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ORIGINAL ARTICLE

Antibacterial and Anti-HIV Metabolites from Marine *Streptomyces albus* MAB56 Isolated from Andaman and Nicobar Islands, India

Radhakrishnan Manikkam[1] · Sangeetha Murthy[2] · Sivasankar Palaniappan[3] · Manigundan Kaari[1] · Amit Kumar Sahu[4] · Madhukar Said[4] · Vijayalakshmi Ganesan[1] · Sivakumar Kannan[3] · Balagurunathan Ramasamy[2] · Somasundaram Thirugnanasambandan[3] · Syed G. Dastager[4] · Luke Elizabeth Hanna[5] · Vanaja Kumar[1]

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Abstract
Marine-derived actinobacteria have tremendous potential to produce novel metabolites with diverse biological activities. The Andaman coast of India has a lot of microbial diversity, but it is still a relatively unknown ecology for isolating novel actinobacteria with beneficial bioactive compounds. We have isolated 568 actinobacterial strains from mangrove rhizosphere sediments and sponge samples. Crude extracts from 75 distinct strains were produced by agar surface fermentation and extracted using ethyl acetate. In the disc diffusion method, 25 actinobacterial strains showed antimicrobial activity; notably, the strain MAB56 demonstrated promising broad-spectrum activity. Strain MAB56 was identified as *Streptomyces albus* by cultural, microscopic, and molecular methods. Conditions for bioactive metabolites from MAB56 were optimized and produced in a lab-scale fermenter. Three active metabolites (C1, C2, and C3) that showed promising broad-spectrum antimicrobial activity were isolated through HPLC-based purification. Based on the UV, FT-IR, NMR, and LC–MS analysis, the chemical nature of the active compounds was confirmed as 12-methyltetradecanoic acid (C1), palmitic acid (C2), and tridecanoic acid (C3) with molecular formulae $C_{14}H_{28}O_2$, $C_{16}H_{32}O_2$, and $C_{13}H_{26}O_2$, respectively. Interestingly, palmitic acid (C2) also exhibited anti-HIV activity with an IC50 value of < 1 µg/ml. Our findings reveal that the actinobacteria from the Andaman marine ecosystems are promising for isolating anti-infective metabolites.

Keywords Actinobacteria · Andaman Islands · Antibacterial · Anti-HIV · Bioactive metabolites

✉ Radhakrishnan Manikkam
mrkactinos@gmail.com

Extended author information available on the last page of the article

Introduction

Infectious diseases remain one of the primary causes of human illness and death. The continuous rise in the evolution of multidrug resistance among bacterial pathogens has caused the WHO and CDC to express grave worry [1]. The effective prevention and treatment of an expanding variety of illnesses brought by bacteria, parasites, viruses, and fungi are threatened by antimicrobial resistance (AMR).

Members of the phylum Actinobacteria are commercially and ecologically significant prokaryotes. They are the most numerous gram-positive bacterial group with a high content of guanine plus cytosine (G + C) in their genome. It is generally known that there are many different actinobacterial genera that are widely reported from both common and uncommon habitats. They produce numerous bioactive compounds that have a variety of biological activities [2]. About 13,700 of the 33,500 microbial bioactive compounds discovered between 1940 and 2010 are produced by actinobacteria. The development of new, wide-spectrum antibiotics has dramatically decreased over the last 20 years, according to numerous major pharmaceutical corporations. This has prevented new antibiotic classes with novel mechanisms of action from entering the market [3]. In search of Actinobacteria, common terrestrial sources are skipped, and motivated researchers look for possibly novel bioactive compounds in unusual and severe settings, such as the marine environment [4–6].

In the marine environment, actinobacteria have a significant ecological role in recycling and they tend to possess new natural compounds with potential for use in pharmaceuticals [7, 8]. In recent years, marine actinobacteria have produced more new secondary metabolites than their terrestrial counterparts [9]. The actinobacterial genus *Streptomyces* continues to be the primary source for novel natural compounds [10]. Studies on certain marine actinobacterial species like *Salinispora* and *Verrucosispora*, which generate salinosporamide and abyssomycin, respectively [11], revealed that marine actinobacteria also occupy a major position in marine natural product drug discovery. In addition, marine-derived antibiotics from Actinobacteria are more effective against clinical pathogens because the antibiotics from terrestrial microbes are losing their potential [12]. In the current work, actinobacteria isolated from the Andaman marine environment in India were used to evaluate and isolate antibacterial and anti-HIV metabolites.

Materials and Methods

Sample Collection

Mangrove sediments and sponges were collected from the Andaman and Nicobar Islands as well as the Lakshadweep Islands (Fig. 1 and Supplementary Fig. 1). The collected samples were packed in plastic bags and sent to the laboratory immediately. After the moisture content was removed by air drying at ambient temperature for a couple of days, the samples were preserved at 4 °C until further study.

Marine Actinobacteria Isolation

The heat pre-treatment and serial dilution approach was used for the isolation of actinobacteria from the collected marine samples [13]. Briefly, 4 ml of sterile seawater and 1 g

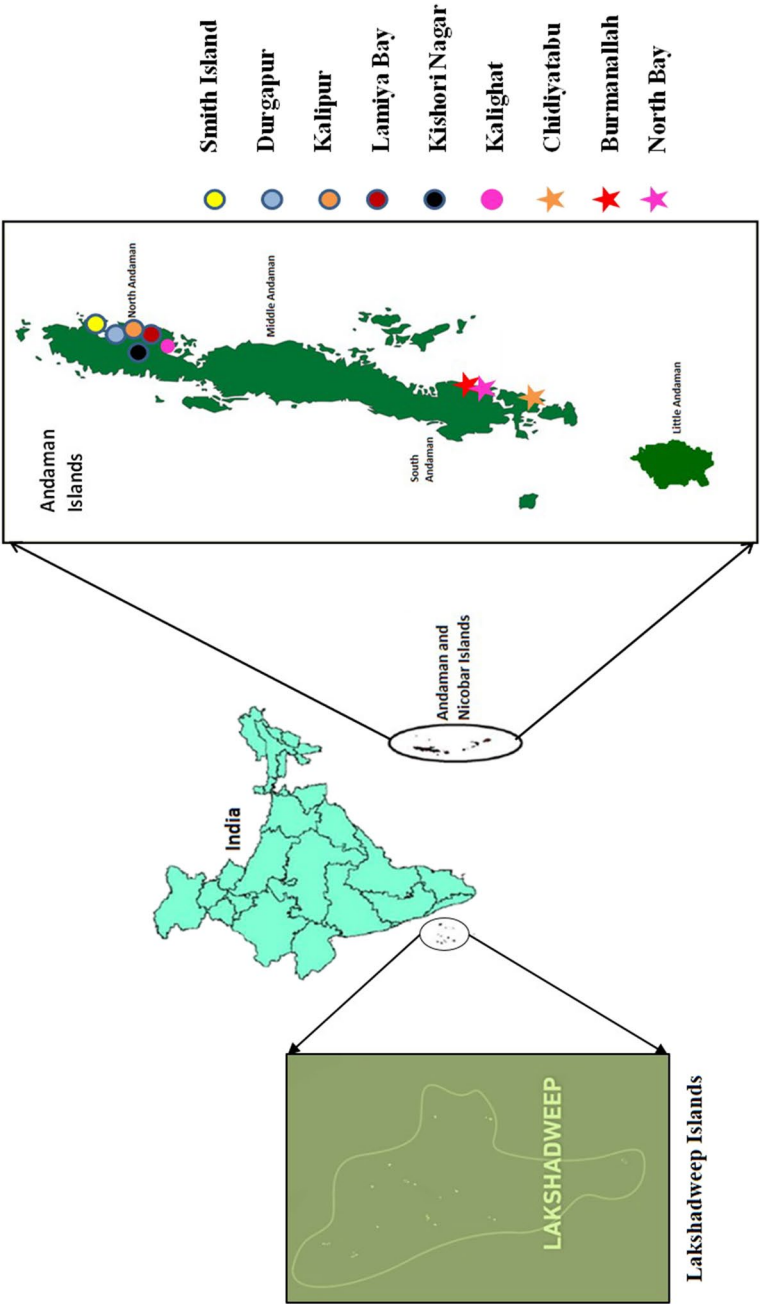

Fig. 1 Locations of marine sediment and sponge samples collected from the Andaman and Nicobar Islands and the Lakshadweep Islands

of sediment sample were combined, and the mixture was heated at 55 °C for 6 min. After a thorough shaking, the samples were further diluted (1:4) in sterile seawater. Spread plating was used to inoculate 50 µl of each dilution onto various isolation media.

The sponge samples were fully homogenized in a sterile mortar using sterile seawater [14]. Crushed samples were tenfold diluted, and 100 µl of 10^{-3}–10^{-5} dilutions was plated in triplicate on 12 different agar plates (Medium M1-M12 (Hi-media)). Cycloheximide (100 µg/ml) (Sigma Aldrich) and nystatin (25 µg/ml) (Sigma Aldrich) were added to all the medium to prevent the development of undesirable bacteria and fungi, respectively. To get observable actinobacterial colonies, the plates were incubated at 28 °C for 2 to 6 weeks. Actinobacterial strains with dissimilar morphologies were chosen, sub-cultured, and stored for future research on yeast extract malt extract (YEME) agar (Hi-media) slants as well as in 20% glycerol broth, respectively, at 4 °C and – 80 °C.

Small Scale Production of Crude Marine Actinobacterial Extracts

The actinobacterial strains were cultured on YEME agar plates for 7–14 days at 28 °C depending on the growth of the isolates for the production of extracellular bioactive compounds. Solid–liquid extraction method was employed to extract the bioactive metabolites from the isolates. After incubation, the mycelium of the strains was discarded and the bioactive compounds-containing agar was sliced and soaked overnight in ethyl acetate (1:3 ratio). The solvent portion, which contains the secreted bioactive compounds, was collected and concentrated using a rotary evaporator [15].

Screening for Antibacterial Activity

According to Radhakrishnan et al. [15], the disc diffusion method was used to test the antibacterial activity of actinobacterial extracts against a set of pathogens including *Staphylococcus aureus* (ATCC 29,213), *Streptococcus pyogenes* (clinical strain), *Escherichia coli* (ATCC 25,922), and *Klebsiella pneumoniae* (ATCC 13,882). On a 6-mm sterile filter paper disc, 100 µg of crude actinobacterial extracts was loaded. The nutrient agar medium was swabbed with test pathogens. Discs loaded with the extracts were then placed on top of the pathogen swabbed nutrient agar plates. After incubation for 24 h at 37 °C, the zone of inhibition of the test pathogens surrounding the disc was determined.

Characterization and Taxonomy of the Potent Strain MAB56

Actinobacterial strain MAB56 that showed wide-spectrum activity against the test pathogens was chosen as the potential candidate for further isolation and characterization of bioactive compounds. Microscopic, cultural, and physiological properties of strain MAB56 were examined by adopting standard methods [16]. Isomers of diaminopimelic acid (DAP) and sugars in whole-cell hydrolysates were examined for chemotaxonomic analysis in accordance with Hasegawa et al. [17].

DNA isolation, PCR amplification, and 16S rRNA gene sequence analysis of MAB56 were carried out by following the protocol described by Gopikrishnan et al. [18]. The taxonomy was confirmed by comparing the nearly complete 16S rRNA gene sequence of strain MAB56 with the non-redundant database of nucleotide sequences deposited at the NCBI web server through the Basic Local Alignment Search Tool (BLAST) program and

the closely related homologs were also identified through phylogenetic analysis. The phylogenetic tree was constructed using the aligned sequences by the neighbor-joining method using Kimura-2-parameter distances in the MEGA 7 software. To determine the support of each clade, bootstrap analysis was performed with 1000 replications. The obtained 16S rRNA sequence was submitted to GenBank to get the accession number.

Production of Bioactive Metabolites

Effects of Solid-State and Submerged Fermentation

Studies were conducted to determine the impact of solid-state and submerged fermentation on the production of bioactive compounds. Spores of strain MAB56 were inoculated onto five YEME agar plates and 100 ml of YEME broth. While YEME broth-containing flasks were incubated in a rotary shaker with 95 RPM at 28 °C for 10 days, YEME agar plates were incubated at 28 °C for 10 days. After incubation, the strain MAB56 from YEME agar plates was evaluated against *S. aureus* (ATCC 29,213) using the agar plug technique [15]. By using the well diffusion technique, the cell-free supernatant from the YEME broth was tested against *S. aureus* (ATCC 29,213) [19]. After 24 h of incubation at 37 °C, the zone of inhibition was measured in diameter.

Solvent Effects on Extraction

To investigate the effect of solvents on extraction, bioactive compounds from strain MAB56 were extracted for 24 h in equal volumes of ethyl acetate and n-hexane. The crude extracts obtained from both solvents were concentrated, quantified, and screened for antimicrobial activity.

Optimization of Bioactive Metabolite Production

One factor at a time (OFAT) approach in the shake flask method was used to investigate the effects of medium components and fermentation conditions on the synthesis of bioactive metabolites from the strain MAB56. The carbon source, nitrogen source, minerals, pH, and temperature are among the variables that are evaluated. Yeast extract (1%), peptone (1%), and NaCl (0.1%) are employed as the basic media components. Exactly, 10% of the inoculum for each variable was inoculated into 100 ml of the reaction medium and incubated for 7 days at 28 °C. After 10 days of fermentation, the amount of crude extracts produced and their antibacterial activity were determined using the disc diffusion technique.

Metabolite Production in Lab Fermenter

Bioactive compounds from the potential strain MAB56 were produced in large quantities using YEME medium in a lab fermenter with a 10-l capacity. Ten percent of the 96-h-old inoculum was inoculated into a 5-l batch of YEME broth in a 10-l fermenter. Adapted incubation conditions include a temperature of 28 °C, 150 rpm of agitation, and 1.5 lpm of aeration. The fermentation process continued for 10 days. After fermentation, cells were separated by centrifugation at 10,000 rpm for 10 min (Eppendorf AG, Hamburg, Germany). Extracellular compounds from the cell-free supernatant were extracted overnight using ethyl acetate at a 1:1 ratio. The crude bioactive compounds present in the organic

extract were concentrated using a rotary evaporator and quantified. The disc diffusion technique was used to assess the antibacterial efficacy of crude extract against *S. aureus* (ATCC 29,213).

Bioassay-Guided Isolation of Active Compounds

Active compounds were partially purified using preparative column chromatography with silica gel (Merck Art. 5735, Kiesselgel 60F 254). The compounds were completely recovered in the first run using a gradient of dichloromethane (DCM) and methanol as the mobile phase, with a steady rise of 5% methanol. A second-level run was conducted using a gradient of pet ether and ethyl acetate as the mobile phase. The polarity of the molecule was changed by gradually adding 5% more ethyl acetate to the mobile phase. In HPLC (Thermo Scientific UltiMate 3000), the C18 column was used for further purification, and the peaks were identified by UV spectroscopy at 220 nm at an elution flow rate of 1 ml/min using a continuous gradient solvent system from 20 to 100% acetonitrile in water. After the peaks were collected individually, concentrated, and categorized, the antibacterial activity was determined against *S. aureus* (ATCC 29,213) and *E. coli* (ATCC 25,922).

Characterization and Structure Elucidation

The spectral properties of purified compounds were studied using nuclear magnetic resonance (NMR, 500 MHz, AVANCE III Bruker spectrometer), liquid chromatography, mass spectrometry (LC–MS, Agilent, USA), and Fourier-transform infrared spectroscopy (FTIR, Perkin Elmer instrument). All the spectral studies and structure elucidation work were carried out at the state-of-the-art analytical facility available at the CSIR-National Chemical Laboratory, Pune, India.

Determination of MIC

The MIC of the three bioactive compounds against *S. aureus* (ATCC 29,213) and *E. coli* (ATCC 25,922) was determined using the microbroth dilution technique in a 96-well plate with slight modifications [20]. Streptomycin (0.1 g/ml) was used as a positive control, while untreated bacterial culture was used as a negative control. The compounds were diluted twice to achieve a final concentration range of 100 to 0.78 g/ml. Pathogenic bacterial strains were grown to a logarithmic phase of 0.1 OD at 600 nm and 100 µl was added to each well of the microtiter plate. Then, each well received an aliquot (100 µl) of the extract at a different concentration and was incubated at 37 °C for 24 h. The optical density (OD) was calculated at 595 nm using a microplate reader (Epoch 2, BioTek Instruments, Agilent Technologies, CA, USA).

Anti-HIV Activity

Adopting the steps described in Vaishnavi et al. [21], the anti-HIV activity of isolated bioactive metabolites was evaluated against the HIV-1 virus. An antiviral drug named nevirapine (0.1 µg/ml) was used as a positive control in this assay. GraphPad Prism was used to get the EC50 value.

Statistical Analysis

One-way analysis of variance (ANOVA) method was used for the statistical analysis of antimicrobial and anti-HIV data. The data are presented as means and standard deviations, and Duncan's multiple range tests (DMRT) were used using SPSS statistical software version 16.0 to identify significant differences between mean values.

Results

Isolation of Marine Actinobacteria

Totally, 568 actinobacterial cultures were isolated from mangrove rhizosphere sediments and sponge samples. Then, 97 morphologically distinct actinobacterial cultures were selected for further characterization. These include 76 cultures from mangrove sediments and 21 cultures from intertidal sponge samples. Upon further sub-culturing, 23 out of 76 sediment-derived actinobacterial cultures failed to grow, whereas the rest of the 74 cultures grew well on ISP2 agar medium supplemented with 50% seawater (Table 1 and Supplementary table 1).

Antimicrobial Screening

An average of 20–25 mg of crude extract per 100 ml of YEME agar was obtained from each strain when extracted using ethyl acetate. In antimicrobial screening, 7.4% of actinobacterial extracts inhibited *S. pyogenes*, 9.62% of the actinobacterial extracts showed activity against *S. aureus* (ATCC 29,213), and 2.22% of the actinobacterial extracts showed activity against *K. pneumonia* (ATCC 13,882). Out of 74 actinobacterial extracts, only the extracts of MAB56 showed 19 ± 0.28 mm zone of inhibition against *E. coli* (ATCC 25,922). Ethyl acetate extracts from four *Streptomyces* and 21 strains of rare actinobacteria were found to be active against at least one bacterial pathogen tested. Extracts from five actinobacterial cultures showed 11–26 mm inhibition against the tested pathogens (Supplementary table 2).

Identification of Potential Actinobacteria MAB56

Strain MAB56, which showed broad-spectrum antibacterial activity against the bacterial pathogens, was selected for further isolation and characterization of active compounds. The strain MAB56 is a mesophilic actinobacterium that forms an extensively branched substrate mycelium and aerial hyphae (Fig. 2a) that differentiate into rectiflexible spore chains (Fig. 2b). The spore surface is smooth. A light gray spore mass was formed on ISP2 agar (Fig. 2c). Reverse side pigment was not formed and soluble pigment was produced on peptone yeast extract iron agar. In addition, melanin pigment was produced on ISP7 medium. The strain grew well when the medium was supplemented with carbon sources such as arabinose, xylose, mannitol, and raffinose (Table 2).

The 16S rRNA gene PCR amplification produced approximately 1400 base pair sequence, which was deposited in NCBI-GenBank under the accession number

Table 1 Sampling location, physio-chemical parameters, and the number of actinobacterial strains isolated from Indian marine ecosystems

S. no	Sampling location	Latitude and longitude	Sample type	Temperature (°C)	pH	Salinity (ppm)	No. of strains
Andaman and Nicobar Islands							
1	Smith Island	Lat: 13.333, Long: 93.066	Sediment-1	29	9	29	45
			Sediment-2	29	7	5	
			Sediment-3	29	9	30	
2	Durgapur	Lat: 13.275, Long: 93.032	Sediment-4	33	7	5	50
			Sediment-5	33	7.5	17	
			Sediment-6	30	9	32	
3	Kalipur	Lat: 13.223, Long: 93.044	Sediment-7	32	8.5	30	47
			Sediment-8	30	9	30	
4	Lamiya Bay	Lat: 13.221, Long: 93.042	Sediment-9	29.5	8	26	52
			Sediment-10	29	7.5	18	
5	Kishori Nagar	Lat: 13.210, Long: 92.853	Sediment-11	29.5	7.5	2	43
6	Kalighat Mangroves	Lat: 13.123, Long: 92.946	Sediment-12	28	7.3	4.5	60
7	Chidiyatapu	Lat: 11.481, Long: 92.709	Sediment-13	30	8	30	48
8	Burmanallah	Lat: 11.554, Long: 92.731	Sediment-14	30	7.5	30	132
			Sediment-15	28	9	17	
			Sediment-16	29	8	30	
			Sediment-17	29	8.5	10	
9	North Bay		Sponge (1–13) (12 deep-sea sponges and 1 intertidal sponge)	30	-	-	56
Lakshadweep Island							
10	Lakshadweep		Sponge (14–17)	28	-	-	35
Total number of actinobacterial strains isolated							568

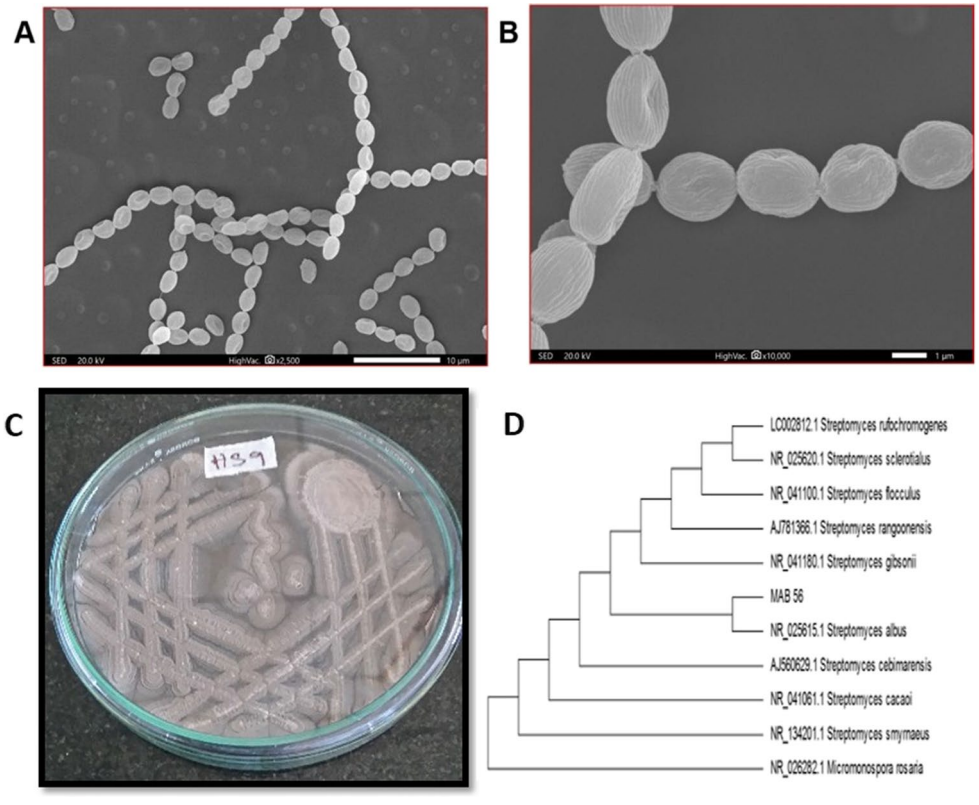

Fig. 2 Taxonomy of potential actinobacteria MAB56 (**a**). Spore chain morphology (**b**). Spore surface morphology (**c**). Cultural morphology (**d**). Phylogenetic placement of MAB56 with its closest phylogenetic members

Table 2 Cultural, morphological, and biochemical characteristics of the strain MAB56 and the closely related *S. albus*

Characters	MAB56	*Streptomyces albus*
Color of aerial mycelium	Light gray	Whitish gray
Melanoid pigment	+	+
Reverse side pigment	-	-
Soluble pigment	+	+
Spore chain	Rectiflexible	Spiral
Spore surface	Smooth	Smooth
Carbone source assimilation		
Arabinose	+	±
Xylose	+	+
Inositol	-	-
Mannitol	±	+
Rhamnose	-	-
Sucrose	-	-
Raffinose	+	±
Cell wall amino acids		
LL-DAP	+	+
Meso-DAP	-	-
Whole-cell sugar	-	-

KF668666. The phylogenetic analysis using previously obtained GenBank sequences revealed that the strain MAB56 forms a 100% similar clade with *Streptomyces albus* (Fig. 2d). A comparison was made between the strain MAB56 and its closest phylogenetic member, *S. albus* (100%), for the cultural, morphological, biochemical, and chemotaxonomical properties (Table 2). Results showed that except for spore chain morphology, strong utilization of arabinose and raffinose, and weak utilization of mannitol, other characters were similar to those of the reference strain and, hence, the strain MAB56 was identified as a species of *Streptomyces* having very close affinity with *Streptomyces albus*. A culture of the strain MAB56 has also been deposited in the Marine Microbial Repository of the National Institute of Oceanography, Kochi, under the Culture Collection number MMRF-1174.

Production of Bioactive Metabolites

Strain MAB56 grown on YEME agar produced 32 mg/100 ml of medium, whereas 21 mg of crude extract was obtained from 100 ml of YEME broth. In addition, agar plug from MAB56 YEME agar plate showed 25 mm of inhibition against *S. aureus* (ATCC 29,213) when compared to the 19 mm of inhibition exhibited by the cell-free supernatant obtained from MAB56 YEME broth. Results revealed that the strain MAB56 produced more antimicrobial compounds in solid culture when compared to liquid culture. In the laboratory fermenter, MAB56 produced around 300 mg of crude bioactive compounds per 1000 ml of medium.

Optimization of Bioactive Metabolite Production

All of the sugars, yeast extract, peptone, minerals other than $ZnSO_4$, pH ranges, and temperatures between 25 and 30 °C were found to have an impact on the production of antimicrobial compounds by MAB56 in the optimization experiment (Supplementary table 3).

Characterization and Identification of Active Compounds

In order to achieve a dark brown residue, the culture filtrate from the fermentation process was extracted with ethyl acetate, concentrated in vacuo, and then processed for silica gel column chromatography employing a gradient solvent system of dichloromethane: methanol. The third and fourth collected fractions out of the seven showed strong antibacterial activity against *S. aureus* (ATCC 29,213) and *E. coli* (ATCC 25,922) (Supplementary table 4). Then, after being combined and re-chromatographed on a silica gel column, the active fractions produced one active sub-fraction. Additionally, the active sub-fraction was purified using an HPLC semi-preparative column, which identified three active substances, namely, 12-methyltridecanoic acid (C1), hexadecanoic acid (C2), and tridecanoic acid (C3). These three compounds showed antimicrobial activity against *S. aureus* (ATCC 29,213) and *E. coli* (ATCC 25,922) (Supplementary table 5).

The bioactive compounds C1, C2, and C3 were found as yellow solids. C1 had IR spectra V*max* of 3302 cm^{-1} and 3234 cm^{-1} (OH), 2916 cm^{-1}, 2850 cm^{-1} (aliphatic C-H), 1730 cm^{-1} (C-O), 1463 cm^{-1} and 1477 cm^{-1} (CH_2 and CH_3), and 1245 cm^{-1} (C-O), whereas C2 had 3333 cm^{-1} (OH), 2916 cm^{-1}, 2849 cm^{-1} (aliphatic C-H), 2323 cm^{-1} (aliphatic C-H), 1733 cm^{-1} (C-O), 1462 cm^{-1} and 1387 cm^{-1} (CH_2 and CH_3), and 1243 cm^{-1} (C-O), and C3 had 3302 cm^{-1} (OH), 2918 cm^{-1}, 2851 cm^{-1} (aliphatic C-H), 1732 cm^{-1} (C-O), 1461 cm^{-1} (CH_2), and 1243 cm^{-1} (C-O).

The ^1H NMR (200 MHz, $CDCl_3$) of C1 depicted 15 proton signals, while those of C2 and C3 showed 15 and 16 proton signals, respectively. The three compounds C1, C2, and C3 had a total of 18, 13, and 16 carbons, respectively. They were also identified by signals appearing at 179.83, 179.82, and 179.80 with the most distant chemical shift indicating carbon at the carboxyl group. Based on these FT-IR, NMR, and LC–MS (Supplementary figs. 2–13), the chemical nature of C1, C2, and C3 was confirmed as 12-methyltetradecanoic acid, palmitic acid, and tridecanoic acid with molecular formulae $C_{14}H_{28}O_2$, $C_{16}H_{32}O_2$, and $C_{13}H_{26}O_2$ respectively (Fig. 3).

Minimum Inhibitory Concentration

The purified compounds C1, C2, and C3 were shown to be more efficient against *S. aureus* (ATCC 29,213), with MICs of 3.125 µg/ml, 6.25 µg/ml, and 12.5 µg/ml respectively. They also showed moderate activity against *E. coli* (ATCC 25,922), with MICs of 12.5 µg/ml, 25 µg/ml, and 25 µg/ml respectively (Fig. 4).

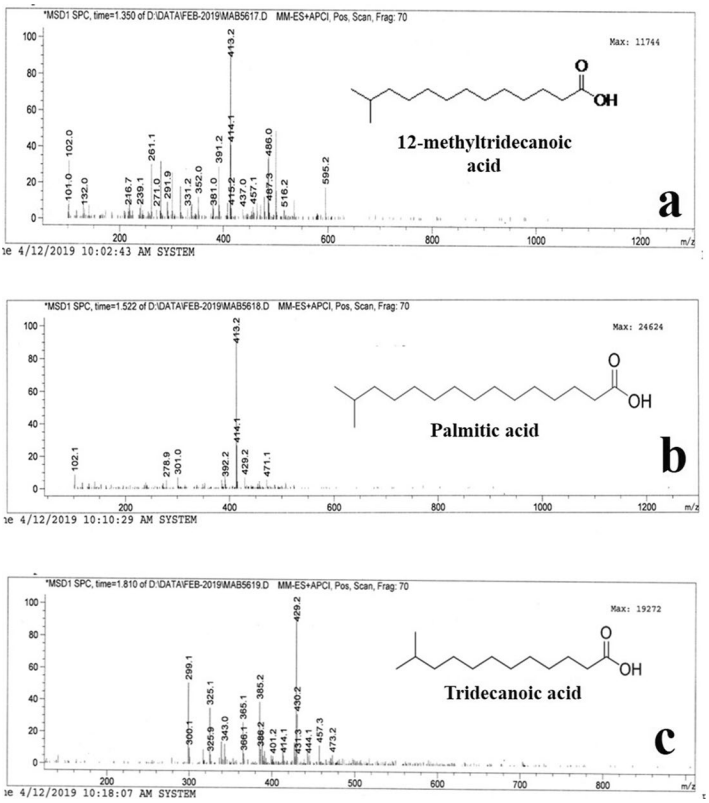

Fig. 3 LC–MS analysis of the bioactive compounds **a** C1, **b** C2, and **c** C3

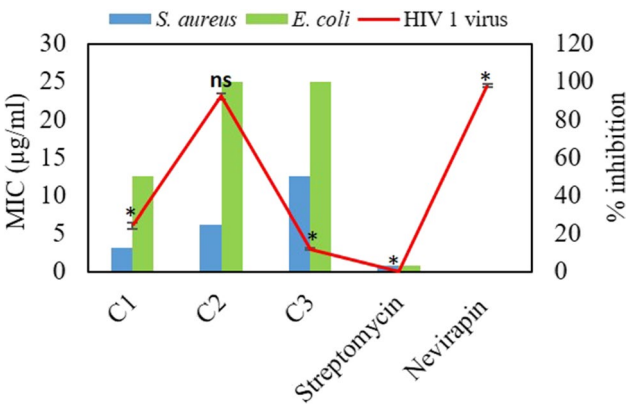

Fig. 4 Antibacterial MIC and anti-HIV activity of the bioactive compounds. "ns" and "*" indicate absence and presence of significant differences (≤ 0.05) using the least significant difference (LSD) among treatment

Anti-HIV Activity

Among the three compounds from MAB56 tested for activity against HIV-1, only the compound C2 (palmitic acid) showed the highest inhibition of 92.21% at 25 µg/ml (Fig. 4). The compound C2 showed an IC50 value of < 1 µg/ml.

Discussion

The continuous pursuit of natural bioactive products to tackle human diseases has paved the way for the discovery of novel drugs and drug leads from microbes, especially those of marine and coastal origin [22]. Actinobacteria from the mangrove ecosystem have an unparalleled capability to biosynthesize chemically diverse and biologically active secondary metabolites [23]. The mangrove rhizosphere is recognized as a prolific source for novel actinobacteria and bioactive metabolites. The mangrove ecosystem in the Andaman and Nicobar Islands is reported to harbor actinobacterial genera exhibiting antibacterial activity against a wide range of pathogens [24, 25]. However, there are only limited studies on the isolation of bioactive metabolites from actinobacteria isolated from this ecosystem. In particular, no reports are available on the antiviral properties of actinobacteria from the Andaman marine ecosystem. With this view, the present study attempted to isolate bioactive metabolites from actinobacteria isolated from mangrove sediments as well as from sponge samples collected from the Andaman and Nicobar Islands, India.

In this study, 79% of morphologically distinct cultures selected produced good growth on ISP2 agar supplemented with 50% seawater. However, 23% of the 96 cultures failed to show growth during subsequent sub-culturing on ISP2 agar supplemented with 50% seawater. This may be due to the obligate marine nature of those actinobacterial cultures. Similar observations were also made in previous studies [26]. As in other ecosystems, *Streptomyces* is reported as the dominant actinobacterial genus in mangrove ecosystems [27–29]. In this study too, the majority of the actinobacterial cultures resembled the morphology of *Streptomyces*. However, some metagenomic-based uncultured actinobacterial studies revealed that there are several rare actinobacterial genera predominantly present in the mangrove sediments [30–32]. Hence, metagenomics-guided isolation may lead to the successful isolation of rare actinobacteria from mangrove sediments. Based on the BLAST analysis, the actinobacterial isolate MAB56 showed high similarity with the *Streptomyces albus* strain. Similarly, *Streptomyces levis*, isolated from marine sediment, showed 98% similarity in blast analysis [33].

Actinobacterial metabolites are produced at various scales depending on the needs of the production vessel, ranging from microtiter plates to larger fermenters. In this study, bioactive compounds from actinobacterial cultures were produced by agar surface fermentation, a variant of solid-state fermentation. This method is simple, less expensive, and easy to perform, and it has been adopted in several studies at academic and research settings [15]. *Streptomyces* isolated from marine environments showed higher production of bioactive secondary metabolites possessing various biological activities, including antimicrobial, anticancer, antioxidant, antitumor, and antiviral [34, 35]. In the present study, *Streptomyces* strains isolated from marine samples showed antimicrobial activity against Gram-positive and Gram-negative bacterial pathogens, in which *Streptomyces albus* MAB56 showed higher activity against *S. pyogenes* and *S. aureus* (ATCC 29,213), among others. Similarly,

an ethyl acetate extract of *Streptomyces* NMF6 isolated from marine sponges showed better antimicrobial activity against Gram-positive and Gram-negative pathogens [34].

Based on the spectroscopy and NMR characterization, the purified compounds were identified as 12-methyltridecanoic acid (C1), palmitic acid (C2), and tridecanoic acid (C3). Also, we found that the spectroscopic results were identical to those of the known compounds, which were also isolated from deep-sea *Streptomyces* sp. UST040711-290 [36] and red sea *Streptomyces* sp. 1S1 [37].

The serious threat to human health posed by viral illnesses has resulted in millions of deaths globally. The treatment of viral diseases is very difficult owing to the ease with which viruses may change their genome and the speed with which they propagate due to urbanization, international travel, and migration. In order to treat viral infections, it is now important to investigate new antiviral agents with various modes of action due to viral resistance and the harmful side effects associated with current antiviral medications. Numerous antiviral secondary metabolites are produced by actinobacteria that are isolated from marine environments. Additionally, a number of studies have shown that marine *Streptomyces* are very effective sources for antiviral chemicals. The bioactive molecule palmitic acid, which was isolated from *S. albus* MAB56 for the current investigation, showed potential antiviral activity against HIV-1, with the maximal percentage of inhibition at 25 µg/ml. Similarly, the *Streptomyces* sp. NMF6 isolated from marine samples also showed potential antiviral activity against the hepatitis A, CoxB4, and HSV-1 viruses [34]. Because viruses are prevalent in the marine environment and interact with microbial populations, the strain MAB56 may have developed the antiviral secondary metabolite palmitic acid as a protective molecule in response to viral assault on the microbial community or the marine host species.

Conclusions

The study has demonstrated that the marine samples host bioactive actinobacteria, and the potent *Streptomyces albus* MAB56 strain was observed to be a good source of bioactive secondary metabolites. *S. albus* MAB56 produces three distinct metabolites, 12-methyltridecanoic acid (C1), palmitic acid (C2), and tridecanoic acid (C3), all of which have antimicrobial activity, with palmitic acid (C2) showing the greatest percentage inhibition of antiviral activity. To move these compounds further, more research is needed on their mechanism of action, in vivo cytotoxicity, pharmacodynamics, and pharmacokinetics.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12010-023-04493-y.

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Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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Authors and Affiliations

Radhakrishnan Manikkam[1] · Sangeetha Murthy[2] · Sivasankar Palaniappan[3] · Manigundan Kaari[1] · Amit Kumar Sahu[4] · Madhukar Said[4] · Vijayalakshmi Ganesan[1] · Sivakumar Kannan[3] · Balagurunathan Ramasamy[2] · Somasundaram Thirugnanasambandan[3] · Syed G. Dastager[4] · Luke Elizabeth Hanna[5] · Vanaja Kumar[1]

1 Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai 600119, Tamil Nadu, India

2 Department of Microbiology, Periyar University, Salem 636011, Tamil Nadu, India

3 CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India

4 Microbial Resource Centre, National Chemical Laboratory, Pune, India

5 National Institute for Research in Tuberculosis, Chennai 600031, Tamil Nadu, India