Anti-MRSA and Anti-VRE Activity of Compounds Extracted from Marine Streptomyces sp. VITBRK3

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The aim of the present study was to assess the anti-MRSA and anti-VRE activity of ethyl acetate (EA) extract of Streptomyces sp. VITBRK3 and identification of bioactive compounds. The EA extract obtained from the isolate was screened for anti-MRSA and anti-VRE activity by well diffusion method against six drug resistant strains. The isolate was identified by molecular taxonomic characterization. The EA extract was subjected to HPLC-DAD analysis for the identification of antibiotics. The EA extract of VITBRK3 showed the inhibition zone of 19 mm against Staphylococcus aureus 21 mm against Enterococcus faecalis. Similarly 20 and 18 mm zone of inhibition was observed against Entrococcus faecium-BM4107 and Entrococcus faecium-Bm4147/ (Van A) respectively. The EA extract also showed 16 mm zone of inhibition against MRSA strain, Staphylococcus aureus U2A 2150 and 20 mm against the clinical isolate Staphylococcus aureus. HPLC-DAD analysis showed the presence of strepturidine, anthranilic acid, indole-3-acetic acid and germicidin B compounds. The observed activity of Streptomyces sp. VITBRK3 may be due to the presence of strepturidine, anthranilic acid, indole-3-acetic acid and germicidin B compounds in the EA extract. The results of this study suggest that secondary metabolites produced by Streptomyces sp. VITBRK3 could be used against MRSA and VRE strains.

Key words: Methicillin resistant Staphylococcus aureus (MRSA), Vancomycin resistant Enterococci (VRE), Streptomyces sp. VITBRK3.

Gram-positive cocci are the most important cause of both community-acquired and nosocomial infections. Antimicrobial resistance is an increasing problem. Gram-positive microorganisms become resistant to antimicrobial agents, intrinsic resistance is acquired either by mutation or by the acquisition of new DNA, most often by resistance plasmid (R plasmid) acquisition. Evolution of resistance occurs through dissemination, transposition and plasmid exchange. The appearance of resistance to multiple antibiotics in nosocomial isolates of streptococci and staphylococci is increasing. Studies showing in vitro transfer of extra chromosomal DNA and demonstrating specific homologies between resistance genes from diverse species support the concept of either direct gene exchange or a common ancestral origin for the resistance. Enterococci, along with approximately 450 other taxa of anaerobic and aerobic bacteria, are part of the normal intestinal flora. Before the identification of multiple-antibiotic-resistant strains in the late 1970s, enterococci were considered relatively innocuous organisms. Over the past two decades, enterococci have been identified as the agents of nosocomial infection with increasing frequency, paralleling the accretion of antimicrobial resistance to most currently approved agents. As a result, enterococci have emerged as one of the leading clinical challenges for physicians when identified as the
cause of serious or life threatening infections.

The emergence of vancomycin-resistant enterococci (VRE) is a global threat. Enterococcal acquisition of vancomycin resistance leaves few options for disease management. Conjugation experiments have confirmed vancomycin resistance gene transfer from enterococci to Staphylococcus aureus. The limited successes over the past decade of prevention and control strategies for containing vancomycin resistance as well as methicillin resistance in staphylococci forced researchers to look for better control measures. Clinical significance of enterococci is complex; although they are commensal microorganisms they are opportunistic pathogens in elderly, immune-compromised and patients who are hospitalized for prolonged period or those who undergo invasive procedures. Enterococci are the second most common cause of UTI and wound infections in worldwide and third leading cause of bacterimia. Enterococci cause an estimated 5 to 15% of bacterial endocarditis. However, enterococcal bacteremia is much more common than enterococcal endocarditis. Enterococcal prostatitis and perinephric abscess have also been reported.

In the late 1999, quinupristin/dalfopristin became available for the treatment of vancomycin-resistant E. faecium infections. Most Enterococcus faecalis strains and many Enterococcus spp. other than E. faecium are intrinsically resistant to quinupristin/dalfopristin. In 2000, a study, from Linezolid, the first of a new class of antimicrobial agents, the oxazolidinones can be administered orally or intravenously, and is active against E. faecium, E. faecalis, E. casseliflavus and E. gallinarum. Oritavancin, tigecycline and daptomycin, are currently being evaluated in clinical trials in spite of which there is a continued need for the development of new antimicrobial agents. MRSA and VRE, the so called superbugs are largely responsible for the increase in numbers of hospital acquired infections.

The rate of discovery of new compounds from existing genera obtained from terrestrial sources has decreased, while the rate of re-isolation of known compounds has increased. The rise in the number of drug-resistant pathogens and the limited success of combinatorial chemistry in providing new agents is proof that the discovery of new anti-infective agents is indispensable. Therefore the exploration of new groups of actinomycetes from unexplored and unique habitats is pursued for exploring therapeutic agents. The streptomycetes, producers of more than half of the 10000 documented bioactive compounds. Streptomycetes are Gram-positive aerobic members of the order Actinomycetales within the class Actinobacteria and have a DNA G-C content of 69±78 mol %.

Streptomycetes produce an extensive branching substrate and aerial mycelium. The substrate hyphae are approximately 0.5-1.0 µm in diameter and often lack cross-walls during the vegetative phase. The diversity of actinomycetes secondary metabolites are one of the most significant medical discoveries. Structurally and functionally diverse bioactive compounds have been isolated as antibiotics with antimicrobial, antiviral, and antitumor activities. Studies of Streptomyces led to the discovery of many antibiotics including, streptomycin, neomycin, tetracycline and chloramphenicol. In the present study we report the Streptomyces isolate capable producing antibacterial antibiotics strepturidine, anthranilic acid, indole-3-acetic acid and germicidin compounds against drug resistant MRSA and VRE strains.

**MATERIALS AND METHODS**

**Sample collection and isolation of actinomycetes**

The isolate was collected from Marakkanam, (Latitude [N] 128200; Longitude [E] 798950) sediment samples at a depth of 400 cm in the southeast coastal region of the Bay of Bengal, India. The sediment samples were dried in laminar air flow for 8-12 h and then kept at 48°C for 10-30 days in a sterile Petri dish and these preheated samples were used for the isolation of actinomycetes. The International Streptomyces Project (ISP) No. 1 media, Starch casein agar and Bennett’s agar with 25% sea water, 25% soil extract was used for the isolation of actinomycetes. The International Streptomyces Project (ISP) No. 1 media, Starch casein agar and Bennett’s agar with 25% sea water, 25% soil extract was used for the isolation of actinomycetes. The International Streptomyces Project (ISP) No. 1 media, Starch casein agar and Bennett’s agar with 25% sea water, 25% soil extract was used for the isolation of actinomycetes. The International Streptomyces Project (ISP) No. 1 media, Starch casein agar and Bennett’s agar with 25% sea water, 25% soil extract was used for the isolation of actinomycetes. The International Streptomyces Project (ISP) No. 1 media, Starch casein agar and Bennett’s agar with 25% sea water, 25% soil extract was used for the isolation of actinomycetes.
Screening for antibiotic production

Antibacterial activity of the potential isolate was studied by agar plate diffusion assay, 10µl of the culture filtrate was applied to filter disks (6mm in diameter). Inhibition zones were expressed as diameters and measured after incubation at 37°C for 24h. Four ATCC strains, two drug resistant pathogens and a clinical isolate were used for the screening. *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Enterococcus faecium* BM4107 were obtained from ATCC culture collection centre. The drug resistant strains *Enterococcus faecium* (BM4147-Van A), *Staphylococcus aureus* (U2A 2150- MRSA) were obtained from Institut Pasteur; Paris and the strain *Staphylococcus aureus* (MRSA) was a clinical isolate. The drug resistance pattern of the clinical isolate was confirmed by studying its sensitivity against selected group of antibiotics. Influence of different culture media on the antibacterial potential of the isolate was studied by cylinder plug method using ISP 1 and sea water, Marine agar, Actinomycetes isolation agar, Starch casein agar (Himedia, Mumbai, India).

Optimization of nutritional and culturing conditions

To determine the optimal nutritional and culturing conditions and to identify the suitable media for growth, the isolate was inoculated in different culture media (SCA, ISP 2, ISP 3, ISP 4, ISP 5, ISP 6, ISP 7, modified Bennett’s agar, sucrose/nitrate agar, and nutrient agar) and the growth was investigated. The effect of cultural conditions like different incubation temperatures (15, 25, 37 and 50 °C), different pH (5.0, 6.0, 7.4 and 9.0) and NaCl concentrations (2, 5, 7, 9 and 12%) on the growth of the isolate was also studied. The carbon and nitrogen sources required were also studied by inoculating the isolates into mineral salt agar with different sugars substituted to starch (D-glucose, sucrose, starch, Dxylose, D-galactose, maltose, L-arabinose, fructose, lactose, and glycerol), organic nitrogen sources like peptone, yeast extract, casein and inorganic sources like ammonium sulphate, ammonium nitrate and urea. The concentrations of carbon sources and carbon utilization tests were done as described earlier [20]. After incubation the dry weight of the mycelium was measured and correlated with the growth of the isolate. Based on the growth of the isolate in different media the cultural conditions were optimized.

Taxonomy

The morphological, cultural, physiological and biochemical characterization of the isolate was carried out as described in ISP [14]. The morphology of the spore bearing hyphae with entire spore chain along with substrate and aerial mycelium was examined under light microscope as well as scanning electron microscope (Hitachi, S-3400N). DNA was isolated from the isolate by using HiPurA bacterial DNA isolation and purification kit (Himedia, India) and amplified by PCR using a master mix kit, Medoxmix (Medox, India) as per user manual. The primers and the PCR conditions were adapted from previous reports21. The design of the sequencing primers and the methodology for the sequencing were adapted from previous reports22,23. The 16S rDNA sequence of the isolate was determined on both strands using dideoxy chain termination method. The similarity and homology of the sequence was compared with the existing sequences available in the data bank using BLAST search. The DNA sequences were aligned and phylogenetic tree was constructed by neighbor joining method. The phylogenetic tree based on Maximum-parsimony method was also carried out using MEGA 4.0.2 -biologist-centric software for evolutionary analysis of DNA and protein sequences24. For G + C content determination DNA was isolated by the method of Marmur24 and the G + C content was determined using the thermal denaturation method of Marmur and Doty25. Based on phylogeny and molecular taxonomy the isolate was identified.

Extraction of active compounds

Spores at 10^7/ml of the isolate were used to inoculate 1000ml Erlenmeyer flasks containing 200 ml of ISP 1 broth supplemented with 1% (w/v) of glucose and magnesium. After incubation at 30°C for 24 h in an orbital incubator with shaking at 200 rpm, this pre-culture was used to inoculate (5% v/v) a total volume of 15 L culture medium having the same composition as the pre-culture. After six days of incubation the culture broth was filtered to separate mycelium and supernatant, which were treated separately as follows: the mycelium was lyophilized, extracted with acetone and the solution concentrated on a rotary evaporator. The supernatant was extracted twice.
with equal volume of ethyl acetate and the combined organic layers were evaporated. The brown gum (EA extract) obtained from the extract was dissolved in 100 ml methanol/cyclohexane (v/v). The EA extract was screened for antimicrobial activity.

**Assay of anti MRSA activity**

The MRSA strains used in this study was sub cultured overnight at 37°C in Mueller Hinton Broth and adjusted to obtain turbidity comparable to 0.5 McFarland standards (1.0 x 10^8 CFU/ml) before MIC tests. The EA extract was assayed for the anti-MRSA activity using broth micro dilution method in sterile glass test tubes. Prior the MIC test, each EA extract was diluted in sterile ultra pure water. The EA extract was then diluted by doubling dilutions at concentrations ranging from 32-1024 mg/ml. The tested strains were added to sterile Mueller Hinton broth into the test tubes before the EA suspension prepared as described above were added. The bacterial suspension without the EA was used as positive control and extracts in sterile broth were used as negative control. The MIC values were taken as the lowest concentration that showed no turbidity after 24 hours of incubation at 37 °C. The turbidity was interpreted as visible growth of the microorganisms. The minimum bactericidal concentration (MBC) was determined by subculture of the tube showing no apparent growth in a sterile Muller Hinton agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value.

**Characterization of secondary metabolite**

The crude extract was dissolved in ethyl acetate extract and it was loaded on silica gel column chromatography and eluted with chloroform, methanol and acetone as the solvent system. The separation of fractions were visualized by the thin layer chromatography with different ratio of chloroform: methanol: acetone (6:0.5:1.5) as a solvent system. The separated fractions in TLC were further confirmed by high performance liquid chromatography (HPLC) using reverse phase column with acetonitrile and water. In phase A, 100% of acetonitrile and phase B, 10% of acetonitrile, 90% of water and 0.1% of formic acid were used. The separation pattern of fractions in HPLC was also confirmed by GLC. The ethyl acetate extract was analyzed using GC, equipped with HP-5 capillary column. The GC oven was held at 70°C for 1 min and then increased to 250°C at 10°C per min for 5 min. Sample (1µl) was injected with the injection temperature of 220°C. Helium was used as the carrier gas with a flow of 30 ml/min.

**HPLC-DAD analysis of the EA extract**

The fractions of the EA extract was subjected to HPLC-DAD screening (University of Tubingen, Germany). The HPLC-DAD chromatographic system consisted of an HP 1090M liquid chromatograph equipped with a diode-array detector and HP Kayak XM 600 ChemStation (Agilent Technologies). Multiple wavelength monitoring was performed at 210, 230, 260, 280, 310, 360, 435 and 500 nm. The UV-visible spectrum was measured from 200 to 600 nm. Sample (5 µl) was injected onto an HPLC column (125 X 4.6 mm, guard column 20 · 4.6 mm) filled with Nucleosil-100 C-18 (5 m). Separation was performed by a linear gradient using 0.1% orthophosphoric acid as solvent A and acetonitrile as solvent B. The gradient was from 0 to 100% solvent B in 15 min at a flow rate of 2 ml/min. Limitations of the method are as follows, polar compounds cannot be separated because of non-retention behaviour on the reversed-phase column. These compounds show front elution. Only compounds having a UV active chromophore can be detected. Sugar type compounds (e.g. aminoglycosides) or peptides containing aliphatic amino acids cannot be detected by this method.

**RESULTS**

**Isolation and screening of actinomycetes**

A total of hundred and twenty actinomycetes were isolated from the sediment samples. The antibiotic used in the isolation media act as selective agents. White powdery and dried colonies suspected to be actinomycetes were sub cultured on ISP-1 agar and sea water collected at the site. Microscopic identification was done to confirm the isolates were actinomycetes before screening for antibacterial activity. Twelve actinomycetes isolates showed antagonistic activity against MRSA and VRE strains. The isolate VITBRK3 was subjected to further characterization.
Table 1. Cultural characters of *Streptomyces* sp. VITBRK3 on different culture media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth</th>
<th>Spore</th>
<th>Reverse colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch Casein agar</td>
<td>Good</td>
<td>White</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>ISP medium 1</td>
<td>Good</td>
<td>Grey white</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>ISP medium 2</td>
<td>Good</td>
<td>White</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>ISP medium 3</td>
<td>Moderate</td>
<td>Grey white</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>ISP medium 4</td>
<td>Moderate</td>
<td>Grey white</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>ISP medium 5</td>
<td>Good</td>
<td>Grey white</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>ISP medium 6</td>
<td>Moderate</td>
<td>White</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>ISP medium 7</td>
<td>Good</td>
<td>Grey</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>ISP medium 1 + Sea water</td>
<td>Abundant</td>
<td>Grey white</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Modified Bennett’s agar</td>
<td>Moderate</td>
<td>White</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>Moderate</td>
<td>Grey</td>
<td>No colour</td>
</tr>
<tr>
<td>Marine agar</td>
<td>Moderate</td>
<td>Grey</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Actinomycetes isolation agar</td>
<td>Abundant</td>
<td>Grey</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Muller-Hinton agar</td>
<td>Good</td>
<td>Grey white</td>
<td>No colour</td>
</tr>
<tr>
<td>Sabouroud Dextrose agar</td>
<td>Poor</td>
<td>White</td>
<td>No colour</td>
</tr>
<tr>
<td>Potato Dextrose agar</td>
<td>Poor</td>
<td>White</td>
<td>No colour</td>
</tr>
</tbody>
</table>

Table 2. Antibiotics sensitivity pattern of drug resistant clinical isolate *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration(µm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>30</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>30</td>
<td>R</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>30</td>
<td>R</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>R</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>R</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>R</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30</td>
<td>S</td>
</tr>
<tr>
<td>Linezolid</td>
<td>30</td>
<td>S</td>
</tr>
</tbody>
</table>

Interpretation of zone of inhibition according to CLSI guidelines (2007)
R- resistant; I- intermediate susceptibility; S-susceptible.

Table 3. Antagonistic activity of VITBRK3 against drug resistant MRSA and VRE strains

<table>
<thead>
<tr>
<th>Microbial strains</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 29213</td>
<td>19</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>19</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>21</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> BM4107</td>
<td>20</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> BM4147-Van A</td>
<td>18</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> U2A 2150- MRSA</td>
<td>16</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (MRSA)</td>
<td>20</td>
</tr>
</tbody>
</table>

Phenotypic characterization

VITBRK3 was grown on oat meal agar medium (ISP 3), Yeast extract malt extract agar (ISP 2), Inorganic salt starch agar (ISP 4). The colour of the mature sporulating aerial mycelium was determined to be greyish white. Reverse side pigments and melanoid pigments were absent. Spira- Spirales spore chain morphology was determined by optical microscope at 1000X magnification and the spore surface morphology was found to be smooth by scanning electron microscopic (SEM) analysis (Fig 1).

Physiological and biochemical characterizations

The ability to utilize various carbon compounds as source of energy was studied. VITBRK3 assimilated arabinose, xylose, inositol, mannitol, fructose, sucrose and raffinose, however the strain did not utilize rhamnose. The isolate utilized 0.1% of L-Asparagine, L-Phenylalanine, L-Histidine and L-Hydroxyproline as nitrogen source. The actinomycetes isolate could be considered as a halophilic as it showed good growth, sporulation and antimicrobial activity when cultured using 2% to 12% NaCl (w/v). The isolate showed maximum growth after 14 days when cultivated on ISP medium with 25% sea water, at 28°C and pH 7.4. The cultural and morphological characteristics of VITBRK3 in different media are given (Table 1). The isolate showed ²-haemolysis on blood agar containing 5% sheep blood.
Taxonomy

The isolated strain is a gram positive, non-acid fast, nonmotile, aerobic actinomycete. The aerial mycelium is branched, white to grey in color and the substrate mycelium is branched. Comparison of biochemical and cultural characteristics of the isolate with those of known actinomycetes species described in Bergey’s manual of Systematic Bacteriology strongly suggests that the strain belongs to the genus *Streptomyces*. The partial sequencing of 16 S rRNA gene of the strain on both directions yielded 16 S rDNA nucleotide sequences with 646 bases. The 16 S rDNA sequence of the strain was deposited in the GenBank (NCBI, USA) under the accession number GQ848482. The BLAST search of 16 S rDNA sequence of the isolate showed 93 % similarity with the strain *Streptomyces* sp. 35005 (GU263853) phylogenetic tree was constructed with bootstrap values (Fig. 2). Based on phylogeny and molecular taxonomy the isolate was identified to be belonged to the genus *Streptomyces* and designated as *Streptomyces* sp. VITBRK3.

Antibiotic sensitivity Test

The drug resistance pattern of the clinical isolate (MRSA strain) was tested and observed that it was resistant to all the antibiotics tested erythromycin (30 µg/disc), cefoxitin (5 µg/disc), ampicillin (10 µg/disc), gentamycine (10 µg/disc), ceftriaxone (30 µg/disc), cefazolin (30 µg/disc) and cefotaxime (30 µg/disc). The isolate was sensitive...
to vancomycin (30 µg) and linezolid (30 µg); 18 mm and 23 mm zone of inhibition was observed respectively (Table 2).

**Antibacterial activity**

The EA extract of VITBRK3 showed activity against Gram positive bacteria. An inhibition zone of 19 mm was observed against *Staphylococcus aureus* (ATCC 29213 and ATCC 25923) 21 mm against *Enterococcus faecalis*. The EA extract of VITBRK3 showed 20 mm zone of inhibition against *Enterococcus faecium*-BM4107 and 18 mm zone of inhibition against *Enterococcus faecium*-Bm4147/ (Van A). The EA extract also showed 16 mm zone of inhibition against MRSA strain of *Staphylococcus aureus* U2A 2150 and 20 mm zone of inhibition against the clinical isolate *Staphylococcus aureus* (Table 3).

**Characterization of secondary metabolite**

The thin layer chromatographic separation of ethyl acetate extract showed four distinct bands when eluted with chloroform: methanol: acetone in the ratio of 6: 0.5: 1.5. The GC separation also confirmed the presence four major compounds which supported TLC and HPLC separation pattern (Fig. 3). FTIR analysis showed the presence of functional groups 3455 cm\(^{-1}\) corresponds to O-H stretch (alcohol), 2840 cm\(^{-1}\) corresponds to C-H stretch (alkanes) and 1667 cm\(^{-1}\) C=O stretch (unsaturated aldehydes and ketones); 1513 cm\(^{-1}\) indicates N-O (Nitro compounds; 950 cm\(^{-1}\) O-H bend (carboxylic acids), 979 cm\(^{-1}\) represents alkenes.

**Identification of compounds by HPLC-DAD analysis**

HPLC-DAD matching of the EA extract of *Streptomyces* sp. VITBRK3 was carried out. The peaks of the chromatogram was matched with the reference compound UV-Visible spectrum. The peaks in the chromatogram having the same UV-Visible spectrum and retention time with that of the reference compound was identified and named. In the UV-Visible spectrum peak 1 corresponds to strepturidine, a nucleoside antibiotic (Figure 4 A), peak 2 corresponds to anthranilic acid (Figure 4 B), peak 3 corresponds to indole-3-acetic acid (Figure 4 C) and peak 4 corresponds to germicidin B (Figure 4 D).
Fig. 4 (a) The HPLC-DAD chromatogram of ethyl acetate extract of *Streptomyces* sp. VITBRK3 peak 1 corresponds to strepturidine antibiotic. (b) The HPLC-DAD chromatogram of ethyl acetate extract of *Streptomyces* sp. VITBRK3 peak 2 corresponds to anthranilic acid. (C) The HPLC-DAD chromatogram of ethyl acetate extract of *Streptomyces* sp. VITBRK3 peak 3 corresponds to indole-3-acetic acid. (d) The HPLC-DAD chromatogram of ethyl acetate extract of *Streptomyces* sp. VITBRK3 peak 4 corresponds to germicidin B.

**DISCUSSION**

In this study the isolate *Streptomyces* sp. VITBRK3 showed anti-MRSA and anti-VRE activity and the EA extract of the isolate have been found to contain antibiotics such as strepturidine, anthranilic acid, indole-3-acetic acid and germicidin. The molecular taxonomy and phylogeny of the isolate showed VITBRK3 is belonged to the genus *Streptomyces*. Chromogenicity of the aerial mycelium is considered to be an important character for the grouping and identification of actinomycetes.27 The antibiotic strepturidine was reported to be produced by *Streptomyces albus*.
and has been shown to be active against azotobacteraceae. Four new antitumor pyranones, PM050511, PM050463, PM060054, and PM060431, were reported from the cell extract of the marine-derived Streptomyces albus POR-04-15-05328. Peak 6 corresponds to anthranilic acid and has been shown to possess DPPH free radical scavenging activity29. Peak 9 corresponds to indole-3-acetic acid and it has been shown to be produced by soil Streptomyces CMU-H00930. Peak 11 corresponds to germicidin B and it has been shown to be produced by Streptomyces viridochromogenes NRRL B-155131 which has an inhibitory effect on the germination of its own arthrospores at a concentration as low as 200 pM (40 pg/ml).

Secondary metabolites produced by actinomycetes were reported to be active against drug resistant bacterial pathogens such as MRSA and VRE strains32, 33. Two compounds TLN-05220 and TLN-05223 (echinosporomycin-type antibiotics) extracted from Micromonospora echinospora ssp and Challisensis NRRL 12255 have been shown to possess anti MRSA and VRE activity34. Recently few compounds having anti MRSA and anti VRE activity have been reported from marine actinomycetes. Dichloromethane extracts of 3 isolates (I-400A, B1-T61, M10-77) showed strong inhibitory activity against MRSA (ATCC 43300) and vancomycin-resistant E. faecalis (ATCC 51299)35. A new thiopeptide antibiotic TP-1161 with rare aminoacetone moiety was extracted from Nocardiopsis sp. CNT-373 was reported to be active against MRSA. Merochlorin A, a novel compound isolated and purified from a marine-derived actinomycete strain CNH189 exhibit antagonistic activity against multi-drug resistant MRSA strain36. The results of this study showed that Streptomyces sp. VITBRK3 is potential actinomycetes isolate capable of producing antibacterial compounds strepturidine, anthranilic acid, indole-3-acetic acid and germicidin B capable of acting against drug resistant MRSA and VRE strains.

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