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ORIGINAL ARTICLE

Antimicrobial potentiality of a halophilic strain of *Streptomyces* sp. VPTSA18 isolated from the saltpan environment of Vedaranyam, India

Ramasamy Vijayakumar · Kanesan Panneer Selvam · Chinnasamy Muthukumar · Nooruddin Thajuddin · Annamalai Panneerselvam · Rengasamy Saravanamuthu

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Abstract A total of 68 morphologically different actinomycetes were isolated from the saltpan soils of Vedaranyam, Tamilnadu, India and tested for their antimicrobial activity by the cross-streak method. Among the strains tested, VPTSA18 showed strong antimicrobial activity. The culture filtrate was extracted with different solvents and tested against 11 bacterial and two fungal pathogens using the well-diffusion method. Of the solvent extracts assessed, the ethyl acetate extract showed high antimicrobial activity against all of the pathogens tested. Based on morphological, physiological, biochemical, cultural, chemo–taxonomical and molecular characteristics (16S rDNA sequencing), this strain was identified as *Streptomyces* sp. VPTSA18.

Keywords *Streptomyces* sp. VPTSA18 · Halophilic actinomycete · Antimicrobial activity · 16S rDNA sequencing

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R. Vijayakumar (✉)
Department of Microbiology,
Bharathidasan University College for Women,
Orathanadu 614 625, India
e-mail: rvijayakumar1979@gmail.com

K. Panneer Selvam
Department of Microbiology, M.R. Govt. Arts College,
Mannargudi 614 001, India

C. Muthukumar
Department of Botany & Microbiology, College of Science,
King Saud University,
P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia

N. Thajuddin
Department of Microbiology, Bharathidasan University,
Tiruchirappalli 620 024, India

A. Panneerselvam
Department of Botany and Microbiology,
A.V.V.M. Sri Pushpam College (Autonomous),
Poondi 613 503, India

R. Saravanamuthu
Department of Botany, A.V.C. College (Autonomous),
Mannampandal 609 305, India

Introduction

The genus *Streptomyces* was originally proposed by Waksman and Henrici (1943) for Gram-positive, aerobic and spore-forming actinomycetes. These microorganisms have a high G+C content in the DNA and contain LL-diaminopimilic acid in their cell wall, but they have no diagnostic sugars (chemotype I according to Lechevalier and Lechevalier 1970; Williams et al. 1989). Members of genus *the Streptomyces* are abundant in terrestrial environments and are easy to isolate on simple laboratory media. Strains can be readily differentiated on the basis of differences in the colours of their aerial and substrate mycelia and based on physiological tests (Goodfellow et al. 1992). *Streptomyces* includes a vast array of species, and the 2002 German Collection of Microorganisms and Cell Cultures (DSMZ) listed approximately 650 species (Wilkins and Scholler 2009) that had been identified based on conventional serological methods, phage typing, protein profiling (Ridell et al. 1986; Kon-Wendish and Schneider 1992; Ochi 1995; Taguchi et al. 1997) and genetic methods, such as DNA–DNA hybridization and 16S rRNA gene sequence analyses (Takeuchi et al. 1966;

Hain et al. 1997; Kataoka et al. 1997; Kim et al. 1999; Anderson and Wellington 2001).

During the past decades, the actinomycetes have provided many important bioactive compounds of high commercial value. Consequently, they are continuing to be routinely screened for new bioactive substances. These searches have been remarkably successful, and approximately two-thirds of naturally occurring antibiotics have been isolated from actinomycetes (Dhanasekaran et al. 2008, 2009). Remarkably, about 61% of all current bioactive microbial metabolites has been purified from streptomycetes, and some from rare actinomycetes (non-streptomycetes) (Moncheva et al. 2002). However, the emergence of drug- and multidrug-resistant pathogens necessitates a continuing search for new antimicrobial compounds with potent anti-pathogenic activity. In this context, the search for new clones of actinomycetes producing novel and superior antimicrobial metabolites is inevitable. Additionally, attempts to explore the actinomycetes of saltpan regions of marine environments in India have been limited to date. Against this background, the aim of the study reported here was to screen the less explored saltpan soils of Vedaranyam, Tamil Nadu, India so as to isolate and identify actinomycetes producing novel antimicrobial compounds possessing potent activities.

Materials and methods

Soil sample collection

Soil samples were collected from saltpans of the Vedaranyam seashore region (10°22′N, 79°51′E), Tamil Nadu, east coast of India. A total of 25 samples of soil were collected at random, brought to the laboratory in sterile polythene bags, and stored for further use.

Isolation of actinomycetes

Starch casein agar (SCA) medium (g/l: starch 10; casein 0.3; KNO_3 2; NaCl 2; K_2HPO_4 2; $MgSO_4$ $7H_2O$ 0.05; $CaCO_3$ 0.02; $FeSO_4$. $7H_2O$ 0.01; agar 18) (Kuster and Williams 1964) was prepared, sterilized at 121°C for 15 min and then added with amphotericin B (50 μg/ml) and tetracycline (20 μg/ml) (Himedia, Mumbai, India) to prevent fungal and bacterial growth, respectively. The collected soil samples were diluted up to 10^{-6}, and 0.1 ml of each diluted sample was spread over the agar plates; triplicates of each dilution were maintained. The inoculated plates were incubated at 28±2°C for 7–10 days, following which the actinomycete colonies were observed, purified, and maintained in SCA medium for further investigation (Vijayakumar et al. 2007).

Screening of actinomycetes producing antimicrobial compounds

The actinomycetes were initially screened to determine their ability to produce antimicrobial compounds by the cross-streak method (Egorov 1985). Briefly, a single streak of the actinomycetes sample was made on the surface of a modified nutrient agar medium (g/l: yeast extract 3; NaCl 5; peptone 5; glucose 5; agar 15; pH 7.1) and incubated at 28±2°C. Following the appearance of a well-defined ribbon of growth marking the original actinomycetes streak on the plates, test pathogens (*Escherichia coli*, *Bacillus subtilis* and *Candida albicans*) were streaked at right angles to the original streak of actinomycetes and incubated at 37°C (bacteria) or 27°C (yeast). On the basis of the presence of an inhibition zone, measured after 24–48 h, the strains producing antimicrobial compounds were selected.

Antimicrobial efficacy

The 25 selected strains were tested for their spectrum against pathogenic bacteria and fungi obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. These isolates were first inoculated into starch casein broth and shaken at 28±2°C and 250 rpm for 7–10 days. After incubation, the staling substances were filtered through filter paper (No.1; Whatman, Maidstone, Kent, UK) and then through a Seitz filter (G5; Pall Corp, Port Washington, NY). The filtrate was transferred aseptically into conical flasks and stored at 4°C for further assay. An equal volume of five different solvents (alcohol, chloroform, distilled water, ethyl acetate and methanol) was separately added to the cell-free culture filtrates and shaken for 2 h, followed by extraction of the antimicrobial compounds (Gandhimathi et al. 2008). Antimicrobial activity of actinomycetes was determined by using a well-diffusion method (Remya and Vijayakumar 2008; Raja et al. 2009). Eleven different pathogenic bacteria were inoculated onto Muller–Hinton agar, and two fungal pathogens were inoculated into Sabouraud's dextrose agar. After solidification, wells were made over the lawn cultures of bacteria and fungi using a sterile well cutter (6 mm), and 25 μl of each solvent extracts were added to each well. The plates were incubated at 37°C (bacteria) or 27°C (fungi). After 24–48 h of incubation, the diameter of the zone of inhibition was measured to evaluate the antimicrobial activity of isolates of actinomycetes.

The active solvent extracts were combined and evaporated to dryness under reduced pressure. The extracts were dissolved in a small amount of methanol, then filtrated and precipitated with an acetone/ether mixture (10:1, v/v). The precipitate was left to stand for 24 h at 5–10°C, filtered, and

then washed with acetone and ether. Five grams of crude powder was obtained from 5 l of culture broth. A methanolic solution of the powder (1.0 g) was chromatographed on silica gel 60 (70–325 mesh) columns. The compound was eluted with the lower phase of a mixture of chloroform/methanol/water (175:100:50). The active eluates were combined and evaporated in vacuum to dryness. A methanolic solution was precipitated with an acetone/ether mixture (10:1 v/v) and filtered to give 200 mg of the compound (Dhanasekaran et al. 2008).

Purification of the antimicrobial compound

The obtained antimicrobial compound was purified by silica gel column chromatography. A 2-g sample of crude powder was dissolved in 10 ml of ethyl acetate. The solution was passed through a silica gel column in benzene, and the active fractions were pooled and subsequently subjected to analytical thin layer chromatography (TLC). For the TLC plates, about 200 g of silica gel was stirred into 500 ml of distilled water and shaken mechanically for 0.5 h before being left to stand. Fifty milliliters of mixed slurry was used to coat the five 20×30-cm glass plates. The coated plates were left to stand until the slurry set, then oven dried. Using a capillary tube a row of spots of the sample was applied along a line 1.5 cm above from the bottom of the TLC plate. The spots were left to dry and then the TLC plate was placed vertically in a trough containing the solvents (n-butanol/ethylacetate/water, 9:9:1). When the solvents had ascended 80% of TLC, the plate was taken out of the trough, dried, and then sprayed with ninhydrin (Stackebrandt et al. 1997).

Characteristics of the active compound

The solubility, melting point, thermo-stability, and pH stability of the antimicrobial compound were analyzed and characterized by the standard methods of Harindran et al. (1999). The ultraviolet spectral measurement of the pure compound was made at 200–400 nm using an UV1601 instrument (Shimadzu, Kyoto, Japan) with ethanol as the solvent. The Fourier Transform infrared (FT–IR) spectrum of the antimicrobial compound was analysed by the methods of Fukuda et al. (1990). The IR spectrum was recorded on a Bruker Optics (Ettlingen, Germany) FT–IR instrument equipped with AT-XT Golden gate accessories. The mass spectrum of the compound was recorded using a Finnigan MAT 8230 mass spectrometer (Thermo Finnigan, San Jose, CA) at a current of 100 MA and 90°C. Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) spectra were analysed by the Ivanova and Schlegel (1997) method and measured in CDCI3 on a JEOL GSX-400 NMR spectrophotometer (JEOL, Tokyo, Japan) at 400 MHZ for ^{1}H.

Taxonomic characterization of strain VPTSA18

Morphological characteristics of the strain were observed by the slide culture technique on SCA medium, and the slides were observed under the light microscope (Leitz Diaplan; Leitz Microsystems, Wetzlar, Germany) and scanning electron microscope (Williams and Cross 1971; Pridham and Tresner 1974). Cultural characteristics of the strain were determined following incubation for 10–15 days at 28±2°C on culture media, as recommended by the ISP (Shirling and Gottlieb 1966), including yeast extract malt extract agar (ISP medium 2), inorganic salt starch agar (ISP medium 4), glycerol–asparagine agar (ISP medium 5), peptone–yeast extract iron agar (ISP medium 6), tyrosine agar (ISP medium 7), asparagine–mannitol agar, Kenknight's agar, nutrient agar, starch nitrate agar, SCA and potato dextrose agar (Himedia). The growth, colour of the spore mass, reverse side colour, and diffusible pigment production were observed after incubation. Isomers of diaminopimelic acid (DAP) in cell-wall hydrolysates and whole-cell sugars of actinomycetes were determined by the methods of Waksman and Henrici (1943) and Boone and Pine (1968).

PCR amplification of 16S rDNA

The DNA of the strain VPTSA18 was extracted by the standard methods of Wilson (1990) and subjected to PCR amplification. A mixture of 39 μl of sterile distilled deionized water, 1 μl of upstream primer (100 pmols) (5′-AGAGTTTGATCCTGGCTCAG-3′), 1 μl of downstream primer (100 pmols) (5′-AGGGCTACCTTGTTACGACTT-3′), 5 μl of 10× PCR buffer, 1 μl of 25 mM $MgCl_2$, 1 μl of dNTP mix (10 mM), 1 μl of actinomycete template DNA (50 ng), and 1 μl of *Taq* DNA polymerase (3 U/μl) was prepared and placed in a 0.5-ml microcentrifuge tube. The total mixture of 50 μl in the tube was gently spun for 10 s and the contents allowed to settle. The sample was kept in an Eppendorf PCR thermal cycler (Eppendorf, Hamburg, Germany), and the amplification carried out as follows: 35 cycles of denaturation for 60 s at 92°C, primer annealing for 60 s at 54°C and polymerization for 90 s at 72°C. Finally, the tubes were ensured complete polymerization at 72°C for 15 min (Weisburg et al. 1991). A 10-μl aliquot of PCR products with 2 μl of loading dye was mixed and loaded onto a 1.2% agarose gel and analyzed electrophoretically at 60 V for 45 min. The gel was observed on a UV transilluminator and compared with a 1-kb DNA ladder. The PCR product was purified using a Microcon PCR centrifugal filter device and sequenced (Applied Biosystems, Foster City, CA) at the facility of the National University of Singapore, Singapore. The 16S rRNA gene sequences of strain VPTSA18 have been deposited in

various GenBanks, including the National Centre for Biological Institute (NCBI; http://www.ncbi.nlm.nih.gov/genebank), European Molecular Biological Laboratory (EMBL; http://www.embl.com) and DNA Data Bank of Japan (DDBJ; http://www.ddbj.com), under sequence accession number DQ845204.

Phylogenetic analysis

The reference sequences required for comparison were downloaded from the EMBL database by accessing the site (http://www.ncbi.nlm.nih.gov/genebank). All of the sequences were aligned using the multiple sequence alignment program CLUSTAL W developed by Higgins et al. (1992). The aligned sequences were then manually checked for gaps, arranged in a block of 250 bp in each row and saved as a format in software MEGA v 2.1. The pairwise evolutionary distances were computed using the Kimura 2 parameter model, as developed by Kimura (1980). The original data set was resampled 1,000 times using the bootstrap program of PHYLOGENY and subjected to bootstrap analysis. The bootstrapped data set was used directly for constructing the phylogenetic tree by using the MEGA program or used for calculating the multiple distance matrix. The multiple distance matrix obtained was then used to construct phylogenetic trees using the neighbour-Joining method of Saitou and Nei (1987). All of these analyses were performed using MEGA v 2.1 (Kumar et al. 2001).

Results and discussion

The filamentous bacteria, especially streptomycetes, are common to almost all habitats. We isolated 68 isolates of actinomycetes from the saltpan soils of Vedaranyam, Nagapattinam district, South India. It has been well established that most actinomycetes exhibit antimicrobial activity. Based on our results, of these 68 actinomycetes isolates, 25 (36.8%) demonstrated antimicrobial activity against various pathogens tested: 22 (88%) with antibacterial activity, 16 (64%) with antifungal activity and 13 (52%) with both antibacterial and antifungal properties. Fifteen (68%) of the 22 isolates with antibacterial activity inhibited the growth of Gram-positive bacteria, 16 (72.7%) isolates had activity against Gram-negative bacteria and nine (40.9%) isolates showed activity against both Gram-positive and Gram-negative bacteria (Fig. 1).

Both quantitative and qualitative variations in the antibiotics produced by different genera and species have been reported. In a study of 47 strains of actinomycetes, Moncheva et al. (2002) found that only 19 isolates showed antagonistic activity and that the antimicrobial compounds

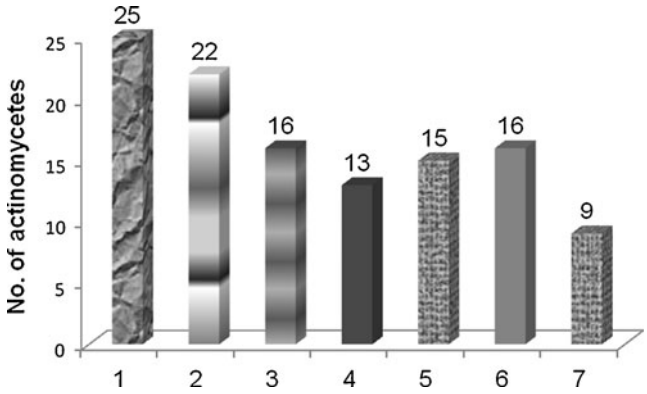

Fig. 1 Antimicrobial activity pattern of actinomycetes isolates. *1* Total antagonist, *2* antibacterial, *3* antifungal, *4* both antibacterial and antifungal, *5* against Gram-positive bacteria, *6* against Gram-negative bacteria, *7* against both Gram-positive and Gram-negative bacteria

produced by the different isolates varied in quality or quantity, or both. The antimicrobial activity of an organism is generally influenced by the nature of the habitat and differences in the composition of the substrate. In addition, there can be variations in terms of different strains and the test organisms. For example, we found that the isolate *Streptomyces* sp. VPTSA18 was comparatively more active than the other antagonistic isolates and interestingly showed a higher antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria. This type of variation has also been reported with reference to activity against dermatophytes in a study performed by Deepika and Kannabiran (2009). These authors isolated about ten saltpan isolates of actinomycetes from Ennore saltpan coastal regions of Tamilnadu, India, and determined the antidermatophytic activity of these isolates by the cross-streak and well-diffusion methods. Three isolates exhibited potential activity against the dermatophyte *Trichophyton rubrum*.

The antimicrobial activity of the different solvent extracts of strain VPTSA18 against 13 pathogens (11 species of bacteria, 2 species of fungi) was remarkable. The ethyl acetate-treated compound (extract) was highly active against *Vibrio cholerae* (26 mm) followed by *Salmonella typhi* (24 mm), *Proteus vulgaris* (23 mm), *Staphylococcus epidermidis* (18 mm), *Cryptococcus neoformans* (18 mm), *Bacillus subtilis* (17 mm), *Salmonella typhimurium* (17 mm), *Salmonella paratyphi* B (17 mm), *Candida albicans* (17 mm), *Klebsiella pneumoniae* (16 mm), *Proteus mirabilis* (15 mm), *Staphylococcus aureus* (15 mm) and *Escherichia coli* (14 mm). The other solvent extracts had a moderate to minimum inhibitory effect against all of the pathogens tested. The petroleum ether extract did not show any activity against the tested pathogens (Table 1). In contrast to these results, Vimal et al. (2009) reported that the petroleum ether extract obtained from the marine actinomycete showed significant antibac-

Table 1 Antimicrobial efficacy of *Streptomyces* sp. VPTSA18

Name of the pathogens (MTCC no.)	Zone of inhibition (mm)				
	Alcohol	Chloroform	Distilled water	Ethyl acetate	Methanol
Bacillus subtilis (121)	8	9	7	17	10
Escherichia coli (43)	8	6	9	14	10
Klebsiella pneumoniae (39)	10	6	7	16	11
Proteus mirabilis (425)	12	7	8	15	10
P. vulgaris (426)	10	7	6	23	9
Salmonella typhi (733)	13	6	12	24	12
S. typhimurium (98)	10	7	6	17	13
S. paratyphi B (735)	8	10	8	17	12
Staphylococcus aureus (87)	8	8	9	15	9
S. epidermidis (2639)	16	11	10	18	10
Vibrio cholerae (3904)	17	15	17	26	13
Candida albicans (183)	10	9	10	17	11
Cryptococcus neoformans (4410)	11	14	11	18	12

MTCC, Microbial Type Culture Collection and Gene Bank (Chandigarh, India)

terial activity against *E. coli* (20 mm), *Pseudomonas aeruginosa* (18 mm), *K. pneumoniae* (15 mm), *Enterococcus faecalis* (20 mm), *Bacillus cereus* (13 mm) and *S. aureus* (6 mm). The ethyl acetate extract showed a high antifungal activity against *Aspergillus fumigatus* (23 mm), *A. flavus* (15 mm) and *A. niger* (12 mm) when compared with petroleum ether and chloroform extracts. The chloroform extract was very effective against yeasts, *Candida krusei* (18 mm), *C. tropicalis* (15 mm) and *C. albicans* (14 mm). Thus, the antimicrobial compounds produced by actinomycete isolates vary depending on the genetic makeup of the isolate, inoculum size, incubation temperature and the solvents used for the extraction.

A single separate band of the antimicrobial compound was observed by TLC. The Rf value of the compound produced by *Streptomyces* sp. VPTSA18 was 0.48 cm. The colour of the separated antimicrobial compound was reddish brown and the melting point was 245°C. The compound was stable in the pH range 6.0–9.0 and temperature range 20–50°C. Analysis of the antimicrobial compound revealed that the absorption maximum was 200 and 205 nm in ethyl acetate. The maximum peaks of the UV spectrum of the VPTSA18 compound were at 200 and 205 nm in ethyl acetate. The IR spectrum of the VPTSA18 compound showed one absorption peak in the region of 1088.0 cm^{-1}, a peak in the region of 1644.4 cm^{-1}, indicating the presence of a C=C– (alkenes) group and another peak in the region of 3311.0 cm^{-1}, indicating that the presence of an NH_2 (amines) group. The mass spectrum showed that the molecular ion peak recorded was 215 m/z, and there were also a large number of peaks throughout the δ value of 1–4 in the ^1H NMR spectrum of purified *Streptomyces* sp. VPTSA18 compound (Table 2). Based on the spectral studies, the antimicrobial compound was identified as a highly oxygenated derivative of carbohydrates. Similar type of work has also been reported by a larger number of researchers (Bordoloi et al. 2001; Wu et al. 2007; Dhanasekaran et al. 2008; Vijayakumar et al. 2011).

Many researchers have tested the antimicrobial efficacy of the compounds obtained from marine actinomycetes against a variety of clinical pathogens. It has been reported that the antimicrobial activity of the compounds from different strains of actinomycetes vary depending on the strains from which the compound obtained, the solvent

Table 2 Characteristics of the antimicrobial compound of *Streptomyces* sp. VPTSA18

S. No.	Criteria	Result
1.	TLC Rf value (cm)	0.48
2.	Colour	Reddish brown
3.	Nature	Viscous
4.	Solubility	Alcohol, chloroform, distilled water, ethyl acetate and methanol
5.	Melting point (°C)	245
6.	pH stability	6-9
7.	Temperature stability (°C)	20-50
8.	UV-spectrum (200 – 400 nm)	200–205
9.	IR spectrum (cm^{-1})	1088.9, 1444.4, 3311.0
10.	Mass (m/z) ion peak	215
11	^1H NMR (1–10 ppm)	1–4

TLC, Thin layer chromatography; NMR, nuclear magnetic resonance

Table 3 Cultural characteristics of *Streptomyces* sp. VPTSA18 on different culture media

Medium	Growth	Sporulation	Diffusible pigment	Colony colour	
				Aerial mycelium	Substrate mycelium
Yeast extract malt extract agar (ISP 2)	Poor	Poor	Pink	White	Dull white
Inorganic salt starch agar (ISP 4)	Poor	Poor	Nil	White	Colourless
Glycerol asparagine agar (ISP 5)	Good	Good	Brown	White	Yellow
Peptone yeast extract agar (ISP 6)	Poor	Poor	Rose	White	Dull white
Tyrosine agar (ISP 7)	Good	Good	Dark brown	White	Yellow
Asparagine mannitol agar	Poor	Poor	Nil	Dull white	White–brown
Kenknight agar	Moderate	Moderate	Pale yellow–brown	White	Light yellow
Nutrient agar	Moderate	Moderate	Brown	Sandal white	Brown
Potato dextrose agar	Moderate	Moderate	Pale pink	Sandal white	Light yellow
Starch casein agar	Excellent	Excellent	Brown	White	Brown
Starch nitrate agar	Moderate	Moderate	Pink	Light yellow	Sandal white

used for the extraction and the nature of the pathogens tested against such compound (Omura et al. 1982; Saadoun and Al-Momani 2000; Sahin and Ugur 2003; Narayana et al. 2005). It is evident that the antimicrobial efficacy of the bioactive compound is the expression of the genetic potentiality of the organisms, and the sensitivity of the test organisms is also one of the genetic properties of the organism. Hence, studies on the genetic relationship between the organisms involved in the microbial interaction could throw more light on the underlying mechanisms.

The results of our identification protocol of the antagonistic isolate are shown in Table 3. Morphological characteristics, such as the formation of a whitish aerial spore mass, brownish reverse side, brown-coloured diffusible pigment with no melanin pigment, development of both aerial and substrate mycelium, development of straight/branched and looped spore chains and a smooth spore surface (Fig. 2a, b), and chemo-taxonomic studies, such as the presence of L-DAP in the cell wall and absence of characteristic sugars in the cell, allowed the cell wall of the strain to be definitively categorized as cell wall type-I. Cultural studies revealed that strain VPTSA18 grew well on several media, including SCA, ISP 5 and ISP 7, and developed whitish aerial mycelium and that the reverse side of most of the media tested became yellow to white. Strain VPTSA18 also produced a diffusible brown- and pink-coloured pigment on most of the media, especially starch nitrate agar media (Table 3). Thus, the morphological, cultural and chemo-taxonomical characteristics confirmed the isolate as *Streptomyces* sp. VPTSA18. Further, the characterization study of isolate VPTSA18 was in accordance with the previous reports of Waksman (1967) and Liu et al. (2005) on *S. griseus*. These researchers reported that the spore chains of *S. griseus* were rectiflexibiles and the surface was smooth. Aerial spore mass colour placed the organism in the grey colour series; the reverse side of colonies was grey to yellow. Melanin pigment was not formed nor was yellow–brown colour soluble pigments produced. Thus, isolate VPTSA18 of our study, which possessed these features, was identified as *Streptomyces* sp. VPTSA18.

Overall, based on all suitable standard taxonomic approaches, the recorded characteristics of the halophilic strain VPTSA18 isolated from the saltpan regions of Vedaranyam were critically analyzed, leading to the conclusion that the isolate should belong to the genus *Streptomyces* sp. Processing of the 842-bp region of the 16S rRNA gene sequence (deposited in various gene banks

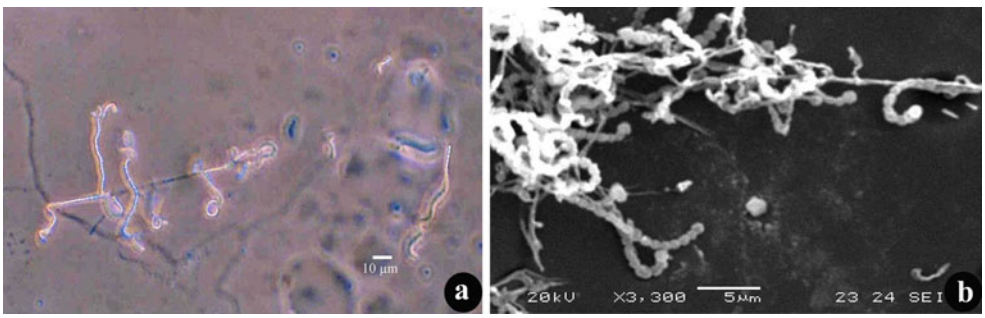

Fig. 2 Micrographs of *Streptomyces* sp. VPTSA18 grown on starch casein agar for 7 days at 28±2°C. **a** Phase contrast micrograph of branched/looped spore chains, **b** scanning electron micrograph of smooth spore surface

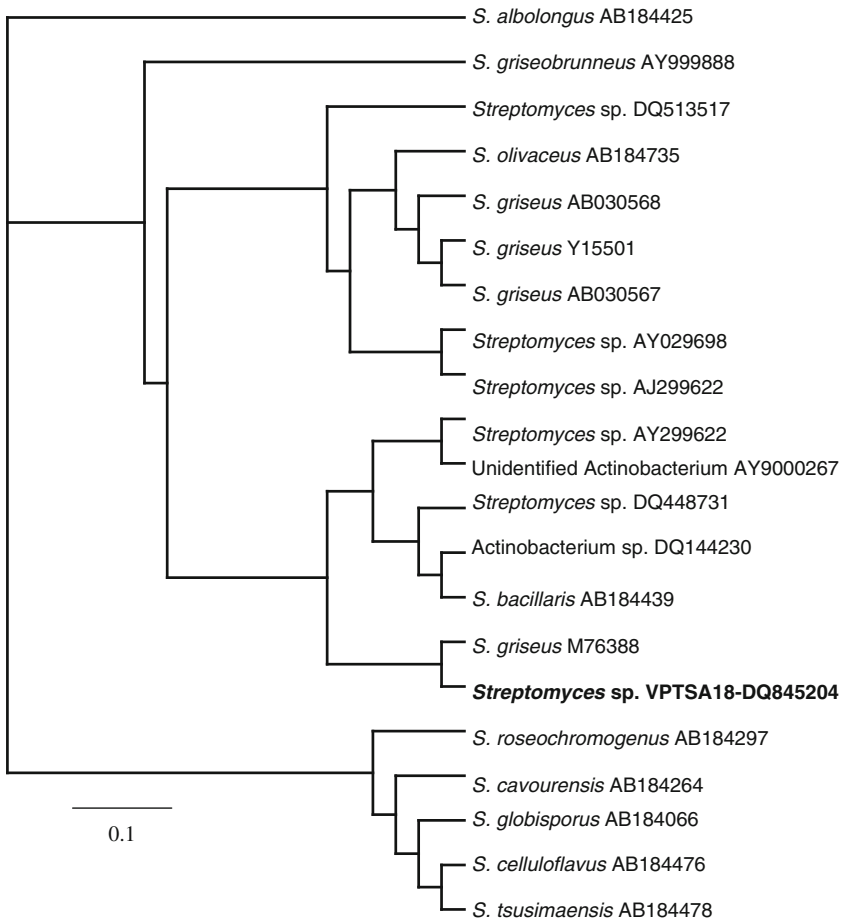

Fig. 3 Phylogenetic tree showing the relationship between the *Streptomyces* sp. VPTSA18 isolate identified in this study and related actinomycetes based on 16S rRNA gene sequences

with accession number DQ845204) belonging to *Streptomyces* sp. VPTSA18 and a phylogenetic evaluation revealed a higher similarity to existing reference species of *S. griseus* M76388 (Fig. 3). Based on these results, the isolate VPTSA18 was tentatively confirmed to be *Streptomyces* sp. Although several researchers have reported the utility of 16S rRNA gene sequence comparison/evaluation as a tool to confirm the identity of actinomycetes (Kreuze et al. 1999; Davelos et al. 2004; Song et al. 2004), the *Streptomyces* VPTSA18 isolate of our study needs to be subjected to other precise methods of identification to check the species fidelity.

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 an useful alternative for the identification of bacterial isolates, especially actinomycetes, that are slow growing and therefore require specialized identification techniques. Based on the results of our study, we conclude that sequencing of the 842-bp 16S rRNA gene provides enough information for the species-level identification of this actinomycetes isolate. However, the accurate assignment of a species to a particular taxa based on a rRNA 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.

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