Bioactive Antifungal Metabolites from Marine Actinomycetes: Isolation, Characterization and their Antibiofilm Activity against *Candida albicans*

T. Rajeshwari[a], P. Jenifer [b*], D. Roselin Jenifer [c], N. Dhanam[d], K. Sowmya[e], S. Seema[e]

a) Department of Biotechnology, Deen College of Arts and Science, Mayiladuthurai, Tamil Nadu, India.

b) Institutional Research Board, Trichy SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India.

c) Department of Biotechnology, Sathyabama Institute of Science and technology, Chennai, Tamil Nadu, India.

d) Department of chemistry, DKM College for women, Vellore, Tamil Nadu, India

e) PG & Research Department of Biotechnology and Bioinformatics, Holy Cross College (Autonomous), Trichy, Tamil Nadu, India.

Corresponding Author:

P. Jenifer

Institutional Research Board,

Trichy SRM Medical College Hospital and Research Centre,

Trichy, Tamil Nadu, India.

ABSTRACT:

The increasing incidence of fungal infections in humans, combined with the limited efficacy of current antifungal therapies and the emergence of drug-resistant fungal pathogens need for the discovery and development of novel antifungal agents. Marine actinomycetes have served as prolific producers of biologically active secondary metabolites for over four decades and continue to represent a valuable and promising source for the identification of new antifungal compounds with potential therapeutic applications. In this study, actinomycetes were isolated from marine soil sediments collected from Akkarai Beach, ECR, Chennai (Tamil Nadu, India). The isolated strains were cultured through fermentation, and the resulting metabolites were extracted and characterized. Antibiofilm activity was evaluated against *Candida albicans*, and the secondary metabolites were characterized by FTIR spectroscopy and GC-MS analysis. Results confirmed that the Streptomyces sp. extract exhibited significant antibiofilm activity (up to 72% inhibition at 50 µl mL^{-1}).

Keywords: actinomycetes, secondary metabolites, FTIR, GC-MS, C.albicans

INTRODUCTION

Actinomycetes are filamentous, Gram-positive bacteria that form branching filaments at some stage of their development. These organisms colonize diverse habitats including soil, aquatic environments, plant litter, composts, and food products. They mediate the degradation of complex polymers such as chitin, lignin, and cellulose, aid in the biocontrol of bacteria and fungi, and synthesize valuable metabolites including antibiotics, pigments, and enzyme inhibitors.

Actinomycetes are classified into more than 150 genera based on morphological and chemical criteria, with nine distinct cell wall chemotypes distinguished by the type of diaminopimelic acid (DAP) present. Cosmopolitan by nature, they occur in terrestrial and aquatic habitats worldwide at concentrations of approximately one million cells per gram of soil. Their distribution is influenced by temperature, oxygen availability, and ecological factors such as socioeconomic conditions. In soil, they are critical agents of organic matter recycling and suppress pathogenic microorganisms. Their role in marine habitats—including the decomposition of cellulose, alginates, and hydrocarbons has increasingly drawn scientific attention.

Oceans cover 71% of the Earth's surface and harbor an enormous, largely unexplored diversity of microorganisms. Marine-derived actinomycetes possess unique metabolic pathways shaped by their distinctive environment and have demonstrated the capacity to produce a wide range of structurally novel secondary metabolites. By 2010, a total of 13,700 bioactive metabolites had been reported from actinomycetes, of which 10,400 originated from Streptomyces species and 3,300 from rare actinomycete strains. Secondary metabolites, unlike primary metabolites, do not contribute directly to basal cellular functions but serve critical ecological roles—repelling predators, inhibiting competing organisms, and ultimately enhancing survival.

Candida albicans is a major opportunistic fungal pathogen in humans, capable of colonizing virtually every tissue and organ and causing serious invasive infections, particularly in immunocompromised patients. As a member of the normal human microbiota, it can transition from commensal to pathogen under conditions of immune suppression. The growing prevalence

of C. albicans biofilm-associated infections and increasing antifungal resistance underscore the need to identify novel inhibitory compounds.

A substantial body of evidence supports the potential of marine actinomycetes as sources of novel bioactive compounds. Zheng, Zeng et al. (2000) demonstrated antitumor activity in marine actinomycetes: 20.6% exhibited cytotoxicity against P388 cells and 18.6% against KB cells, with Micromonospora showing the highest induction rates. Asha Devi, Jeyarani et al. (2006) reported that Streptomyces sp. produced effective antibacterial and antifungal compounds against pathogens including Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Klebsiella sp., and Aspergillus niger.

Basha, Rekha et al. (2007) found that marine actinomycetes could produce L-asparaginase—an anti-leukemia agent—with optimum activity at pH 7.5. Ramesh and Mathivanan (2009) reported that of 208 marine actinomycetes tested, 111 isolates exhibited antimicrobial activity against human and plant pathogens, while several produced industrially relevant enzymes including lipase, gelatinase, and cellulase.

Hameş-Kocabaş (2012) identified new actinomycete species and novel metabolites from marine sponges and soil sediments, emphasizing the importance of enrichment techniques, selective media, and extended incubation periods. Ambavane, Tokdar et al. (2014) identified Caerulomycin from a marine actinomycete as a novel antifungal compound for treating infectious diseases. Nandhini, Bharathy et al. (2014) identified Streptomyces rubralavendulae strain SU1 as a promising candidate against fungal infections.

Abdelfattah et al. (2016) evaluated actinomycetes isolated from the Red Sea coast, demonstrating cytotoxicity towards breast cancer cell lines. Davies-Bolorunduro et al. (2019) purified two novel anticancer compounds, ULDF4 and ULDF5, from actinomycetes isolated from Lagos Lagoon. Palla et al. (2018) characterized Streptomyces hydrogenans from mangrove soil as a producer of a thermostable antifungal metabolite, highlighting the value of chromatographic and spectral characterization for compound isolation.

MATERIALS AND METHODS

Sample Collection and Isolation

Marine soil sediments were collected from Akkarai Beach, ECR, Chennai (Tamil Nadu, India), following the modified methodology of Rajivgandhi et al. (2016). Samples were packed in polythene bags and transported immediately to the laboratory. The soil was air-dried and held at

45°C for 10 minutes to reduce fungal and bacterial contamination. Approximately 1 g of soil was transferred to 99 mL of sterile 50% seawater and serially diluted to 10^{-5}. One milliliter of diluted suspension was plated on Starch Casein Agar (SCA) containing amikacin (2.5 mg mL^{-1}) and fluconazole (7.5 mg mL^{-1}). Plates were incubated for 21 days at 28°C. From day 5 onward, morphologically distinct colonies were purified by streak-plate sub-culturing on SCA and stored in SCA slants at 4°C.

Morphological Characterization

Macroscopic characteristics (size, shape, aerial and substrate mycelium, pigmentation, and spore morphology) were recorded. Microscopic observations employed the coverslip technique: a coverslip was inserted at 45° into solidified SCA and incubated at room temperature. After removal, coverslips were placed on clean glass slides and examined microscopically. Gram staining was also performed to confirm organism identity.

Fermentation and Extraction of Secondary Metabolites

A fully grown Streptomyces sp. culture was inoculated into 250 mL of culture medium (0.5% dextrose, 0.2% peptone, 0.25% NaCl, 0.4% $CaCO_3$, 0.2% potassium nitrate) in a 500-mL Erlenmeyer flask. The flask was incubated at 28°C in a shaker incubator for 7–10 days. The culture broth was then centrifuged at 5,000 rpm for 20 minutes. The cell-free supernatant was collected and mixed with an equal volume of ethyl acetate, then placed on a rotary shaker at 200 rpm overnight. The extract was concentrated by rotary vacuum evaporation, air-dried, and dissolved in 5% DMSO for antibiofilm testing.

Antibiofilm Activity Assay Against C. albicans

Crystal violet assay was performed using 24-well polystyrene microtiter plates. Each well was washed twice with 200 µL PBS and air-dried for 45 minutes. The assay mixture (1 mL) contained 100 µL of C. albicans suspension (10^6 cells mL^{-1}) and varying concentrations (1–10 µg mL^{-1}) of Streptomyces sp. extract. Control wells received no extract. Plates were incubated at 37°C for 48 hours, then stained with 100 µL of 0.4% crystal violet for 5–6 minutes. After two washes with MilliQ water and air-drying, biofilm was destained with 1 mL of 95% ethanol and optical density was measured at 570 nm.

FTIR Spectral Analysis

FTIR spectrum analysis was performed using a Shimadzu IR-470 Plus instrument (scanning range: 400–4000 cm^{-1}). The bioactive extract (5 mg) was mixed with KBr and pressed into a thin disk.

Scans were acquired at 4 cm^{-1} resolution to identify functional groups and structural features of the Streptomyces sp. ethyl acetate extract.

GC-MS Analysis

Gas chromatography–mass spectrometry (GC-MS) analysis was performed on an Agilent GC-MS system with an AOC-20i/s autosampler and a capillary column coupled directly to the MS. Analysis conditions: initial temperature 100°C for 20 minutes, then ramped to 280°C at 5°C/min; injector port temperature maintained at 250°C; helium used as carrier gas. Compounds were identified with the NIST spectral library.

RESULTS AND DISCUSSION

Isolation and Culture

A total of nine marine actinomycetes were isolated from the ECR beach sediment sample. Colonies displaying the characteristic morphology of Streptomyces sp. were selected, sub-cultured to obtain pure forms, and preserved at 4°C for downstream experiments.

Morphological Characterization of Pure Culture

Table 1 summarizes the macroscopic and microscopic characteristics of the pure isolate. The strain grew optimally at 28°C (mesophilic) with a pH of 7.2, displayed whitish-grey coloration, produced a yellow diffusible pigment in the medium, and formed spiral spore chains with smooth spore surfaces. Microscopic examination confirmed that the isolate forms extensively branched aerial and substrate mycelia terminating in arthrosporic chains, consistent with the Streptomyces genus of Actinomycetes.

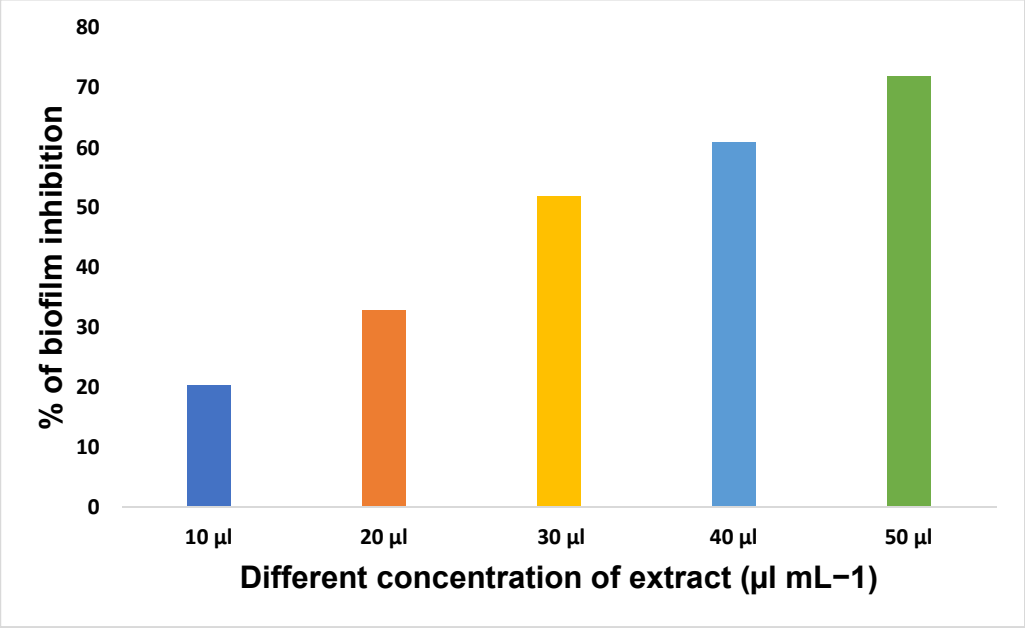
Table 1. Morphological characteristics of the pure Streptomyces sp. isolate.

Morphological Characteristic	Observation
Temperature	28 °C (Mesophilic)
pH	7.2
Color	Whitish grey
Aerial mycelium	Present

Substrate mycelium	Present
Pigmentation in medium	Yellow diffusible pigment
Spore chain	Spirals
Spore surface	Smooth

Antibiofilm Activity Against *C. albicans*

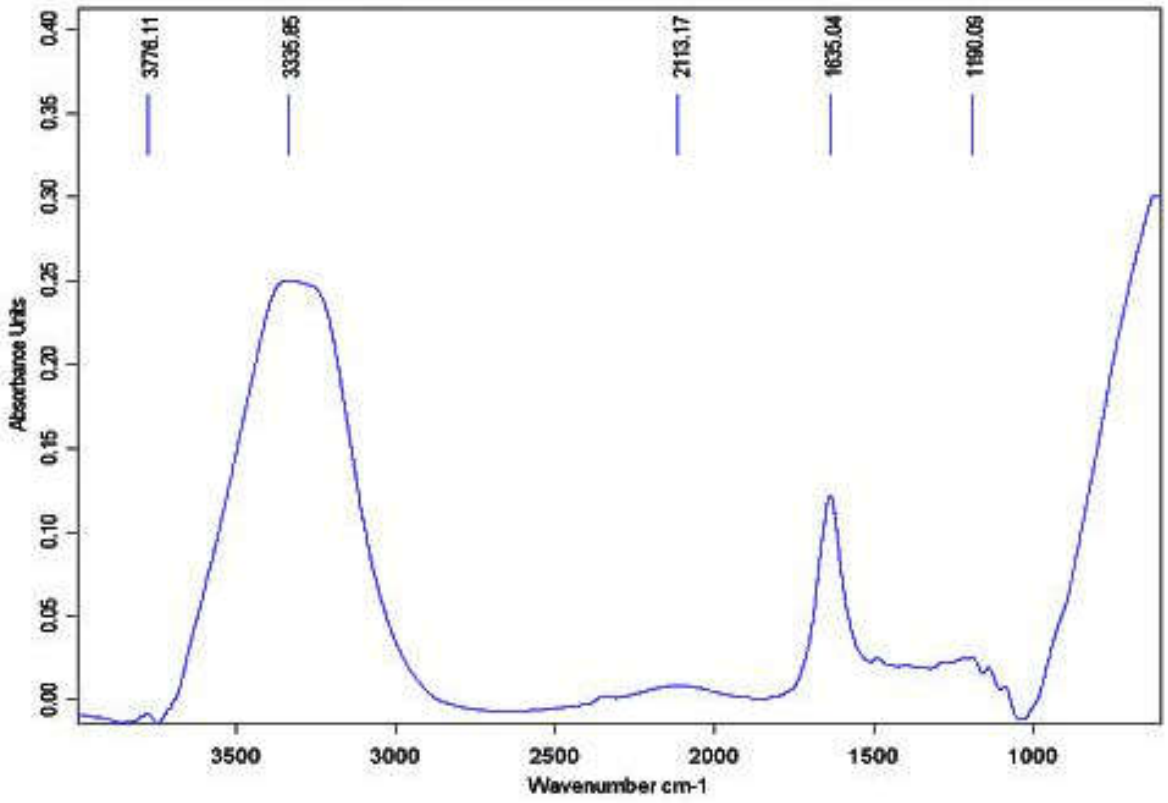
The ethyl acetate extract of Streptomyces sp. demonstrated dose-dependent inhibition of C. albicans biofilm formation. At 10 µl mL^{-1}, biofilm inhibition was 20%; at 20 µl mL^{-1}, it reached 33%; at 30 µl mL^{-1} (designated the biofilm inhibitory concentration BIC_{50}), 50% inhibition was observed; and at 50 µl mL^{-1}, the maximum inhibition of 72% was achieved. These results indicate that the Streptomyces sp. secondary metabolites act specifically on biofilm formation rather than on overall cellular growth of C. albicans.



FTIR Spectral Analysis

The FTIR spectrum of the Streptomyces sp. extract exhibited characteristic absorption peaks at 3776.11 cm^{-1} (O–H stretching, hydroxyl group), 3335.85 cm^{-1} (N–H stretching, aliphatic secondary amino group), 2113.17 cm^{-1} (C≡N double bond stretching), and 1635.04 cm^{-1} (polygenic compound double bond). These results indicate that the crude extract is rich in hydrocarbons substituted with hydroxyl, amino, aldehyde, or keto functional groups—structural

features commonly associated with biologically active natural products. FTIR analysis confirmed the presence of hydroxyl, amino, and carbonyl functional groups, indicating the occurrence of alcohols, amines, and carbonyl-containing metabolites. Similar FTIR profiles have been reported for bioactive metabolites isolated from marine Streptomyces, suggesting that these functional groups are frequently associated with antimicrobial and antifungal secondary metabolites (Palla et al., 2018; Ravi and Kannabiran, 2018)

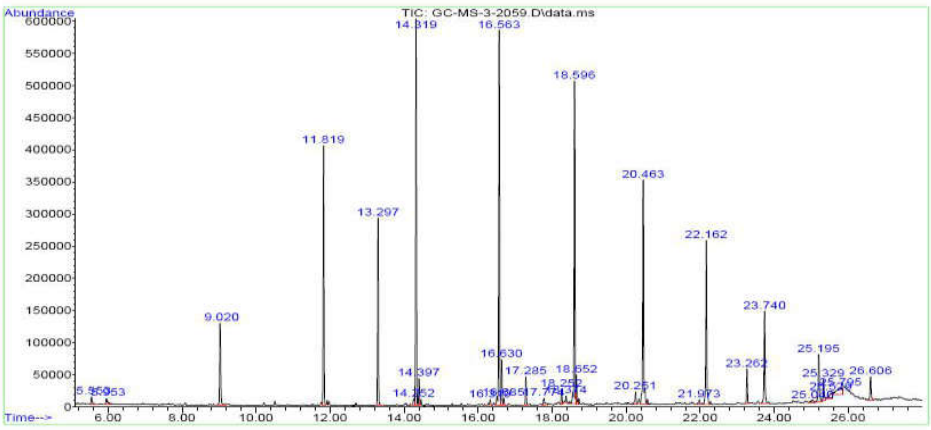


GC-MS Analysis: Compound Identification

GC-MS analysis identified 30 compounds in the Streptomyces sp. ethyl acetate extract (Table 2). Dominant constituents by peak area percentage included 9-Eicosene (14.75%), 2-Tetradecene (13.37%), 1-Octadecene (13.34%), 1-Docosene (10.46%), and 5-Tetradecene (8.69%). Phenol, 2,4-bis(1,1-dimethylethyl) (7.66%) and Heptadecyl heptafluorobutyrate (6.54%) were also notable. The diversity of long-chain alkenes, aliphatic hydrocarbons, and ester derivatives confirms the chemical richness of the marine Streptomyces secondary metabolome. GC–MS

profiling revealed thirty compounds, predominantly long-chain alkenes, phenolic compounds, esters, and fatty acid derivatives. Compounds such as Phenol, 2,4-bis(1,1-dimethylethyl) possess well-documented antioxidant and antimicrobial activities, whereas dibutyl phthalate and eicosane have previously been isolated from Streptomyces species exhibiting antifungal activity (Ahsan et al., 2017; Zhang et al., 2018). The occurrence of these metabolites supports the observed antibiofilm activity against Candida albicans, although purification and structural elucidation are necessary to identify the specific bioactive constituent responsible for the antifungal effect. Eicosane and DBP, isolated from Streptomyces sp., were documented for their antifungal activity against Rhizoctonia solani (Ahsan et al. 2017). Thus, from the GC-MS spectrum, it is evidenced that most of the compounds identified from Streptomyces sp. extract have been expressed for their antimicrobial and antifungal activities.

Table 2. Compounds identified by GC-MS analysis in Streptomyces sp. ethyl acetate extract.



Peak	Area (%)	Retention Time (min)	Compound
1	0.43	5.553	2-Pentene, 4,4-dimethyl
2	0.47	5.953	1-Decene
3	3.87	9.020	11,13-Tetradecadien-1-ol

4	8.69	11.819	5-Tetradecene
5	7.66	13.297	Phenol, 2,4-bis(1,1-dimethylethyl)
6	0.35	14.252	Cyclohexane, (1-hexyltetradecyl)
7	13.37	14.319	2-Tetradecene
8	0.97	14.397	1-Iodo-2-methylundecane
9	0.38	16.319	1-Decanol, 2-ethyl
10	14.75	16.563	9-Eicosene
11	1.66	16.630	Heptadecane
12	0.32	16.685	5-Ethyl-1-nonene
13	1.11	17.285	Phthalic acid, isobutyl cyclohexyl
14	0.53	17.774	1,2-Benzenedicarboxylic acid
15	0.60	18.252	Dibutyl phthalate
16	0.37	18.374	Dodecane, 3-methyl
17	13.34	18.596	1-Octadecene
18	0.93	18.652	Heptadecane, 9-octyl
19	0.68	20.251	Nonadecane
20	10.46	20.463	1-Docosene
21	0.31	21.973	Tetracontane, 3,5,24-trimethyl
22	6.54	22.162	Heptadecyl heptafluorobutyrate
23	1.31	23.262	1,2-Benzenedicarboxylic acid
24	3.89	23.740	Heptadecyl trifluoroacetate
25	0.31	25.040	1-Bromo-11-iodoundecane
26	2.33	25.195	Cyclooctacosane
27	0.98	25.329	2,6,10,14,18,22-Tetracosahexaene
28	0.68	25.529	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)
29	1.54	25.795	n-Propyl 11-octadecenoate
30	1.19	26.606	Heptacosyl acetate

CONCLUSION

This study successfully isolated and characterized a Streptomyces sp. strain from marine sediments at ECR Beach, Chennai. Morphological characterization confirmed the isolate as a member of the genus Actinomycetes, growing optimally at 28°C and pH 7.2 with characteristic spiral spore chains

and branched mycelia. Antibiofilm testing against *C. albicans* demonstrated significant, concentration-dependent inhibition, with maximum efficacy of 72% at 50 µl mL^{-1}. FTIR analysis confirmed the presence of key functional groups—hydroxyl, amino, aldehyde, and keto—in the extract. GC-MS profiling revealed 30 distinct compounds, supporting the chemical diversity of marine Streptomyces secondary metabolites. Collectively, these findings affirm the potential of marine-derived actinomycetes as sources of novel antifungal compounds and warrant further investigation into compound isolation, structural elucidation, and therapeutic application.

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