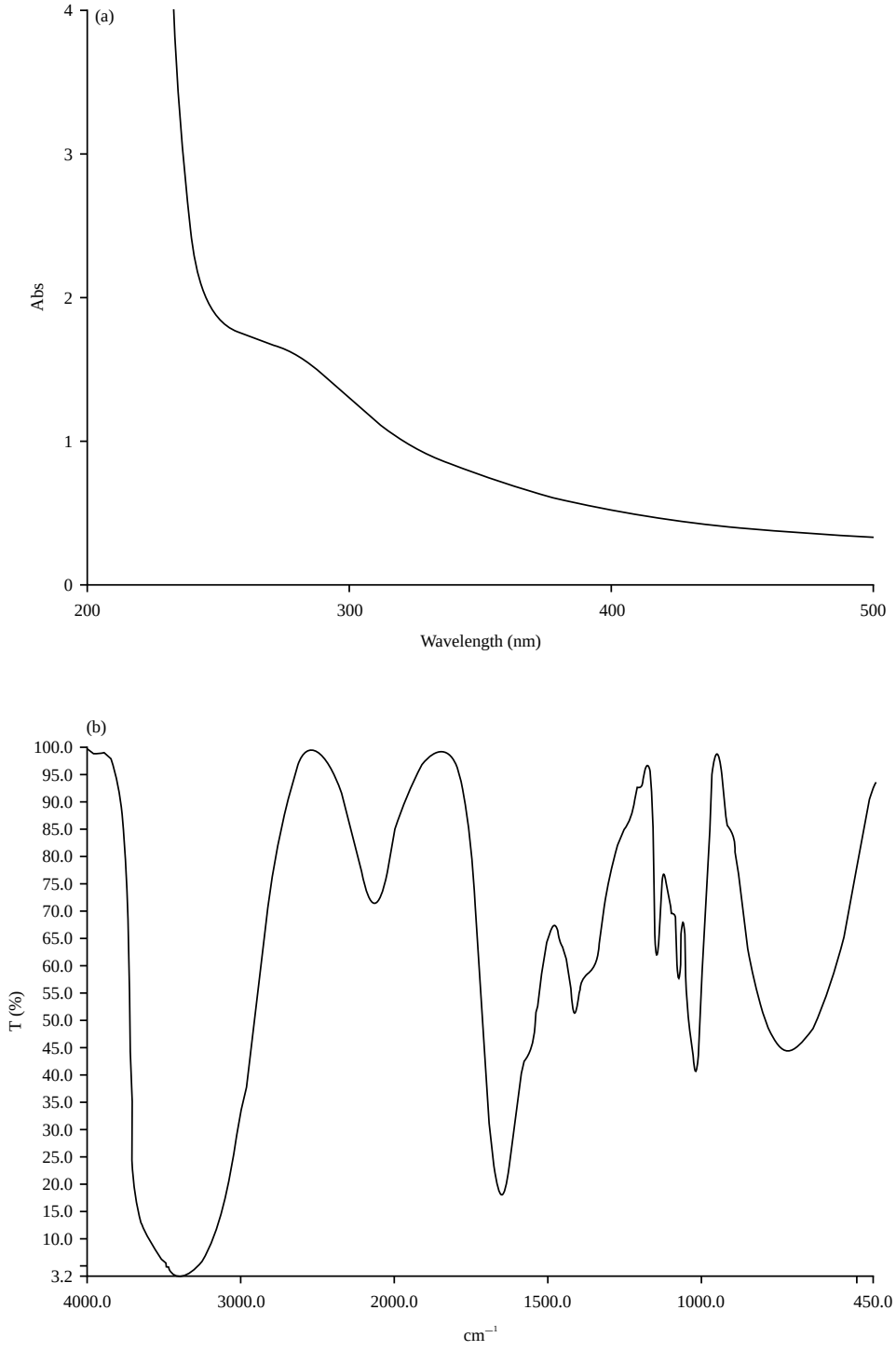

Fig. 2(a-b): Spectral analysis of antimicrobial compound from *Streptomyces* sp., VPTSA1-4, (a) UV spectrum and (b) IR spectrum
X-axis: Absorption peak (cm^{-1})

suggests a compound with C=C-(alkenes) presence, while the C°C-(alkynes) group was formed at 2111.1 cm^{-1} (Fig. 2b). At 228 m/z molecular ion peak was observed (Fig. 3a). In ^{1}H NMR spectrum of the active compound, maximum peaks were concentrated throughout the δ of 1-5 (Fig. 3b). Based on the spectral results of the present study, highly oxygenated derivatives of carbohydrates exhibited a higher degree of antimicrobial activity.

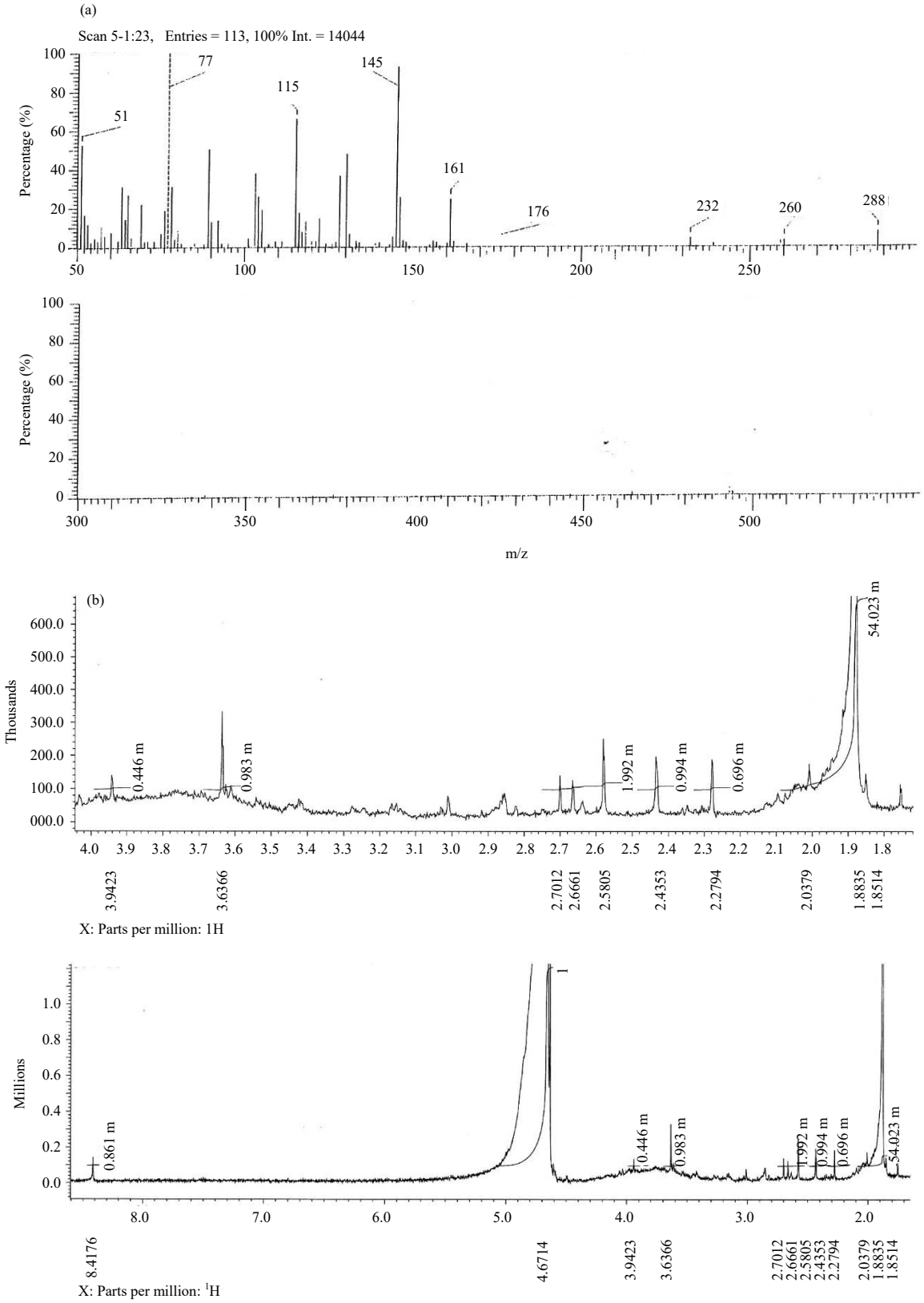

Fig. 3(a-b): Spectral analysis of antimicrobial compound from *Streptomyces* sp., VPTSA1-4, (a) Mass spectrum x-axis: m/z value, y-axis: Intensity period and (b) ^{1}H NMR spectrum x-axis: ppm, y-axis: Number of atoms

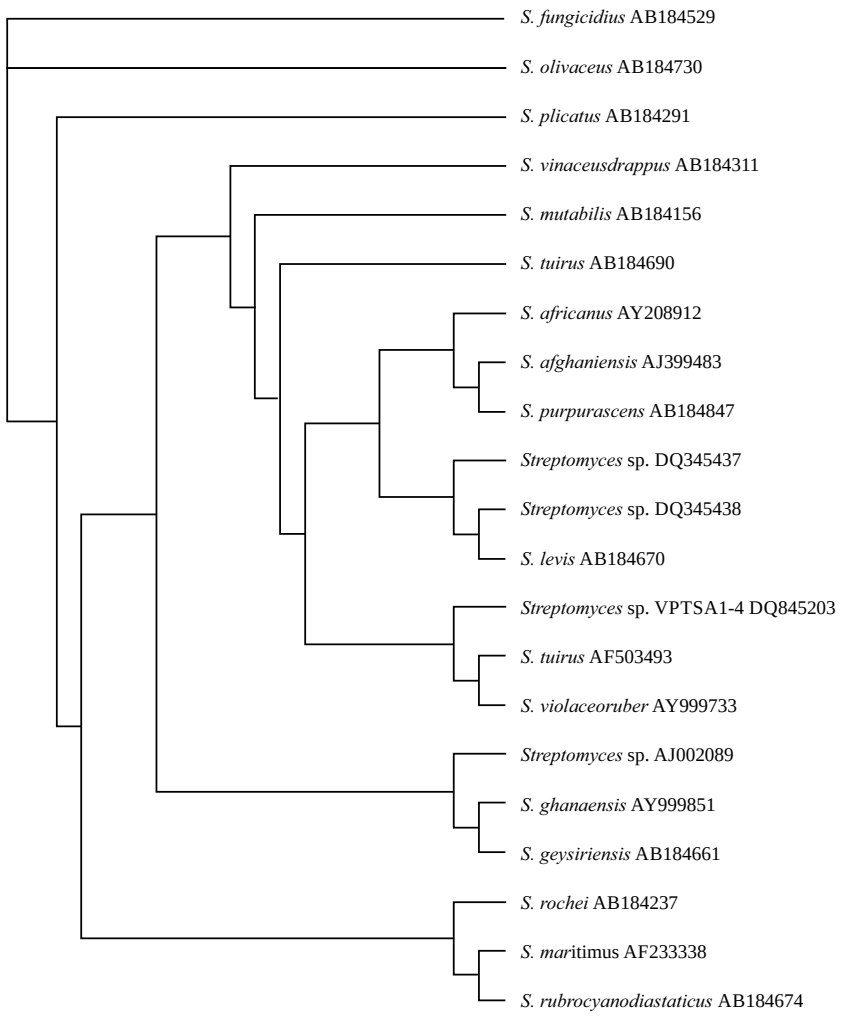

Fig. 4: Phylogenetic tree derived from partial 16S rRNA gene sequence of *Streptomyces* sp. (VPTSA1-4) by neighbour-joining tree method

Molecular characterization of potential actinobacteria: The 16S rRNA gene sequence of the potent isolate *Streptomyces* sp., VPTSA1-4 was analyzed for speciation. The 16S rRNA gene sequence of the active isolate was deposited in Genbank (NCBI/EMBL/DDBJ) with accession number DQ845203. The nucleotide (906 bp) sequence of the isolate matched with existing *Streptomyces* sequences in the Genbank. Phylogenetic relatedness (NJ-Tree) of the isolate was between 98-100% and it was found that the isolate VPTSA1-4 showed higher similarity to *Streptomyces tuirus* AF503493 clone. Based on the results, the active isolate was confirmed as *Streptomyces tuirus* VPTSA1-4 (Fig. 4). The secondary structure of *Streptomyces* sp., VPTSA1-4 showed 38 stems with a free energy of -210.3 kkal mol^{-1}. Moreover, the isolate had 52 numbers of restriction sites and 59% of GC content as found by the NEB cutter program.

DISCUSSION

Of the total (n = 19) actinobacteria isolated, 68.4% of the isolates belong to *Streptomyces* genera followed by *Actinopolyspora* (15.8%) and *Saccharopolyspora*, *Nocardiopsis* and *Actinoplanes* at 5.3% in Coromandel Coast (Bay of Bengal) at Vedaranyam and Thondi, India. The actinobacteria have been reported from almost all parts of the world, which indicate their diversity and adaptability to variable inhospitable habitats. Two earlier studies from the east coast of India have documented 33 morphologically distinct actinobacteria among 117 colonies and the dominance of *Streptomyces* was observed in the marine environment[8,16]. Such predominant diversity of streptomycetes has also been reported on the East Coast of India[17], West Coast of India[18], mangrove sediment in Tanjung

Api-Api, South Sumatra, Indonesia[19]. Thus streptomycetes are ubiquitous occupying almost all ecosystems of the world, which indicate the plasticity and adaptability of their metabolic and genetic system to thrive in extreme environments. However, the absolute picture of actinobacterial diversity cannot be obtained by a single study. It can be achieved by using different media and employing various sample treatments for the isolation from different substrates collected from the habitat with frequent visits to the field.

In this study, 52.6% of marine actinobacterial isolates exhibited antimicrobial activity in preliminary screening against *B. subtilis*, *C. albicans* and *E. coli*. Further, the superior antimicrobial compound producing isolate *Streptomyces* sp., VPTSA1-4 was assessed for its refined antimicrobial activity by the cultivation and extraction with five different organic solvents and it was found that the ethyl acetate extract of *Streptomyces* sp., VPTSA1-4 showed significant activity against all the tested microbial pathogens. The streptomycetes are the treasure house of bioactive principles. However, the screening phenomenon of several actinobacterial isolates is very essential to find out the potential antagonist. Similar to our study, it has been reported that around 30% of actinobacteria isolated from a marine source have antagonistic activity[8,16] whereas, 19% of isolates from the limestone mining area of Mawsmai near Sohra, Meghalaya had antibacterial activity[20], in contrast to these results, 48.1% of actinobacteria from the soils of Kalapatthar (5545 m) Mount Everest Region with antibacterial activity[21] and as much as 53% of the actinobacteria exhibited antimicrobial activity[22]. The capability of ethyl acetate in the solubilisation of antimicrobial compounds obtained from actinobacteria is already reported by many workers[8,16,23]. Available nutrients/substrates of the habitats and complex biochemical pathways may greatly influence the quantity and quality of antibiotic production by actinobacteria.

In the present study, the isolate VPTSA1-4 was selected as a potential strain and optimized at various cultural conditions for further production of the antimicrobial compound. According to the results, it was observed that the isolate grew well in mesophilic temperature (30°C), neutral to slight alkaline (pH 7-8) and halophilic (4-6% of NaCl) conditions on SCA media and triggered the production of antimicrobial compounds. In line with this study, actinomycetes strains isolated from different Regions of Livingston Island, Antarctica were grown at 25°C media with 1% NaCl. Most of them were tolerant to 3% NaCl[1]. Potential antibacterial compound producing marine actinobacterial isolate produced rectiflexibles, sporophores and globose-shaped smooth spore surface[8]. Development of straight/branched and looped spore chains and a smooth spore surface was reported in an active antimicrobial compound producing strain *Streptomyces* sp., VPTSA18[23]. A similar type of phenotypic characteristics of the prominent antimicrobial producing isolates to generic level and growth optimization was reported by many workers[5,9,16].

In the present study, ethyl acetate solvent extracted and purified antimicrobial compound using TLC and column chromatographically was stable at pH 6-9 and temperature 20-50°C. Further, the chemical nature of the antimicrobial compound was studied spectroscopically in UV, IR, Mass and ^{1}H NMR and identified as highly oxygenated derivatives of carbohydrates. Following our study, ethyl acetate was found suitable for attaining maximum separation of alkaloid compound from *Streptomyces* JF714876[11]. In addition to this study, the extraction, purification and characterization of antimicrobial compounds, namely (2R, 3R)-2, 3-butanediol, (3R)-1, 3-butanediol and (2R, 3S)-2, 3-butanediol extracted using ethyl acetate from *Streptomyces* sp., MSL were studied[12]. A similar kind of study has also been reported by many researchers[8,16,23,24]. The discovery and successful production of new classes of the antimicrobial compound would also depend upon the development of appropriate fermentation conditions and downstream processing technologies.

Based on the results of our study, we conclude that sequencing of the 906 bp 16S rRNA gene provides enough information for the species-level identification of *Streptomyces* sp., VPTSA1-4 as *S. tuirus* VPTSA1-4 with 98-100% of evolutionary relatedness. Phylogenetic analysis of preliminarily identified four streptomycetes using 16S rDNA partial sequences was also confirmed as *Streptomyces*. In an earlier study the strain LD-21 was related to *Streptomyces niveus* NRRL2466T (DQ442532) with 99.47% sequence similarity and the other three isolates LD-29, LD-34 and LD-39 were related to *Streptomyces camponoticapitis* 2H-TWYE14T (KP784807) with similarity of 99.26, 99.41 and 99.48%, respectively[20]. Recently, the taxonomic hurdles of actinobacteria are solved by studying nucleic acids, including 16S rRNA gene sequencing. Molecular identification and characterization of actinobacteria by 16S rRNA gene sequencing serves as a more reliable strategy[14,16,25]. The finding of the study also agreed with the report of Khucharoenphaisan *et al.*[26], sequence-based identification is becoming an increasingly important tool of identification. Although the cost of performing such tests limits its application in most clinical laboratories, it is a useful alternative for the identification of actinobacteria. However, the accurate assignment of a species to particular taxa based

on rRNA gene sequence will depend upon the continued generation of sequences from well-characterized isolates, the deposition of these sequences in publicly available databases and additional taxonomic studies to resolve the appropriate classification of presently unnamed species. In addition, such taxonomically unresolved species and strains could be a source of valuable bioactive components that when subjected to optimum fermentation parameters will lead to the most sought-after remedies as evidenced in our study.

CONCLUSION

The study reassertions the fact that the marine ecosystem can be relied upon and extensively explored for potential antimicrobial compounds producing streptomycetes against drug-resistant microbial pathogens. The salt pan environment provides such a rich source of potential metabolite-producing actinobacteria. This study has found differences in secondary structure, G+C composition and restriction enzyme sites within the genome of the isolate. It could be ascertained that the isolates are difficult to be classified by traditional procedures and can be characterized by 16S rRNA gene sequencing. The data could supplement the taxonomy-related knowledge generated through conventional methods guiding the characterization of taxonomically unresolved streptomycetes. The bioactive compound produced by the *Streptomyces tuirus* VPTSA1-4 will be an effective and alternative antimicrobial even against drug-resistant pathogens.

SIGNIFICANCE STATEMENT

The study suggests that the marine ecosystem is a potential source for antimicrobial compounds producing streptomycetes which could be genomically characterized and these agents can be used as antimicrobial drugs against drug-resistant pathogens.

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REFERENCES

1. Moncheva, P., S. Tishkov, N. Dimitrova, V. Chipera, S. Antonova-Nikolova and N. Bogatzevska, 2002. Characteristics of soil actinomycetes from Antarctica. J. Cult. Collect., 3: 3-14.
2. Bérdy, J., 2012. Thoughts and facts about antibiotics: Where we are now and where we are heading? J. Antibiot., 65: 385-395.
3. Nolan, R. and T. Cross, 2012. Isolation and Screening of Actinomycetes. In: Actinomycetes in Biotechnology, Goodfellow, M., S.T. Williams and M. Mordarski (Eds.), Elsevier, Netherlands, ISBN: 9780080984339, pp: 1-32.
4. Lam, K.S., 2006. Discovery of novel metabolites from marine actinomycetes. Curr. Opin. Microbiol., 9: 245-251.
5. Projan, S.J. and P.J. Youngman, 2002. Antimicrobials: New solutions badly needed. Curr. Opin. Microbiol., 5: 463-465.
6. Projan, S.J., 2003. Why is big pharma getting out of antibacterial drug discovery? Curr. Opin. Microbiol., 6: 427-430.
7. Mincer, T.J., W. Fenical and P.R. Jensen, 2005. Culture-dependent and culture-independent diversity within the obligate marine actinomycete genus *Salinispora*. Appl. Environ. Microbiol., 71: 7019-7028.
8. Thirumurugan, D., R. Vijayakumar, C. Vadivalagan, P. Karthika and M.K.A. Khan, 2018. Isolation, structure elucidation and antibacterial activity of methyl-4,8-dimethylundecanate from the marine actinobacterium *Streptomyces albogriseolus* ECR64. Microb. Pathogen., 121: 166-172.
9. Al-Ansari, M., N. Alkubaisi, P. Vijayaragavan and K. Murugan, 2019. Antimicrobial potential of *Streptomyces* sp. to the gram positive and gram negative pathogens. J. Infect. Public Health, 12: 861-866.
10. Djebbah, F.Z., L. Belyagoubi, D.E Abdelouahid, F. Kherbouche, N.A. Al-Dhabi, M.V. Arasu and B. Ravindran, 2021. Isolation and characterization of novel *Streptomyces* strain from Algeria and its *in vitro* antimicrobial properties against microbial pathogens. J. Infect. Public Health, 14: 1671-1678.
11. Vidyasagar, G.M., S. Babanagare and S. Kamradgi, 2020. Purification and characterization of antimicrobial agent from *Streptomyces* sp. JF 714876. Int. J. Pharm. Sci. Nanotechnol., 13: 4952-4959.
12. Madasu, R.H.B., V. Muvva, R.K. Munaganti, K.R. Dorigondla and V. Yenamandra, 2017. Bioactive-guided fractionation of diols from *Streptomyces* sp. MSL. 3 Biotech, Vol. 7. 10.1007/s13205-017-0649-1.
13. Chen, X., Y. Jiang, Q. Li, L. Han and C. Jiang, 2016. Molecular Phylogenetic Identification of Actinobacteria. In: Actinobacteria-Basics and Biotechnological Applications, Dhanasekaran, D. and Y. Jiang (Eds.), InTech, United Kingdom, pp: 141-174.
14. Monciardini, P., M. Sosio, L. Cavaletti, C. Chiocchini and S. Donadio, 2002. New PCR primers for the selective amplification of 16S rDNA from different groups of actinomycetes. FEMS Microbiol. Ecol., 42: 419-429.
15. Kumar, S., M. Nei, J. Dudley and K. Tamura, 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief. Bioinform., 9: 299-306.

16. Thirumurugan, D. and R. Vijayakumar, 2015. Characterization and structure elucidation of antibacterial compound of *Streptomyces* sp. ECR77 isolated from east coast of India. Curr. Microbiol., 70: 745-755.
17. Rengasamy, S. and U. Thangaprakasam, 2018. Isolation, screening and determination of α-amylase activity from marine *Streptomyces* species. Int. J. Pharm. Pharm. Sci., 10: 122-127.
18. Vijayakumar, R., G. Gopika, D. Dhanasekaran and R. Saravanamuthu, 2012. Isolation, characterisation and antifungal activity of marine actinobacteria from Goa and Kerala, the west coast of India. Arch. Phytopathol. Plant Prot., 45: 1010-1025.
19. Rozirwan, H.I. Muda and T.Z. Ulqodry, 2020. Antibacterial potential of actinomycetes isolated from mangrove sediment in Tanjung Api-Api, South Sumatra, Indonesia. Biodiversitas, 21: 5723-5728.
20. Syiemiong, D. and D.K. Jha, 2019. Antibacterial potential of actinobacteria from a limestone mining site in Meghalaya, India. J. Pure Appl. Microbiol., 13: 789-802.
21. Gurung, T.D., C. Sherpa, V.P. Agrawal and B. Lekhak, 2009. Isolation and characterization of antibacterial actinomycetes from soil samples of Kalapattar, Mount Everest Region. Nepal J. Sci. Technol., 10: 173-182.
22. Ramesh, S. and N. Mathivanan, 2009. Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. World J. Microbiol. Biotechnol., 25: 2103-2111.
23. Vijayakumar, R., K.P. Selvam, C. Muthukumar, N. Thajuddin, A. Panneerselvam and R. Saravanamuthu, 2012. Antimicrobial potentiality of a halophilic strain of *Streptomyces* sp. VPTSA18 isolated from the saltpan environment of Vedaranyam, India. Ann. Microbiol., 62: 1039-1047.
24. Rabia-Boukhalfa, Y.H., Y. Eveno, S. Karama, O. Selama, B. Lauga, R. Duran, H. Hacène and V. Eparvier, 2017. Isolation, purification and chemical characterization of a new angucyclinone compound produced by a new halotolerant *Nocardiopsis* sp. HR-4 strain. World J. Microbiol. Biotechnol., Vol. 33. 10.1007/s11274-017-2292-8.
25. Stach, J.E.M., L.A. Maldonado, A.C. Ward, M. Goodfellow and A.T. Bull, 2003. New primers for the class *Actinobacteria*: Application to marine and terrestrial environments. Environ. Microbiol., 5: 828-841.
26. Khucharoenphaisan, K., K. Sinma and C. Lorrungruang, 2013. Efficiency of actinomycetes against phytopathogenic fungus of chilli anthracnose. J. Appl. Sci., 13: 472-478.