

Bio Prospecting of Marine-derived *Streptomyces spectabilis* VITJS10 and Exploring its Cytotoxicity Against Human Liver Cancer Cell Lines

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ABSTRACT

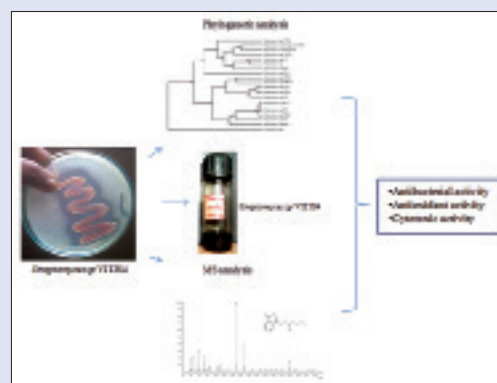
Background: Recently, numerous pathogens have developed resistance due to the indiscriminate use of commercial therapeutic drugs. **Objective:** The main aim of the study was to evaluate the bioactive potential of the *Streptomyces spectabilis* VITJS10 crude extract. **Materials and Methods:** The *S. spectabilis* VITJS10 ethyl acetate extract was tested for antibacterial, antioxidant, and cytotoxic properties. Genotypic characterization was done using 16S rDNA partial gene amplification and sequencing. The authenticity of the crude chemical constituents were determined by gas chromatography–mass spectrometry (GC-MS). **Results:** The antibacterial potential revealed the effective activity against *Shigella flexneri* (MTCC No: 1457) (22 mm), *Salmonella typhi* (MTCC No: 1167) (23 mm), *Escherichia coli* (MTCC No: 1588) (22 mm), *Pseudomonas aeruginosa* (MTCC No: 4676) (22 mm) at 20 mg/mL concentration. Scavenging ability of the extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay revealing its 95% inhibition at 5 mg/mL concentration. Hepatocellular cancer cells (HepG2) cell line was used to evaluate the cytotoxicity by 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay. The extract showed maximum inhibition at IC₅₀ of 250 µg/mL with 53.6% cell viability. The highest 16S rRNA gene sequence homogeneity was observed 99% similar with the novel strain *S. spectabilis* S3-1. The chemical components of the crude extract of VITJS10 were detected with 37 chemical constituents. However three major compounds were identified, namely Sulfurous acid, 2-ethylhexyl tridecyl ester, Phenol, 2,4-bis (1,1-dimethylethyl), and Trans-2-methyl-4-n-pentylthiane, S, S-Dioxide. **Conclusion:** Hence the present study justifies the overwhelming circumstantial evidence as the most bioactive metabolites from the marine origin, which has potential utilization in pharmaceutical industry.

Key words: Bioactivity, cytotoxicity, MARINE actinomycetes, secondary metabolites, *Streptomyces spectabilis*, VITJS10

SUMMARY

- The aim of this study was to explore the bioactive potential of marine *Streptomyces* sp. isolated from marine soil and understand the bioactive properties of the crude extracts. It is clearly evident from the study that the bioactive metabolites produced by *Streptomyces* sp. exhibited good antibacterial, antioxidant and anticancer activity. Our results indicated that

Starch casein medium was the good base for bioactive metabolite production. The taxonomic position of *Streptomyces* sp. isolated revealed unique pattern of phenotypic properties that distinguished it from representatives. The molecular characterization results provided valuable data for establishing the internal taxonomic structure of the genus. Hence high mortality rates, serious side effects, deficiencies of the available chemotherapeutics, and high costs during treatment clearly underscore the need to develop new anticancer agents. With the above significant features the strain could be recommended for its use in medicinal and agricultural sectors, an extensive knowledge on the behavior of natural compounds can be gained for the benefit of health.



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INTRODUCTION

Cancer is a leading cause of death worldwide, accounting for approximately 1/8 of all deaths.^[1] Moreover, antimicrobial resistance causes more extensive morbidity and premature death in infected patients. There is certainly an urgent need for new antimicrobial agents, given the increase in drug resistance in many common bacterial pathogens and changes in the spectrum of pathogens. Research strategies for the discovery of new antimicrobial drugs are heavily influenced by the need to discover and develop new agents active against organisms resistant to earlier generations of drugs. Anticancer drugs from marine origin have received much attention in recent years. It is increasingly evident that the true biological origin of many metabolites originally isolated from certain marine microorganisms. Secondary metabolites from marine actinomycetes have been extensively reviewed recently, demonstrating their structural diversity and emphasizing

their promising potential as pharmaceuticals and to enable enhanced detection of novel chemical class of antibiotics. The discovery of new active metabolites must be followed by adequate biological testing.^[2] Hence the present study was focused to characterize the *Streptomyces spectabilis* VITJS10 crude extract for its biological potential.

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MATERIALS AND METHODS

Sample collection, isolation, and characterization of marine actinomycetes

Marine soil samples were collected from south-east coast of Tamil Nadu, India, Kanyakumari – Chinnamuttom (Lat. 8°5'N and 77°39'E), at the depth of 50 cm at littoral zone. The isolation of *Actinobacteria* was performed on starch casein agar. The morphological and cultural characteristics of the potent strain were determined on various ISP medium.^[3] The spore morphology was observed under a light microscope and scanning electron microscopy (SEM) (FEI QUANTA; FEG 200). The morphological identification was done on the basis of nonomura guidelines.^[4,5]

Cross streak assay

The strain *Streptomyces* sp VITJS10 was cross streaked on modified nutrient glucose agar against wide range of clinical pathogens namely *Shigella flexneri* (microbial type culture collection and Gene Bank [MTCC] No: 1457), *Salmonella typhi* (MTCC No: 1167), *Escherichia coli* (MTCC No: 1588), *Staphylococcus aureus* (MTCC No: 7405), and the zone of inhibition was measured after 2 days of incubation.^[6]

Production

The inoculum of the potent strain *Streptomyces* sp VITJS10 was prepared on starch casein broth at an incubation period of 7 days at room temperature. Simultaneously the culture filtrate was extracted with ethyl acetate and concentrated by using a rotary evaporator.^[7] The extract was then air dried to solid residue and tested for bioactive potential.

Antibacterial activity

The *in vitro* antibacterial activity of the strain *Streptomyces* sp VITJS10 crude extract was determined by agar well diffusion method.^[8]

Antioxidant activity

The antioxidant activity was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. Various concentrations (0.1, 0.5, 1.0, 3.0, and 5.0 mg/mL) of *Streptomyces* sp VITJS10 crude extract was taken in separate tubes. Ascorbic acid was used as reference compound (0.1, 0.5, 1.0, 3.0, and 5.0 mg/mL). A freshly prepared solution of 0.002% DPPH in methanol was added to each tube containing different concentrations of extracts (2.0 mL). The samples were incubated in dark at 37°C for 20 min and read at 515 nm. The data was expressed as the percent decrease in the absorbance compared with the control. The percentage inhibition of radical scavenging activity was calculated.^[9]

Maintenance of cell cultures

The hepatocellular cancer cells (HepG2) were obtained from NCSS, Pune and cultured in RPMI-1640 medium on 10 cm tissue culture dishes (Greiner Bio-one™, Germany) supplemented with 10% heat inactivated fetal bovine serum (FBS). Cells were incubated in humidified incubator with 5% CO₂ at 37°C, and sub-cultured when confluence was reached up to 80%.^[10]

Cytotoxic assay

The 3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide (MTT) assay was used to assess the viability of the cells. The HepG2 cells (5 × 10³) were seeded per well into 96-well plates containing 100 µL Dulbecco's Modified Eagle medium with 10% FBS incubated at 37°C for 24 h, Then, the cells were treated with 100 µL of the of *Streptomyces* sp VITJS10 crude extract in triplicates with different concentrations ranging from 1 to 500 µg/mL. Doxorubicin (5 µg/mL) was used as internal positive control and 100 µL of DMEM was used as negative control; wells without any cells were considered as blank. After incubating in

humidified incubator with 5% CO₂ at 37°C for 48 h, 20 µL of 5 mg/mL MTT diluted in phosphate-buffered saline (PBS) were added to each well and incubated for 4 h. One hundred microliter of 10% Sodium dodecyl sulfate (SDS) in 0.01 M Hydrochloric acid solution was added to each well to dissolve the formazan crystals formed. The plates were covered with aluminum foil and kept in an incubator for 12 h for dissolution of the formed formazan crystals. Amount of formazan was determined measuring the absorbance at 560 nm using a micro plate reader.^[11]

16S r DNA sequencing

Total genomic DNA was isolated using the phenol chloroform method.^[12] Polymerase chain reaction (PCR) amplification of 16S r-DNA was carried out using the primers. Sequencing was performed using big dye terminator cycle sequencing kit (Applied BioSystems, USA). The sequence data was submitted to the GenBank database under the accession number KJ636987. The phylogenetic tree was constructed via the neighbor-joining method using the EvolView program.^[13]

Gas chromatography–mass spectrometry analysis

The crude extract of *Streptomyces* sp VITJS10 was subjected to gas chromatography–mass spectrometry (GC-MS) for the detection of volatile compounds. The analysis was performed using GC SHIMADZU QP2010 system.^[14] NIST08 and WILEY8 database was used for the identification of the separated peaks. The diversity of compounds was indicated by the metabolite profiles.

RESULTS

Marine actinomycetes are the richest source of bioactive compounds used in modern medicine as pharmaceutical intermediates. Microbial derived metabolites have recently attracted great interest, owing to

Table 1: *Streptomyces spectabilis* VITJS10: Morphological, physiological, and biochemical properties

Morphological properties					
Spore morphology	Spore surface	Color of aerial mycelium	Color of substrate mycelium	Gram staining	Motility
Spiral	Warty	Reddish	Pink	+	-
Effect of temperature		Effect of pH		Effect of NaCl tolerance	
15°C	-	5	-	0.5%	++
28°C	++	6	-	1%	+
37°C	+	7	+	2%	-
45°C	-	7.2	++	3%	-
50°C	-	7.5	+	4%	-
60°C	-	8	-	5%	-
Carbon source			Nitrogen sources		
D-glucose	++		Cysteine		-
Sucrose	-		Arginine		+
D-galactose	+		Therionine		-
Mannose	+		Alanine		-
Maltose			Aspartic acid		-
Lactose	-		Glycine		++
Mannitol	-		Histidine		-
L-rhamnose	+		Lysine		-
Arabinose	-		Phenyl alanine		+

their versatile applications. The strain VITJS10 was studied on the basis of its morphology and recognized by their presence of aerial hyphae with reddish orange color [Table 1]. The colonies with powdery appearance had a characteristic feature of warty surface with spiral chains under SEM (magnification 6000 \times) with spore diameter of 0.6 μ m [Figure 1a and b]. The biochemical and taxonomical characterization represented the features of Streptomycetaceae family. The antibacterial activity was screened and confirmed through cross streak assay against the pathogens showing broad spectrum [Figure 2]. The ethyl acetate extract showed broad spectrum antimicrobial activity toward Gram-positive and -negative bacteria at the concentration of 20 mg/well against *S. flexneri* MTCC No: (1457) (22 mm), *S. typhi* MTCC No: 1167 (23 mm), *E. coli* MTCC No: 1588 (22 mm), *Pseudomonas aeruginosa* MTCC No: 4676 (22 mm) [Figure 3A and B]. The crude extract studied for its antioxidant activity at different concentrations displayed highest degree at 5 mg/mL with 95% inhibition [Figure 4a and b]. The hemolytic property of the *S. spectabilis* VITJS10 crude extracts revealed maximum zone of lysis with 15 mm. The *S. spectabilis* VITJS10 crude extracts was also found to exhibit significant anticancer activity against HepG2 with the inhibition rate of 53.6% at concentration of 250 μ g/mL. The positive control doxorubicin (5 μ g/mL), which was used as internal positive

control, showed 94% cell lysis at concentration of 250 μ g/mL. The HepG2 cells was noticed with highest percentage of cell death [Figure 5]. The 16S rDNA sequence revealed the highest similarity to *S. spectabilis* S3-1 with 99%. Further the isolate VITJS10 was named as *Streptomyces spectabilis* VITJS10 [Figure 6]. The chemical constituents of *S. spectabilis* VITJS10 crude extract revealed 37 chemical compounds; out of which, 3 important components were identified. The major chemical structures namely Sulfurous Acid, 2-ethylhexyl tridecyl ester were noticed with the retention time (RT) at 6.16 min showing peak at m/z 376 [M]⁺ in accord with the molecular formula, C₂₂H₄₄O₃S. Phenol, 2,4-bis (1,1-dimethylethyl) (MW-206) Formula- C₁₄H₂₂O, which showed its RT at 13.20 min, Trans-2-methyl-4-n-pentylthiane, S, S-Dioxide, RT at 14.1 min: (MW218) C₁₁H₂₂O₂S [Figure 7]. The study justifies that marine *Streptomyces* provide a number of metabolites when compared with other bacteria. Hence the study showed significance results as promising targets for the development of cytotoxic and antimicrobial drugs.



Figure 1: (a) *Streptomyces spectabilis* VITJS10 on starch casein agar; (b) scanning electron microscopy analysis of *Streptomyces spectabilis* VITJS10 spores



Figure 2: Cross streak assay of *Streptomyces spectabilis* VITJS10

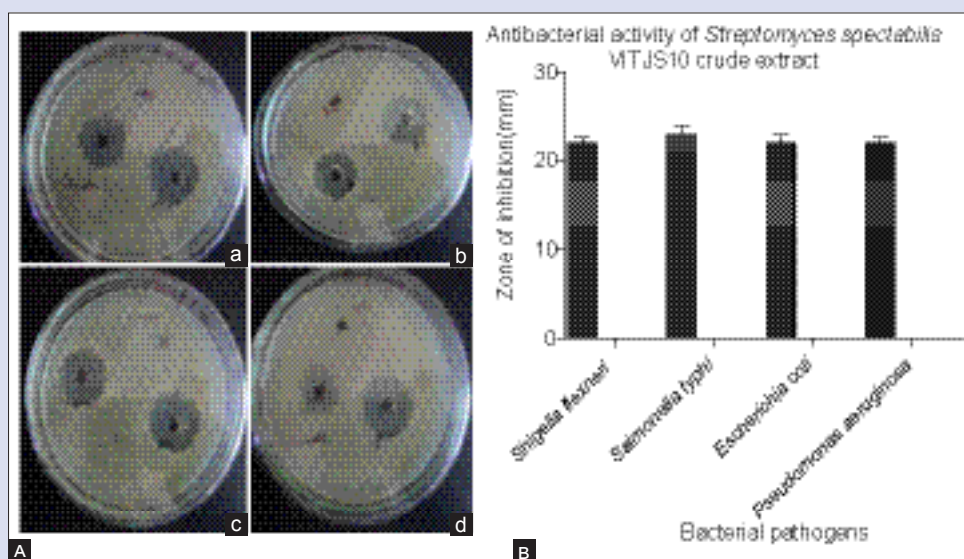


Figure 3: (A) Pictorial view showing the inhibition of bacterial pathogens (a) *Shigella flexneri* MTCC No: (1457), (b) *Salmonella typhi* MTCC No: 1167, (c) *Escherichia coli* MTCC No: 1588, (d) *Pseudomonas aeruginosa* MTCC No: 4676. (B) Graphical representation of antibacterial property *Streptomyces spectabilis* VITJS10 crude extract

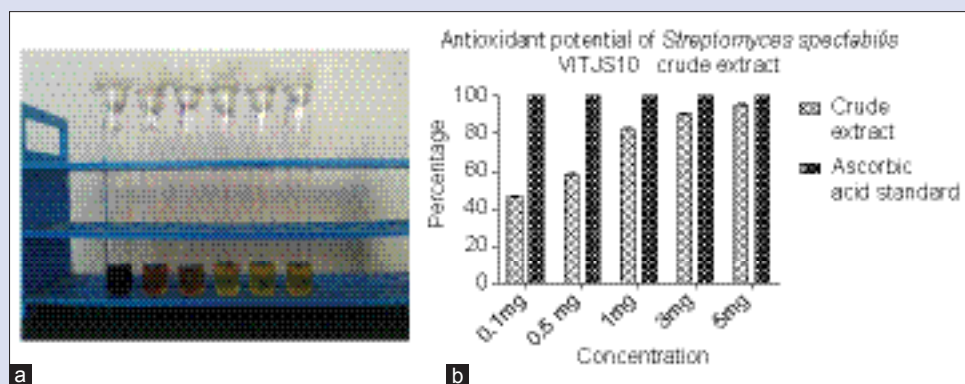


Figure 4: (a) Pictorial view showing antioxidant property of *Streptomyces spectabilis* VITJS10 crude extract (b) Graphical representation of antioxidant property of *Streptomyces spectabilis* VITJS10 crude extract

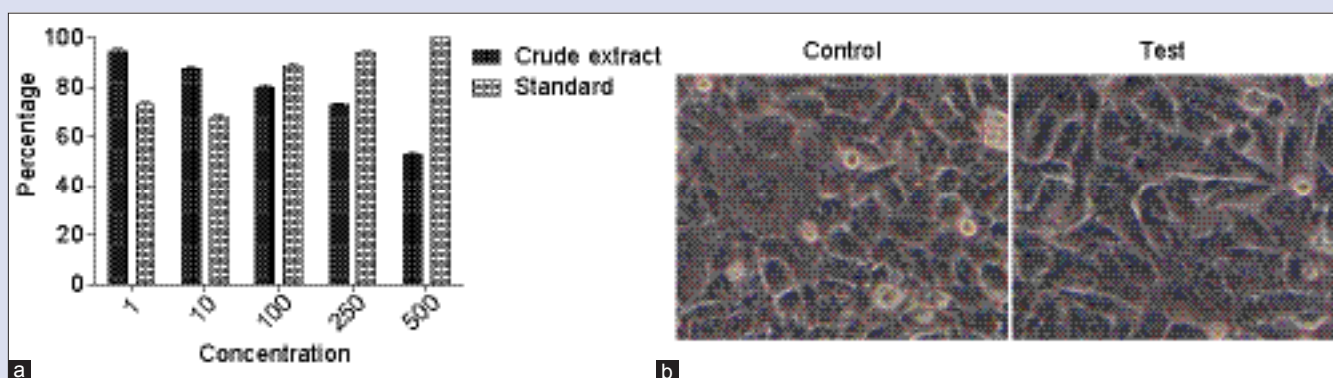


Figure 5: (a) Percentage of cell viability shows the effective drug concentration toxic to HepG2 cell lines. (b) Morphological changes in HepG2 cell lines after treated with standard drug (Doxorubicin) and crude extract of *Streptomyces spectabilis* VITJS10

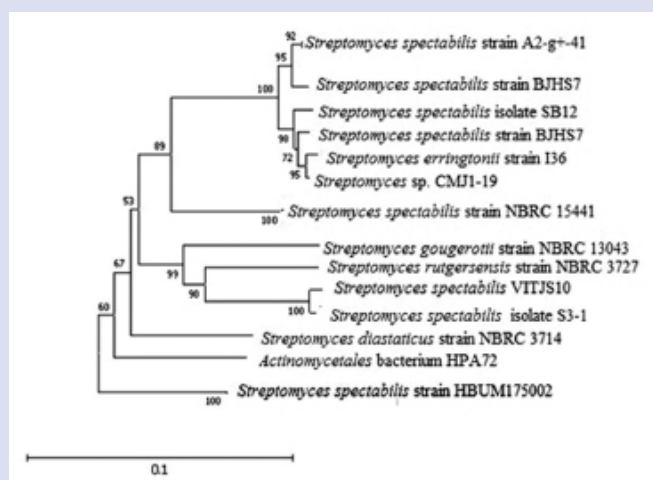


Figure 6: Phylogenetic tree of *Streptomyces spectabilis* VITJS10

DISCUSSION

Recently, much attention has been directed toward biologically active compounds isolated from marine saltern. The natural products have been shown to have antioxidant activity and are capable of scavenging

free superoxide radicals, thus reducing the risk of cancer.^[15] One of the more significant findings emerge from this study is that the *S. spectabilis* VITJS10 extract exhibited a promising anticancer activity on human liver cancer cell lines. Several mechanisms were found to underlie these multifaceted synergistic activities of the active principles. The potential of antimicrobial and anticancer activity perhaps would be due to the presence of the parental compounds in the crude extract showing distinctly synergistic effects. Similarly, most of the bioactive molecules isolated from Actinobacteria are cytotoxic: Streptazone B1 produced by strains HB117, HB122, and HB291.^[16] Fredericamycin A (produced by strains HB116, HB118, and HB157).^[17] The present study has shown the beneficial effects of the *S. spectabilis* VITJS10 synergistic compounds. Further studies are needed to confirm in large, rigorous trials. Hence the advantages of natural compounds are known with fewer side effects in comparison to orthodox medical drugs, which indicate more specific for the positive treatment outcome.

CONCLUSION

Streptomyces spectabilis VITJS10 showed various bioactive properties, which highlighted its importance as potential pharmacological agents. Hence, there could be probability of new bioactive compound in the crude extract, which might provide a basis for further development of novel compound. This also provided a new insight toward the development of good candidates for pharmaceutical and bioactive natural products.

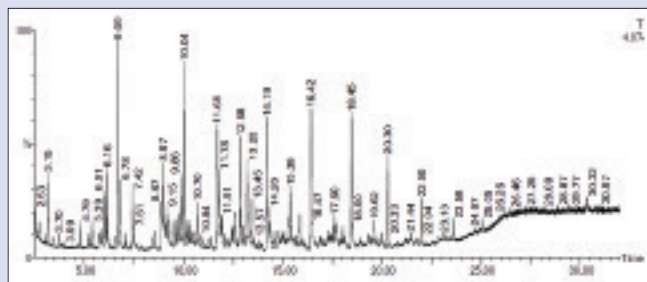


Figure 7: Gas chromatography–mass spectrometry chromatogram of *Streptomyces spectabilis* VITJS10 crude extract

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Conflicts of interest

There are no conflicts of interest.

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