A multifunctional *Streptomyces maritimus* SACC-152 isolated from Andaman and Nicobar Islands, India.

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Abstract

A mangrove derived *Streptomyces* strain SACC-152 isolated from Andaman and Nicobar Islands was investigated for its multiple bioactive and biosynthetic properties. Morphology, cultural, physiological and molecular studies showed that the strain SACC-152 is closely similar to *Streptomyces maritimus*. Bioactive metabolites from the strain SACC-152 were produced by submerged fermentation and extracted using ethyl acetate. The ethyl acetate extract of strain SACC-152 exhibited a broad spectrum of antimicrobial activity against various bacterial and fungal pathogens. Luciferase Reporter Phage (LRP) assay showed more than 80% inhibition against Mycobacterium tuberculosis H37Rv, drug sensitive and multi drug resistant (MDR) *M. tuberculosis* strains. In MTT assay, the extract showed promising activity against HT-29 colon cancer cell lines with the IC50 value of 9.68 µg/ml. In the antioxidant assay, the extract showed maximum of 61.35 ± 2.46% free radical scavenging activity. The extract of SACC-152 caused highest mortality against the mosquito larvae of *Aedes aegypti* (LC50 19.6 µg/mL, r²=0.61). Further, the genomic DNA of strain SACC-152 were found to harbor secondary metabolite biosynthetic genes such as polyketide synthases (PKS-I), the adenylation domains of non-ribosomal peptide synthase (NRPS) and halogenase (*Halo*). Results of this study revealed that, the *Streptomyces maritimus* SACC-152 showed versatile multifunctional properties and hence deserves the potential for the isolation of bioactive secondary metabolites.

Keywords: *Streptomyces maritimus*, Andaman and Nicobar Islands, Mangroves, Biosynthetic genes and anti-tubercular activity.

Introduction

Infectious diseases are the major threat to human health [1]. There were estimated 1.2 million tuberculosis deaths and 1.4 million HIV/AIDS deaths, in 2018; 4,35,000 malaria deaths in 2017 [2-4]. The multi-drug resistant (MDR) organisms play an important role behind these increasing deaths year after year [5]. Due to the emergence of MDR among existing pathogens, current antibiotics are losing their ability to fight against the pathogens [6]. Hence, researchers are endeavoring continuously to search novel and potential bioactive metabolites in order to tackle the serious consequences caused by MDR pathogens. Microbial metabolites persist as a lead hub for the discovery of novel antibiotics to treat most of the human and animal diseases [7].

Due to the reducing percentage of finding novel bioactive metabolites from the soil microbial species, only a few attempts have been made to understand the microbial diversity of marine sediments which are an inexhaustible resource for the search of new drugs [8]. Among the microbial resources, members of the phylum actinobacteria notably *Streptomyces* remains a tremendous source for novel bioactive metabolites [9]. Actinobacteria inhabiting marine environment gain much attention compared to their terrestrial counter parts due to their diverse taxonomic, biosynthetic and bioactive potentials [10].

The Andaman and Nicobar (A & N) Islands are the richest but under investigated source for actinobacteria with special reference to bioactive metabolites [11]. Only few reports on marine actinobacteria from Andaman and Nicobar Islands have
been documented that too mainly for the distribution, antimicrobial, antioxidant and enzymatic activities [11,12].

To the best of our knowledge, except the works reported by Radhakrishnan et al. [13,14] there are no documented studies have been reported the anti-tubercular activity of actinobacteria isolated from A & N Islands. Thus the present study reported that antimicrobial, anti-tubercular, antioxidant, anticancer and larvicidal properties of Streptomyces maritimus SACC-152 isolated from A & N Islands, India

Materials and Methods

Description of Streptomyces strain SACC-152

Streptomyces strain SACC-152 was isolated from the mangrove rhizosphere sediments collected from Andaman and Nicobar Islands (Lat:13° 20’ 24” N, Long: 93° 3’ 0” E), India by standard spread plate method using Starch Casein Agar (SCA) medium prepared in 50% filtered sea water. Viability of strain SACC-152 was maintained on ISP2 agar slants as well as in 20% glycerol broth at -80°C.

Characterization and taxonomy of strain SACC-152

Micromorphological features such as the presence of aerial; substrate mycelium and mycelial fragmentation were observed under the bright field microscope at 40x magnification. Cultural characteristics such as growth, colony consistency, aerial mass colour, production of reverse side and soluble pigment by the strain SACC-152 was studied by growing the culture on ISP2 agar medium. Growth properties of strain SACC-152 on different culture media (ISP1 - ISP7), pH, temperature and NaCl concentrations were performed according to the methods described by Holt et al. [15].

The genomic DNA of strain SACC-152 was isolated using solute ready genomic DNA kit. DNA was analyzed by gel electrophoresis and quantified using a spectrophotometer (Nano Drop ND-1000, Thermo Scientific, and Gloucester, UK). The 16S rRNA gene sequence of strain SACC-152 was amplified using the primers: 27F 5′AGAGTTTGATCMTGGCTCAG3′ (forward) and 1492R 5′TACGGYTACCTTGTTACGACTT3′ (reverse).

The PCR amplified product of strain SACC-152 was sequenced and analyzed at Eurofins Genomics, Bangalore, India. The sequences obtained were aligned with similar sequences available in GenBank using MEGA 7 program [16]. The aligned sequences were used to construct the phylogenetic tree by following neighbor joining algorithm in MEGA 7 program. The bootstrap method was used to determine the confidence of the branches of the phylogenetic tree. The partial 16S rRNA nucleotide sequence of strain SACC-152 has been deposited in GenBank database.

Fermentative production of bioactive metabolites

For the production of secondary metabolites, spores of strain SACC-152 from ISP2 agar plates were taken and aseptically inoculated into 250 mL conical flasks containing 50 mL of ISP2 broth and incubated at 28°C in rotary shaker with 120 rpm speed for 48 hours. Five percent of this seed culture was transferred to 1 L flask containing 200 mL of ISP2 broth as production medium and incubated at 28°C in a rotary shaker with 120 rpm speed for 96 hours. Further the cell free supernatant was separated by centrifugation at 5000 rpm speed at 4°C for 30 minutes.

The extracellular bioactive metabolites present in the cell free supernatant was extracted through liquid-liquid extraction method using ethyl acetate (1:1 v/v) for 24 hours with intermittent manual shaking. Then, the organic layer was collected and concentrated to dryness using rotary evaporator to obtain the crude solvent extract.

Testing for Biological Activities

Antimicrobial activity

Crude ethyl acetate extract of strain SACC-152 was evaluated for antimicrobial activity by disc diffusion method against the pathogens such as (ATCC-29213), Escherichia coli (ATCC-25922), Klebsiella pneumoniae (ATCC-29212), Mycobacterium smegmatis (NCIM-5682), Bacillus subtilis, Aeromonas hydrophila, Salmonella paratyphi, Providencia vermicola, Candida albicans and Cryptococcus neoformans. Bacterial and fungal pathogens with 0.5 McFarlands standard was prepared with sterile nutrient broth and Sabouraud broth. All the pathogens were inoculated onto Muller Hinton Agar (MHA) plates using sterile cotton swab. About 10 mg/ml concentration of SACC-152 extract was prepared as main stock using ethyl acetate and filtered using sterile 0.45 μm syringe filter. One mg per ml of working stock solution was prepared by adding 10 μl of stock extract into 90 μl of ethyl acetate solvent and mixed well.

Antibiotic discs were prepared at 250 μg/disc concentrations from working extract on sterile Whatman No. 1 filter paper disc (5 mm in diameter) and allowed to dry. The extract impregnated discs were placed over the MHA plates previously inoculated with test pathogens. The plates were incubated for 24 hrs at 37°C and then observed for zone of inhibition was measured and expressed in millimetre in diameter. The disc impregnated with only ethyl acetate was used as negative control.

Anti-tubercular activity

Anti-tubercular activity of the ethyl acetate extract was tested against standard laboratory strain Mycobacterium tuberculosis H37Rv, SHRE (Streptomycin, Isoniazid, Rifampicin and Ethambutol) sensitive and multi drug resistant (MDR) (resistant to Rifampicin and Isoniazid) clinical strains of M. tuberculosis by adopting Luciferase Reporter Phage (LRP) assay. Two mg per ml of working concentration of sample was prepared using 10% dimethyl sulfoxide (DMSO) and filtered using 0.45 μm filters. Growth of M. tuberculosis strain was maintained on Lowenstein-Jensen (LJ) medium at 30 ± 2°C.
High titer of mycobacteriophage phAETRC202 (gifted from Department of Bacteriology, National Institute for Research in Tuberculosis (NIRT), Chennai) was prepared using M. smegmatis mc2155 in Middle brook 7H9 complete medium. About 350 μl of G7H9 broth supplemented with 10% albumin dextrose complex and 0.5% glycerol was taken in cryo vials and added with 50 μl of sample in order to get the final concentration of 200 μg/ml. Then hundred microlitre of M. tuberculosis cell suspension was added to all the vials. The above procedure was followed for all the three types of M. tuberculosis strains used. The 10% DMSO was also included in the assay as the solvent control. All the vials were incubated at 37°C for 72 hours.

After incubation, 50 μl of high titer phage phAETRC202 and 40 μl of 0.1 M CaCl₂ solution were added into the test and control vials. All the vials were incubated at 37°C for 4 hours. After incubation, 100 μl from each vial was transferred into a Luminometer cuvette. About 100 μl of D-Luciferin was added and the relative light unit (RLU) was measured in a Luminometer (Berthold).

Control RLU – Test RLU
% RLU reduction= ---------------------------------------- X 100

Control RLU

**Antioxidant activity**

Five microlitre ethyl acetate extract of SACC-152 extract at different concentrations was mixed with 195 μl of 0.016% DPPH solution freshly prepared in 95% methanol. The mixture was kept at room temperature in the dark for 20 min before measuring the reading at 515 nm with microplate reader [17]. Ascorbic acid was used as a positive control. The percentage of free radical scavenging activity of SACC-152 extract was calculated according to the formula expressed below:

\[
\text{Absorbance of control } - \text{Absorbance of sample}
\]

% DPPH scavenging activity= ---------------------------------------- X 100

Absorbance of control

**Anti-cancer activity**

**Cell lines maintenance and growth condition:** Anticancer activity of the ethyl acetate extract was tested against the cancer cell lines such as MCF7 (breast cancer), HT-29 (colon cancer) and HeLa (cervical cancer) by adopting MTT assay. All the cell lines were maintained in RPMI (Roswell Park Memorial Institute)- 1640 (Gibco) supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and Streptomycin (100 μg/ml) at 37°C humidified incubator containing 5% CO₂ and 95% air. The cell cultures were viewed using an inverted microscope to assess the degree of confluence and to confirm the absence of bacterial and fungal contamination.

**MMT:** MCF-7 and H-29 colon cancer cells were seeded onto a sterile flat bottom 96-well plate at a density of 5000 cells/ well and incubated for 48 hours. Twenty microlitre ethyl acetate extract of SACC-152 was added into each well with the final concentration ranging from 5 to 100 μg/ml. About 0.05% (v/v) of sterile DMSO was used as negative control for this experiment. Microtitre plate was further incubated with the extract for 72 hrs. Fifty microlitres of 5 mg/mL MTT (Sigma) solution was then added to each well and the plates were incubated at 37°C in a humid atmosphere with 5% CO₂, 95% air for 4 hrs.

The medium was then gently aspirated and 100 μl of (DMSO) was added to dissolve the formazan crystals. The absorbance of dissolved formazan product was determined spectrophotometrically at 570 nm using a microplate reader [18]. The percentage of cell viability was calculated as follows:

\[
\text{Absorbance of treated cells}
\]

\[
\text{Absorbance of untreated cells (0.05% DMSO only)}
\]

**Larvicidal activity**

Larvae of Culexquinquefasciatus and Aedesaegypti were obtained from the National Center for Disease Control Research Institute, Coonoor, Tamil Nadu, India. The larvae were kept in plastic enamel trays containing de-chlorinated tap water and maintained. Larvicidal assay was performed by placing 25 mosquito larvae into 200 ml of sterilized double distilled water which contain the ethyl acetate extract of strain SACC-152 at different concentrations (100, 300, 500, 700, 900 μg/ml) into a 250 mL beaker.

Distilled water was used as control for each individual concentration. Mortality was estimated after 24 hours to calculate the acute toxicities on larvae of C. quinquefasciatus and A. aegypti [19]. Mosquito larva without ethyl acetate extract of SACC-152 was used as negative control and all conditions were tested in five replicates.

**Molecular detection of secondary metabolite biosynthetic genes**

The isolated actinobacterial strain SACC-152 was investigated for the presence of secondary metabolite biosynthetic genes such as KS domains of polyketide synthase type I (PKS I), KS domains of PKS II, the adenylation domains of non-ribosomal peptide synthase (NRPS), the enzyme aminodeoxyisochorismate synthase PhzE of the phenazine pathways (phzE), the enzyme dTDP-glucose-4, 6-dehydratase (dTGD) glycosylation pathway, the enzyme halogenase (Halo) of halogenation pathway and the enzyme cytochrome P450 hydroxylase (CYP) in polyenepolyketide biosynthesis through
the PCR based method using the degenerate primers as described previously (Table 1) [20].

**Table 1. Cultural and physiological characteristics and the effect of critical medium components on antimicrobial activity of Streptomyces sp. strain SACC-152.**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Variables</th>
<th>Morphological Growth</th>
<th>Zone of inhibition against S. aureus (millimeter in diameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different media</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ISP1</td>
<td>++</td>
<td>15.50 ± 0.50</td>
<td></td>
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<tr>
<td>ISP2</td>
<td>+++</td>
<td>23.00 ± 0.58</td>
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<tr>
<td>ISP3</td>
<td>+++</td>
<td>17.33 ± 0.67</td>
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<tr>
<td>ISP4</td>
<td>+++</td>
<td>19.83 ± 0.93</td>
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<tr>
<td>ISP5</td>
<td>+++</td>
<td>0.00</td>
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<tr>
<td>ISP6</td>
<td>*</td>
<td>0.00</td>
<td></td>
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<tr>
<td>ISP7</td>
<td>***</td>
<td>9.50 ± 0.29</td>
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<tr>
<td>Utilization of carbon source (1%)</td>
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<td></td>
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</tr>
<tr>
<td>Glucose</td>
<td>+++</td>
<td>15.00 ± 0.58</td>
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<tr>
<td>Starch</td>
<td>+++</td>
<td>15.83 ± 0.73</td>
<td></td>
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<tr>
<td>Fructose</td>
<td>++</td>
<td>10.00 ± 0.29</td>
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<tr>
<td>Sucrose</td>
<td>++</td>
<td>9.17 ± 0.73</td>
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<tr>
<td>Xylose</td>
<td>+</td>
<td>0.00</td>
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<tr>
<td>Utilization of nitrogen source (1%)</td>
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<tr>
<td>Yeast extract</td>
<td>+++</td>
<td>14.50 ± 0.50</td>
<td></td>
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<tr>
<td>Malt extract</td>
<td>+++</td>
<td>16.00 ± 0.00</td>
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<tr>
<td>Peptone</td>
<td>++</td>
<td>13.83 ± 0.76</td>
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<tr>
<td>KNO₃</td>
<td>+</td>
<td>9.17 ± 0.73</td>
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<tr>
<td>Ammonium sulphate</td>
<td>-</td>
<td>0.00</td>
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<tr>
<td>Minerals (0.1%)</td>
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<tr>
<td>K₂HPO₄</td>
<td>++</td>
<td>14.17 ± 0.60</td>
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<tr>
<td>MgCl₂</td>
<td>++</td>
<td>13.17 ± 0.44</td>
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<tr>
<td>FeSO₄</td>
<td>+</td>
<td>10.50 ± 0.76</td>
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<tr>
<td>ZnSO₄</td>
<td>-</td>
<td>0.00</td>
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<tr>
<td>KH₂PO₄</td>
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<td>0.00</td>
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<tr>
<td>pH</td>
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<td>*</td>
<td>0.00</td>
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<tr>
<td>7</td>
<td>***</td>
<td>17.33 ± 0.73</td>
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<tr>
<td>8</td>
<td>***</td>
<td>15.17 ± 0.33</td>
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<tr>
<td>9</td>
<td>++</td>
<td>16.33 ± 0.93</td>
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</tr>
<tr>
<td>10</td>
<td>++</td>
<td>0.00</td>
<td></td>
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<tr>
<td>Temperature (°C)</td>
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<td></td>
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<tr>
<td>20</td>
<td>++</td>
<td>10.50 ± 0.50</td>
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<tr>
<td>30</td>
<td>+++</td>
<td>17.50 ± 0.29</td>
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<tr>
<td>30</td>
<td>+</td>
<td>0.00</td>
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<tr>
<td>Sea water (%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>*</td>
<td>8.33 ± 0.73</td>
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<tr>
<td>25</td>
<td>***</td>
<td>12.17 ± 0.44</td>
<td></td>
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<tr>
<td>50</td>
<td>***</td>
<td>15.50 ± 0.50</td>
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</tbody>
</table>
Effect of culture conditions and medium components on secondary metabolites production

Effect of various critical medium components and culture conditions on antimicrobial metabolite production by the strain SACC-152 was studied by adopting classical one-variable-at-a-time method [21]. Factors and variables used in this study are given in Table 1. Effect of carbons and nitrogen sources were studied by replacing glucose and malt extract from ISP-2 agar medium with 1% of different sugars and different nitrogen sources, respectively.

Effect of minerals was studied by supplementing 100 mg of different minerals such as K$_2$HPO$_4$, MgCl$_2$, FeSO$_4$, ZnSO$_4$ and KH$_2$PO$_4$ into ISP-2 agar. Effect of sodium chloride was studied by supplementing different concentration of sea water viz., 0%, 25%, 50% and 100%. Effect of temperature (20°C-40°C) and pH (6-10) was studied by incubating ISP-2 agar plates seeded with 100 μL of strain SACC-152 spore suspension. All the plates were incubated for 7 days at 28°C. Agar plugs from SACC-152 strain grown on different medium components and culture conditions was tested against *S. aureus* by agar plug method [22]. After 24 hours of incubation at 37°C, the zone of inhibition was measured and expressed in millimeter in diameter.

**Results**

Characterization and taxonomy of Streptomyces strain SACC-152

*Streptomyces* strain SACC-152 is Gram-positive and aerobic. The morphological observation of the 15-day-old culture grown on ISP-2 medium revealed the abundant growth of both aerial and vegetative hyphae formed powdery type of colonies which was well developed and not fragmented. Good growth was observed on ISP-1, ISP-2, ISP-3, ISP-5 and ISP-7 agar medium after 7-14 days of incubation at 28°C, whereas it grew poorly on ISP-6 medium. But the strain SACC-152 was unable to produce both reverse side and soluble pigment. Growth on ISP2 agar, the aerial and substrate mycelium were observed under bright field microscope as light gray and pale gray in color, respectively. Based on the cultural and micromorphology, the strain SACC-152 was tentative as *Streptomyces*.

Phylogenetic and genomic analyses

The PCR amplification of the 16S rRNA gene of strain SACC-152 yielded 1489bp sequence. The 16S rRNA sequencing and its BLAST analysis have confirmed that the strain SACC-152 was showed 99.93% similarity with the sequence of *Streptomyces maritimus*. The 16S rRNA gene sequence of strain SACC-152 was deposited in the GenBank database with the accession number MN121692. Phylogenetic tree was constructed based on the 16S rRNA gene sequences showed that strain SACC-152 (Figure 1) formed a distinct clade with type strains *Streptomyces maritimus* BD26T, *Streptomyces levis* VLK-B, *Streptomyces enissocaesilis* A5 with a bootstrap value of 67%, indicating the high confidence level of the association. The 16S rRNA gene sequence of strain SACC-152 has exhibited highest similarity with that of *Streptomyces albidoflavus* DSM 40455T (99.7%), *Streptomyces hydrogenans* NBRC 13475T (99.7%), *Streptomyces somaliensis* NBRC 12916T (99.7%), followed by *Streptomyces koyangensis* VK-A60T (99.5%) and *Streptomyces daghestanicus* NRRL B-5418T (99.5%).

Figure 1. Neighbor-joining phylogenetic tree based on 16SrRNA gene sequences comparing strain SACC-152 to Streptomyces species and other genera in the family Streptomycetaceae. Nocardia sp. SYPA7134 was used as the out-group. The numbers on the branches indicate the percentage bootstrap values of 1000 replicates; only values >50% are indicated.

Antimicrobial activity

The ethyl acetate extract of strain SACC-152 showed broad spectrum activity against both Gram positive and Gram negative bacterial pathogens. Maximum inhibition was observed against *S. aureus* (19.93 ± 0.64 mm) followed by *E. coli* (18.83 ± 0.44 mm) and *S. paratyphi* (18.17 ± 0.60) whereas it showed least activity against *P. vermicola* (9.83 ± 0.60 mm). Moderate activity was observed against fungal strains *C. albicans* (16.83 ± 0.44 mm) and *C. neoformans* (17.50 ± 0.76 mm) (Figure 2).

Figure 2. Antimicrobial activity of ethyl acetate extract of *Streptomyces maritimus* SACC-152.
**Anti-tubercular activity**

In LRP assay, ethyl acetate extract of strain SACC-152 showed more than 80% inhibition against all three *M. tuberculosis* strains tested (Figure 3). It showed maximum level of activity against drug sensitive strain *M. tuberculosis* (91.27 ± 0.36%) followed by *M. tuberculosis* MDR strain (85.3 ± 1.32%) and *M. tuberculosis* H37Rv (84.77 ± 0.45%) at 2 mg/ml concentration.

**Antioxidant activity**

The results of DPPH free radical scavenging assay revealed that the ethyl acetate extract is concentration depended. It showed maximum of 61.25 ± 0.38% DPPH free radical scavenging activity at 500 µg/mL concentration (Figure 4).

**Anticancer activity**

The ethyl acetate extract of SACC-152 at the highest concentration (1000 µg/mL) showed significant cell death (P < 0.05) against all three cancer cell lines compared to the control. Among the panel of cancer cell lines tested, the extract showed maximum activity against the colon cancer cell line HT-29 with the IC50 value of 9.68 µg/mL (Table 2 and Figure 5).

**Mosquito larvicidal activity**

In larvicidal assay, the ethyl acetate extract of SACC-152 was found to exhibit highest mortality against the larvae of *A.aegypti* (LC50=953.6 µg/mL, r2=0.61) followed by the larvae of *C. quinquefasciatus* (LC50=2124.5 µg/mL, r2=0.98) (Table 3).
Optimization of physiochemical parameters

The secondary metabolites of the strain SACC-152 showed promising antimicrobial activity against S. aureus tested when cultured in ISP-2 medium amended with starch (15.83 ± 0.73 mm) and glucose (15.00 ± 0.58 mm) followed by fructose (10.00 ± 0.29 mm) and xylose (9.17 ± 0.73 mm) as carbon source (Table 1). Out of five nitrogen and five mineral sources tested, malt extract (16.00 ± 0.00 mm) and K2HPO4 (14.17 ± 0.60 mm) served as suitable combination in ISP-2 medium for the production of antimicrobial metabolites by the strain SACC-152. Other parameters such as medium pH at 7 (17.53 ± 0.01 mm), 50% saline condition (15.50 ± 0.50 mm) and incubation temperature at 30°C temperature (17.50 ± 0.29 mm) were found to influence the antimicrobial metabolite production against S. aureus (Table 1).

Detection of secondary metabolite biosynthetic genes

PCR using, the DNA isolated from the Streptomyces maritimus SACC-152 was found to contain secondary metabolite biosynthetic genes viz., PKS-I (700 bp), NRPS (500 bp) and Halo (500 bp) primers (Figure 6). This indicates the presence of KS domains of polyketide synthase type I, the adenylation domains of non-ribosomal peptide synthase gene and the enzyme halogenase (Halo) gene belonging to halogenation pathway.

![Figure 6. PCR based amplification and detection of Secondary metabolite genes identification (Lane 1- 100 bp Ladder, Lane 2- PKS1 and Lane 3- NRPS) in Streptomyces maritimus SACC 152.](image)

Optimization of physiochemical parameters

The secondary metabolites of the strain SACC-152 showed promising antimicrobial activity against S. aureus tested when cultured in ISP-2 medium amended with starch (15.83 ± 0.73 mm) and glucose (15.00 ± 0.58 mm) followed by fructose (10.00 ± 0.29 mm) and xylose (9.17 ± 0.73 mm) as carbon source (Table 1). Out of five nitrogen and five mineral sources tested, malt extract (16.00 ± 0.00 mm) and K2HPO4 (14.17 ± 0.60 mm) served as suitable combination in ISP-2 medium for the production of antimicrobial metabolites by the strain SACC-152. Other parameters such as medium pH at 7 (17.53 ± 0.01 mm), 50% saline condition (15.50 ± 0.50 mm) and incubation temperature at 30°C temperature (17.50 ± 0.29 mm) were found to influence the antimicrobial metabolite production against S. aureus (Table 1).

Discussion

Actinobacteria from understudied marine ecosystems are reported as promising source for novel secondary metabolites [8,23]. However, there are only few studies on the marine actinobacteria in various parts of the A & N Islands [12,24]. In the present study, a marine actinobacterial strain isolated from A & N Islands, identified as S. maritimusSACC-152 showed novel multi potential biological properties. There are not many reports documented that a single genus of actinobacteria isolated from A and N Islands having multi potential activity but a very few reports shows that actinobacterial strains having antimicrobial [11,25]; antifungal [24] activity and enzyme production [12]. The strain SACC-152 showed optimum growth and secondary metabolite production at 30°C on ISP2 media. And our results were similar to earlier reports where ISP2 was found as a suitable media for production of secondary metabolites from Streptomyces sp. [26].

The Streptomyces strain SACC-152 showed broad range of antimicrobial activity against various pathogens (Figure 2). Previous reports are also showing that the ethyl acetate extract of the same species of genus Streptomyces maritimus isolated from soil sample having promising antimicrobial activity against various bacterial and fungal pathogens [27]. Similarly, Yuan et al. [20] and Dholakiya et al. [28] reported that Streptomyces strain exhibited strong antimicrobial activity against various pathogens. Also, Xu et al. 2017 documented that the actinobacteria isolated from marine ecosystem showed broad range of antimicrobial activity against multiple gram positive and gram negative pathogens, in which Streptomyces strain showed maximum inhibition against S. aureus.

Due to the emerging resistance strains to current anti-TB drugs, our study also focused on determining strain SACC-152 as a potent anti-TB agent [29,30]. In the present study, the Streptomyces strain SACC-152 was found to exhibit more than 80% inhibition against all three MTB strains tested by LRP assay (Figure 3). Similarly, Chen et al. [31] reported that marine derived Streptomyces showed anti-TB activity against H37Rv. In addition, other actinobacterial genera Amycolatopsis sp., also showed anti-mycobacterial activity isolated from rhizosphere of Cynodondactylon [32]. Our previous studies also clearly confirmed that Streptomyces strains isolated from marine ecosystem showed promising source for anti-TB activity [13].

Antioxidants are used for protection against oxidative stress in cell by blocking the oxidative damage caused by the reactive oxygen species [33]. In this present study, the antioxidant activity of ethyl acetate extract of strain SACC-152 was investigated by assessing its radical scavenging abilities on DPPH radical and the results were proved that the potential strain SACC-152 may possess the ability to donate hydrogen atom to the DPPH radical. It showed up to 61.25 ± 0.38% DPPH radical scavenging activity at 500 µg/ml concentration.

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of ethyl acetate extract of strain SACC-152 (Figure 5). Similarly Karthik et al. [34] and Lee et al. [35] used DPPH assay to study antioxidant activity of Streptomyces strains and results were similar to our finding. Also, ethyl acetate extract of Streptomyces sp. isolated from marine ecosystem showed significant inhibition of DPPH activity [36].

Cancer is a common cause of mortality in the world population and second leading disease of cause of death [37]. Furthermore, the incidence of the development of resistance to chemotherapy has become a major health problem [38]. Thus, there is an urgent requirement for alternative anticancer agents which may overcome the failure of chemotherapy. Thus, the present study investigated the role of ethyl acetate extract of strain SACC-152 and showed maximum inhibitory effect against HT-29 and Hela cancer cell lines followed by MCF-7 breast cancer cell lines (Table 2). Similarly, Tan et al. [36] reported that Streptomyces sp. exhibited significant growth inhibition against various human cancer cell lines including HT29, Hela and MCF-7. Similarly in another study, the anticancer effect of actinobacteria isolated from marine environment showed higher and mild inhibition against human cervical and colon cancer cell lines, respectively [39].

Mosquito borne diseases are the major transmissible diseases and its control is one of the most serious concerns in many countries including India [40]. Important bioactive metabolites from actinobacteria that interferes with the digestion systems of larvae were used as larvicidals [41]. Hence in the present study results showed that the ethyl acetate extract of strain SACC-152 showed a maximum mortality against A. aegypti followed by the larvae of C. quinquefasciatus. Similarly, others finding also suggest that Streptomyces sp. showed larvicial activity against Culex [42], Aedes [43] and Anopheles [44]. Furthermore, marine actinobacteria, Nocardiaalba also showed strong larvicial activity against larvae of A. egypti, C. quinquefasciatus and A. stephensi [45].

Biologically active natural metabolites are identified through encoded by various secondary metabolite genes, in which PKS-I, PKS-II and NRPS are crucial for the biosynthesis of the major active metabolites [46]. Therefore, in our study, the multifunctional Streptomyces maritimus SACC-152, we attempted to PCR amplify the various biosynthetic genes, in which three secondary metabolite genes such as PKS-I, NRPS and Halo genes were detected (Figure 6). PKS and NRPS are responsible for the synthesis of bioactive polyketides and peptides, while halogenase is an enzyme that has been reported to be derived from halo, and all three genes are all renowned for playing vital roles in biological activities [20]. The present study also correlated the production of bioactive compounds to the presence of biosynthetic genes in S. maritimusSACC-152. Similarly, Zothanpuia et al. [47] reported that three biosynthetic genes, PKS-II, NRPS and phzE were detected in many actinobacterial strains that are responsible for antimicrobial activity.

The growth and nutritional parameters of actinobacterial strain highly influences the secondary metabolites production [48]. Our study reviled that ISP-2 medium served as the best culture medium for the production of bioactive metabolites by the potential strain SACC-152. Synthesis of antibiotics depends on the type of nutrients amended in the culture media. Utilization of different carbon, nitrogen and mineral sources in the growth medium by actinobacterial strain has critical for secondary metabolite production [49].

Conclusion

The present study demonstrated that ethyl acetate extract of S. maritimus SACC-152 exhibiting strong antimicrobial, anti-TB, antioxidant, anticancer and larvicial activities. The biosynthetic genes PKS-I, NRPS and Halo present in the strain SACC-152 could be responsible for its diverse biological activities. In conclusion, a novel S. maritimus strain SACC-152, isolated from the unexplored mangrove sediment of Andaman and Nicobar Islands, India showed promising multifunctional biological activities. However, fractionation and further characterization of such active metabolites from this multifunctional strain S. maritimus SACC-152 are needed for their optimum utilization toward multifunctional properties. These finding suggest that S. maritimusSACC-152 could be a novel and unique multifunctional strain with highly versatile applications and hence it merits further development towards wide array of applications. Further study is currently under progress in this regard in our laboratory.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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