

Isolation, Screening, and Characterization of *Streptomyces* Strain from the Marine Soil Sample and Evaluation of Antimicrobial Activities

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Abstract: The study involved isolating and screening *Streptomyces* species from marine soil and evaluating their antibacterial activity. They produce the bulk of antibiotics and differ significantly in appearance, physiology, and metabolic processes. The soil sample collected from marine sources yielded four actinomycete types, and the isolate VR3 from Marakkanam Beach showed strong antibacterial activity and was selected for further investigation. The spore chain structure of the *Streptomyces phaeolivaceus* strain VR3 revealed that the spores were simple, transverse, Gram-positive, and smooth-surfaced. The aerial mycelia of the isolate, while cultivated on various media, were generally white, whereas the substrate was creamy white. The isolated VR3 showed antibacterial activity against *Escherichia coli* (9 mm), *Staphylococcus aureus* (7.5 mm), *Proteus mirabilis* (7.7 mm), *Pseudomonas aeruginosa* (7 mm), and *Klebsiella pneumoniae* (4.5 mm) at a concentration of 3.5mg/ml. The minimum inhibitory concentration of the isolate VR3 was 0.531 mg/ml for *Escherichia coli* and *Pseudomonas aeruginosa*, 1.062 mg/ml for *Staphylococcus aureus*, 0.265 mg/ml for *Proteus mirabilis*, and 2.125 mg/ml for *Klebsiella pneumoniae*.

Keywords: Actinomycetaceae; *Streptomyces* species; antibacterial activity; marine soil.

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1. Introduction

Actinomycetes are commonly found and can live in harsh conditions [1]. Gram-positive, filamentous bacteria with a fungal shape are known as actinomycetes. They are abundantly dispersed in the natural world, especially in soil [2]. Because of their ability to synthesize numerous natural chemicals and to adapt to extreme environments, microbes in extreme habitats have attracted significant interest [3]. Because they develop slowly compared to common conditions, actinomycetes from diverse environments, such as saltwater habitats, are exceedingly challenging to cultivate. The selection of the screening source, the chosen medium, the growing environment, and the isolation and identification of potential colonies are all important considerations in standard isolation approaches [4]. One of the most useful prokaryotic bacteria for biotechnological purposes is the actinomycete. They are well recognized as a source of bioactive compounds and antibiotics. It has been confirmed that the majority of their bioactive compounds exhibit antibacterial (streptomycin, tetracycline, and

chloramphenicol), antifungal (nystatin), antiviral (tunicamycin), and antiparasitic (ivermectin) characteristics [5,6]. The most commercially and biotechnologically useful prokaryotes are actinomycetes, which may create a variety of biologically active secondary metabolites, including enzymes, anticancer agents, antibiotics, and immunosuppressive compounds. Various bioactive compounds are extracted from several *Actinomycetes* species that possess well-documented biological activities, including antifungal, antibacterial, antimalarial, anticancer, and anti-inflammatory properties. Similarly, Actinomycetes can produce various bioactive naturally occurring substances, including cellulose, xylanase, cosmetics, nutritional supplements, insecticides, antibiotics, herbicides, and antiparasitic agents [8]. These are saprophytic, unlimited microorganisms that are a key ingredient in the synthesis of antibiotics [9]. In 1984, the first marine *Actinomycete* was identified [10, 11], and numerous new marine Actinomycete species have since been discovered in aquatic habitats worldwide [12-17]. Actinomycetes have population sizes that vary with physicochemical factors such as pH, temperature, total organic carbon, salinity, pressure, etc. [10]. The diverse chemical structures and conformations of bioactive compounds from marine actinomycetes may facilitate the development of innovative medications with the potential to combat various drug-resistant illnesses [18]. *Actinomycetes* from various settings are being screened for their capacity to produce new bioactive components because the occurrence of novel bioactive substances identified from terrestrial *Actinomycetes* decreases over time. *Actinomycetes* isolated from the marine environment have been shown to be biologically active and adapted to life in water. *Streptomyces* is particularly prolific and may produce numerous bioactive molecules and antibiotics [19, 20]. Because of their unique chemical properties, the bioactive compounds produced by marine actinomycetes may be used to develop novel treatments for various chronic diseases that are resistant to existing drugs [21-24]. The main genus of Actinobacteria, *Streptomyces*, is an aerobic, gram-positive filamentous bacterium that forms spores. They generate a thick, leathery, extensively branched substrate mycelium that produces diffusible pigments [25, 26]. On the other hand, *Streptomyces* produces aerial hyphae that can split to form spores resistant to adverse conditions and readily transported to alternative locations and nutrient sources when conditions are restricted [27]. They have arthrospore chains on their aerial mycelium, and the Guanine and Cytosine composition of *Streptomyces* genomes ranges from 69 to 78% [28]. About 40% of all bioactive secondary compounds are produced by *Streptomyces species*, making it crucial for drug discovery [29]. Due to their inhibitory properties, many *Streptomyces* produce over 6,000 bioactive secondary metabolites, which are used in medical applications, including antimicrobials [30]. The *Streptomyces* genus is recognized for producing medically tested antibiotics and other chemotherapeutic drugs [31-33]. The genomes of *Streptomyces*, which are relevant to ecology, biotechnology, and veterinary medicine, were sequenced, and it was found that their morphological, biochemical, and genetic variability accurately reflects their diversity [34]. In addition to a wide range of naturally occurring bioactive substances, such as antifungals, antivirals, anti-hypersensitivity, and anticancer [35, 36]. They also manufacture other molecules that are harmful to competing bacteria [37,38]. Various studies have recently focused on isolating *Streptomyces*, bacteria that produce antibiotics, from coastal environments [39]. *Streptomyces*-produced bioactive substances are used as biological control agents and to treat human and animal ailments. They are also well-known manufacturers of medicinal, agrochemical, and industrial enzymes [40]. Numerous modes of action are exhibited by *Streptomyces* antimicrobial substances, including membrane function disruption, suppression of cell wall production, and interference with

nucleic acid biosynthesis [41]. They are crucial for the breakdown of organic matter, carbon recycling, and the improvement of soil fertility [42-44]. The emergence of antimicrobial resistance in bacteria to frequently prescribed antibiotics and antifungal medications has created a need for novel chemicals, and members of the genus *Streptomyces* provide possible lead molecules [45]. The study aimed to separate, recognize, and describe the *Streptomyces* species found in marine soil samples.

2. Materials and Methodology

2.1. Isolation of microbial strain.

The soil sample was collected from the coastal region of Marakkanam, Viluppuram district, Tamil Nadu. At a depth of 25 cm, soil samples were aseptically taken, and factors including pH and temperature were recorded at the time of sample collection.

2.1.1. Pre-treatment of soil samples.

After air drying, the collected soils were sieved to remove debris. The dry dirt was covered with 1% calcium carbonate and left overnight. The dirt was isolated, dried for two hours at 60°C, and used for further study.

2.2. Morphological study.

2.2.1. Isolation of actinomycetes from the marine soil sample.

The starch-casein agar medium was prepared and sterilized for 15 minutes at 121 °C. Subsequently, 20 µg/l of tetracycline and 50 µg/l of amphotericin B were added to inhibit bacterial and fungal growth. The SCA media was poured into the sterilized Petri dishes, and then 9 ml of sterile, double-distilled water and 1g of marine soil sample were mixed. The samples were again serially diluted to 10⁻⁶, and 0.1 of the diluted samples were spread on agar plates. The inoculated petri plates were incubated at 30°C for up to one week. The purified isolates were used for streaking colonies on a new plate of SCA medium using sterile wire loops. The isolated pure cultures of *Streptomyces* species were transferred to the SCA slants and stored at 4°C for further use. The fresh bacterial colonies were kept alive in 20% glycerol and stored at freezer conditions for future studies (Figure 1) [46].

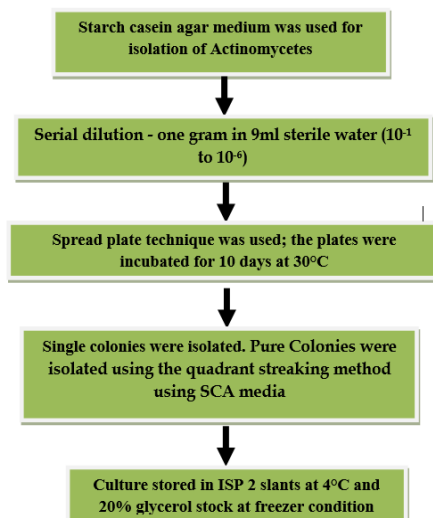


Figure 1. Schematic representation of a *Streptomyces* strain isolated from a marine soil sample.

2.2.2. Morphological characterization.

Actinomycete strains were characterized morphologically using a magnifying glass on a starch-casein agar plate after 5 to 14 days of culture. Colony morphology was observed regarding aerial color, aerial mycelium, size, colony type, reverse side color, and pigmentation. Isolates were examined under a microscope.

2.2.3. Gram staining.

Each isolate was converted into a thin smear and heat-fixed on a clean glass slide. The smear was then stained for 1 minute with crystal violet, rinsed with water, and inspected with Gram stain iodine. The slide was washed with tap water and alcohol used for the study. The smear was decolorized before being counterstained with safranin. The slide was then cleaned, air-dried, and viewed under a microscope.

2.2.4. Acid-fast staining.

The acid-fast staining method was used to visualize the actinomycete colony. A thin coating of the isolate was spread out and heated on a clean glass slide. After three to four repetitions of the procedure for 5 minutes, the slide was washed with water, and the smear was counterstained with methylene blue for 60 seconds. The slide was then decolorized using acid alcohol for 30 seconds. After washing the smear under running water, it was air-dried.

2.2.5. Slide culture method.

The sporulation pattern and chain architectures were studied using the slide culture technique. A 24-hour-old strain of *Streptomyces* species was placed at the spot where the sterile coverslip was inserted over the glass slide, followed by incubation for 7 days at 30°C at an angle of 40° in starch casein agar medium. After that, the glass slide and the *Streptomyces* growth were carefully removed and placed on an additional glass slide that had been previously lactophenol blue-stained. The slide was examined under a 100x light microscope to study the spore surface morphology.

2.3. Molecular characterization.

2.3.1 DNA isolation of the *Streptomyces* isolates

The Actinomycete strains were cultivated twice using Tris EDTA buffer after reaching the late exponential stage in Starch Casein broth at $28 \pm 2^\circ\text{C}$. 500 μl of 5 M solutions of sodium chloride were mixed with 0.5–1.0 g of resuspended cells in 5 ml of lysis buffer (25 mM Tris, 25 mM EDTA, pH 8.0; 10–15 μg lysozyme, and 50 g/ml Rnase) to retrieve chromosomal DNA. This procedure took 30–80 min at 37°C. The suspension was thoroughly stirred in a vortex mixer until it became translucent. The cells were lysed following the injection of 1.2 ml of 10% SDS. For 15 to 30 minutes, the lysates were incubated at 65°C. After adding 2.4 ml of 5 M potassium acetate, the mixture was stirred and allowed to sit on ice for 20 minutes. The resultant solution was centrifuged for 30 minutes at 6,000 rpm to get 8 ml of supernatant. The DNA was extracted by precipitation with 2 liters of isopropanol. The residue was dissolved with 700 l/g of 50 mM Tris and 10 mM EDTA (pH 8.0). After spinning off insoluble materials, the aqueous phase was collected into a 1.5 ml microfuge tube. After adding 500 μl of isopropanol and 75

µl of 3M sodium acetate, the solution was centrifuged for 30 to 2 minutes. After being dried and dissolved in 100 µl of T.E. (10 mM Tris/1 mM EDTA, pH 8.0), the residue was washed with cold 70% ethanol [47].

2.3.2. 16S rRNA PCR amplification.

A combination of 12.5 µl of sterile, deionized, distilled water (28.1 nmol) of the upstream primer (5' AGAGTTTGATCCTGGCTCAG 3') and 32.3 nmol of the downstream primer (5' GGTTACCTTGTTACGACTT 3') Four microliters (µl) of a 10X PCR premix kit, including reaction buffer 1x, gel loading buffer 1X, dNTPs mix (2.5 mM each), i-Star Taq DNA polymerase (2.5 U/µl), and one microliter (50 ng) of actinomycete template DNA, were placed in a 0.5 ml microcentrifuge tubes. The 20 µl mixture was gently spun in a tube for 10 seconds, and then the particles were allowed to settle. The Eppendorf PCR thermal cycler was used to store the samples. The 35 cycles of the amplification were performed as follows: primer annealing for 60 sec at 55°C, denaturation for 60 sec at 94°C, and polymerization for 45 sec at 72°C. At 72°C for 10 minutes, the tube's full polymerization was ensured. 2 µl of PCR products were loaded onto a 1% agarose gel and subjected to electrophoresis for 45 minutes at 50 volts. A UV transilluminator was used for gel observation, and the results were compared with a 1 Kb DNA ladder [48].

2.3.3 Nucleotide sequence accession.

The standard protocol was used to isolate and sequence the DNA of the bacterial species. The morphological and sequencing results confirmed that the selected strains belonged to different genera, and the generated raw data were submitted to GenBank. The submitted gene sequence was confirmed as *Streptomyces phaeolivaceus* strain VR3 Bacterial species, and the accession number was OQ121828.

2.3.4 Phylogenetic analysis

Using the website <http://www.ncbi.nih.gov/genbank>, the reference sequences required for comparison were retrieved from the EMBL database. All sequences were aligned using the multiple-sequence alignment tool Clustal W, developed by Higgins *et al.* (1992). The manually aligned sequences were grouped into blocks of 250 base pairs per row, gap-checked, and then stored in the format used by MEGA 4 [49]. The phylogenetic tree was constructed using the neighbor-joining method, and each clade support was evaluated using a 1,000-replication bootstrap analysis [50, 51].

2.4. Biological analysis.

2.4.1. Antibiotic sensitivity.

Actinomycete lawn cultures were established on starch-casein agar. The media was covered with the antibiotic discs that were chosen. The plates performed room-temperature incubation. The zone of inhibition was measured and classified as sensitive or resistant after 24 hours of incubation [52].

2.4.2. Optimization of growth conditions.

Optimization of culturing conditions was based on the screening results from the secondary screening. The incubation process was carried out at the following conditions: 4°C, 10°C, 15°C, 25°C, 30°C, 37°C, 42°C, 45°C, 50°C, and 60°C. To enhance the salt content, various concentrations of marine salt (20 g, 40 g, 60 g, 80 g, and 100 g per liter) were used, and the cultures were incubated at 28°C. The Optimization of culture development at different pHs as 4, 5, 6, 7, 8, and 9 was noticed at 28°C [53].

2.4.3. Organisms tested for biological analysis.

Bacterial and fungal cultures were obtained from the Microbial Type Culture Collection (MTCC) and American-type culture collections (ATCC). Bacterial cultures (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*), Fungal cultures (*Aspergillus niger*, *Candida albicans*). The cultures were maintained on slants and stored at 4 °C. These strains were subcultured every three months to maintain their viability [54].

2.4.3.1. Screening for biological characterization.

The cross-streak technique evaluated the Actinomycetes' capacity to produce antimicrobials [55]. On the surface of the altered Bennett's agar plate, a single streak of actinomycetes was formed and incubated at 30°C. The pathogens (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, and *Staphylococcus aureus*) were streaked at a right angle to the first streak of actinomycetes and incubated at 37°C after observing a fine ribbon-like formation of the actinomycetes on the Petri dishes. We assessed the zones of inhibition after 24 hours and 48 hours. The actinomycetes that produce antimicrobial chemicals were selected based on the presence or absence of zones.

2.4.3.2 Optimisation of Microbial Growth and Production of Bioactive Metabolites

Bioactive metabolites from Actinomycete were extracted using various media compositions and listed in Table 3.3. 500 ml culture media were initially placed in one-liter Erlenmeyer flasks for fermentation. A loopful of cell culture from a pure colony was utilized as an inoculum. The cell cultures were kept at 28°C and 180 rpm of agitation for 15 days. Every two days, 10–20 milliliters of each sample were collected, and the supernatant was separated by centrifuging the sample for 15 minutes at 10,000 rpm. Subsequently, the supernatant was passed through a Millipore filter (0.45µm) to collect samples free of cells. The well-diffusion assay was used to assess the antibacterial properties of each cell-free supernatant [56]; 50–200µl of the sample was added to each well and examined. All test microbes were used to evaluate the antimicrobial activity, as shown in Table 3.0. The well-diffusion assay modified the Schillinger and Lucke (1989) approach. The actinomycete strains with the strongest antagonistic activity were selected and tested against harmful bacteria and fungi. The selected strains were cultured individually for 7 days at 30°C and 180 rpm in 500 ml conical flasks containing 200 ml of starch casein broth. The microbial residue was separated through a Millipore filter (0.45 m) followed by incubation to get cell-free extraction. The filter was used under 33 aseptic conditions to preserve it for further testing in the conical flasks. Three yeast-like fungi and five clinically significant pathogenic bacteria were inoculated into S.D.A. and

M.H.A., respectively. On top of the identically grown media, the bacterial isolates were dispersed. The sterile cork borer wells, 5 mm in diameter, were obtained after solidification. A separate quantity of cell-free filtrate (25 µl) was placed into the wells and incubated at 37°C for 24 hours. After incubation, the antimicrobial activity of the actinomycete strain was analyzed by measuring the inhibition zone diameter around the wells [59].

2.5. Preparation of organic extracts.

Cultures of *Streptomyces* isolates were inoculated into 25 mL of yeast tryptone extract medium (ISP1) and grown for 21 days. The cell-free broth was filtered through 0.22 µm syringe filters, and the metabolites were extracted with an equal volume of ethyl acetate. After shaking vigorously for 30 min, the mixture was allowed to stand in a separatory funnel to separate the 2 phases. The organic layer was removed and heated at 50°C [60]. For additional examination, the dried extract was weighed, reconstituted in 1 mL of double-distilled water, and sterilized using 0.22 µm syringe filters [61].

2.6. Determination of minimal inhibitory concentration.

The M.I.C. was ascertained in a 96-well microplate using Muller-Hinton broth after dissolving 40 mg of crude extract in 200 µl of DMSO [57].

3. Results and Discussion

3.1. Isolation of actinomycetes from a marine soil sample.

Five different media, broth-like starch casein broth, yeast malt extract broth (ISP-2), oatmeal broth (ISP-3), inorganic salt starch broth (ISP-4), glycerol asparagine broth (ISP-5), and nutrient broth were used for the isolation of the actinomycetes isolates from the sample. On starch casein agar, the actinomycete isolates were seen as morphologically different colonies (Figure 2). The majority of the actinomycete isolates belonged to the genus *Streptomyces*, and some were *Micromonospora* species (Table 1).



Figure 2. Isolation of marine Actinomycetes.

Table 1. Cultural characteristics of *Streptomyces phaeolivaceus* VR3 on various culture media.

Si. No	Test	Properties	Media
1.	Growth	Excellent	Starch casein agar (SCA)
	Aerial mycelium	White	
	Substrate mycelium	Yellowish brown	
	Pigmentation	Nil	
2.	Growth	Moderate	Yeast malt extract broth (ISP-2)

Si. No	Test	Properties	Media
	Aerial mycelium	White, Pale grayish	
	Substrate mycelium	Pale yellow, brown	
	Pigmentation	Nil	
3.	Growth	Good	Oatmeal broth (ISP-3)
	Aerial mycelium	Whitish	
	Substrate mycelium	Pale yellow	
	Pigmentation	Nil	
4.	Growth	Excellent	Inorganic salt starch broth (ISP-4)
	Aerial mycelium	Whitish	
	Substrate mycelium	Yellow to slightly brown	
	Pigmentation	Nil	
5.	Growth	Excellent	Glycerol asparagine broth (ISP-5)
	Aerial mycelium	White to pale creamy	
	Substrate mycelium	Yellowish brown	
	Pigmentation	Nil	

3.2. Physiological characterization.

The growth of *S. phaeolivaceus* VR3 isolate was assessed at five different temperatures. The isolate could not grow at the following tested temperatures: 4°C, 10°C, 50°C, and 60°C. Instead, it developed optimally at 20°C, 30°C, and 40°C. Up to 6% salinity (NaCl) and pH 7.0 were also favorable growth conditions for the isolate. Among the six antibiotics tested, the isolate was sensitive only to Bacitracin and Chloramphenicol, and showed resistance to Amikacin, Ampicillin, Streptomycin, and Tetracycline (Table 2).

Table 2. Antibiotic sensitivity test.

Si. No	Tests	<i>Streptomyces phaeolivaceus</i> VR3
1.	Chloramphenicol	Resistant
2.	Amoxicillin	Resistant
3.	Gentamicin	Sensitive
4.	Streptomycin	Sensitive
5.	Ampicillin	Sensitive
6.	Tetracycline	Sensitive

3.3. Morphological characterization.

Actinomycetes display a noteworthy variety of macroscopic characteristics, including the coloration of the spores, aerial and substrate mycelium, and diffusible extracellular pigments. Actinomycetes are distinguished from the other actinomycete groupings primarily by their morphology. The Actinomycete was observed in tight spirals with smooth spore surfaces in both normal and scanning electron microscopic views, as shown in Table 3.

Table 3. Identification and characterization of *Streptomyces phaeolivaceus* VR3.

Si. No	Properties	Actinomycetes
1.	Sporophore Morphology	Spirally twisted
2.	Spore mass	White
3.	Colour of Substrate mycelium	Dull yellowish brown color
4.	Colour of aerial mycelium	Dull white
5.	Spore surface	Smooth
6.	Acid-fast	Non-acid-fast
7.	Gram staining	Positive

3.4. Molecular characterization of *Streptomyces* species.

Streptomyces sp. molecular characteristics were assessed by amplification of the 16S rRNA gene using PCR. In an agarose gel, the amplifying and genomic DNA products were

segregated. A unique 16S rRNA sequence primer (5' AGAGTTTGATCCTGGCTCAG 3' - forward primer and 5' GGTTACCTTGTTACGACTT 3' - reverse primer) was used to partially sequence the 16S rRNA gene of the Actinomycete isolated from the marine coastal region. After processing, the actinomycete 16S rRNA sequence was submitted to GenBank (NCBI) under accession OQ121828, corresponding to the strain *Streptomyces phaeolivaceus* VR3. The analysis involved comparing the marine *Streptomyces* sequence with different sequences found in the EMBL database. The marine isolate *Streptomyces* sp. 1404 bp sequence matched the extant *Streptomyces phaeolivaceus* subsp. Species, according to phylogenetic analysis (neighbor-joining tree).

3.4.1. Identification of actinomycetes.

Based on the morphological, physiological, and molecular properties, the marine isolate of Actinomycete was identified as *Streptomyces phaeolivaceus* (Figure 3). Bergey's Manual of Systematic Bacteriology [52], Bergey's Manual of Determinative Bacteriology [54], and a phylogenetic study utilizing the multiple sequence alignment tool Clustal W all validated the species identification.

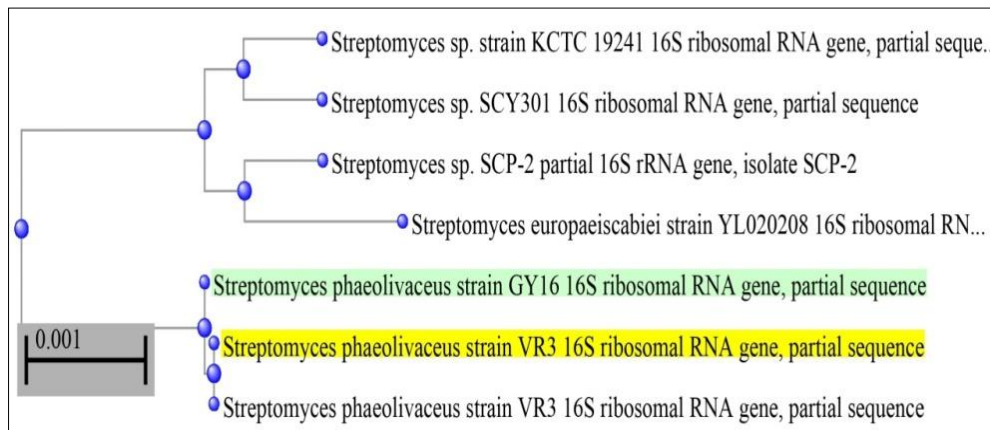


Figure 3. Associations between the streptomycete isolates *Streptomyces phaeolivaceus* VR3 and related species of the genus *Streptomyces* sp. are depicted in neighbor-joining clusters based on an almost full 16S rRNA gene sequence.

3.5. Minimal inhibitory concentration (M.I.C.) of VR3 strain.

The isolated *Streptomyces* VR3 strain was also tested using M.I.C.s, and we discovered that the M.I.C.s for all species ranged from 0.2 to 2.1g/mL. Significant susceptibility to the strain was observed in the tested Bacteria, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Klebsiella pneumonia*. The minimum inhibitory concentration of isolate VR3 against *Klebsiella pneumonia* was 2.125 mg/mL, demonstrating greater resistance among the gram-negative bacteria tested. Whereas *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* demonstrated the lowest M.I.C. values of 0.531 mg/ml and 0.265 mg/ml, *Staphylococcus aureus* was determined to be the gram-positive bacterium that responded to the VR3 the most; the M.I.C. value was 1.062 mg/ml.

3.6. Discussion

Although there is a remarkable variety of life on land, the seas have the highest biodiversity [58]. A few of the 18 major lineages now recognized as belonging to the domain Bacteria, containing five subclasses and 14 suborders, are Actinobacteria [59]. Around 7,000

compounds listed in the Glossary of Natural Products are generated by actinobacteria, bacteria belonging to the Order Actinomycetales (also known as actinomycetes), one of the five subclasses. Actinomycetes develop as aerial mycelia and exhibit a high G+C content in their deoxyribonucleic acid (DNA) [62]. They produce approximately half of the known bioactive molecules, including enzymes, antibacterials [63-65], anticancer properties, and immunosuppressives [66-69]. Over the last few decades, many actinomycetes have been isolated and screened from soil [70]. Recently, the pace of isolating active constituents has grown while the rate of discovering new molecules from terrestrial actinomycetes has dropped [71, 72]. The distribution in the ocean is largely unknown, and the existence of native coastal actinomycetes in the ocean remains a mystery. Research on the diversity of actinomycetes in marine environments has increased significantly over the last 10 years. *Streptomyces* strains are found worldwide in marine and terrestrial environments [73, 74] and are of industrial interest due to their ability to produce new compounds. Some filamentous bacteria can degrade intricate biological polymers and are highly adapted to the marine ecosystem. The family Streptomycetaceae, which contains gram-positive aerobic representatives of the order Actinomycetales and suborder Streptomycineae inside the new class Actinobacteria, would include the genus *Streptomyces*. The DNA G+C content of this family ranges from 68 to 79 mol% [75]. It is well recognized that actinomycetes, particularly *Streptomyces*, have antibacterial properties. In addition to saltwater and sediment, marine *Streptomyces* is extensively found in microbial sources such as mollusks, fish, mangroves, sponges, and seaweed. These microbes are becoming increasingly significant not only from a taxonomic and environmental perspective but also because they produce novel bioactive substances, such as enzymes, antibiotics, enzyme inhibitors, and pigments, and because they have biotechnological uses, such as producing probiotic strains and single-cell proteins [76]. Several types of actinomycete isolation media were used in the procedure; S.A., SCA, and I.S.P. showed promising medium parameters for the isolate's development [77]. Only one marine Actinomycete, *Streptomyces phaeolivaceus* VR3, was shown to have antibacterial action against Gram-positive, Gram-negative, and fungal strains in the current experiments of four actinomycete isolates. The findings demonstrated the huge potential for discovering and characterizing novel chemicals for medicinal uses. It has been found that several human pathogens have up to ten distinct resistance-generating genes [78]. The morphological, biochemical, metabolic, cultural, physiological, and molecular characteristics can all be used to identify *S. phaeolivaceus* VR3. Bacterial colony formation, substrate vegetative and aerial mycelium, formation of 57 sporophores, and spores are among the key features of the characterizing *Streptomyces* strains [79-81]. Since the identification of nucleic acid sequences using sequencing methods, molecular systematics, which involves classification and identification, has taken on a new role. Because it reveals details about the species' phylogenetic position, 16S rRNA sequencing has become increasingly significant as a technique for identifying bacterial strains [82-84]. In this study, of the five isolated actinomycetes, only one marine Actinomycete, *Streptomyces phaeolivaceus* VR3, was identified based on morphological, biochemical, and physiological characteristics. Hence, the current investigation demonstrates that *Streptomyces phaeolivaceus* subsp. and the isolate *S. phaeolivaceus* VR3 is closely related. A polyphasic taxonomic methodology, incorporating morphological, ecological, biochemical, cultural, physiological, and molecular criteria, can only aid in creating an effective taxonomic recognition system for all microbes, not just actinomycetes.

4. Conclusion

The final findings of the present research work isolated, screened, and identified *S. Phaeolivaceus* VR3 from the marine soil sample collected from the Marakkanam Coastal Area, which showed good antibacterial activity. The spore chain structure of the strain *Streptomyces phaeolivaceus* VR3 revealed that the spores were simple, transverse, Gram-positive, and smooth-surfaced. The aerial mycelia of the isolate, while cultivated on various media, were generally white, whereas the substrate was creamy white. The isolate VR3 showed antibacterial activity against *Escherichia coli* (9 mm), *Staphylococcus aureus* (7.5 mm), *Proteus mirabilis* (7.7 mm), *Pseudomonas aeruginosa* (7 mm), and *Klebsiella pneumonia* (4.5 mm) at a concentration of 3.5mg/ml. The minimum inhibitory concentration of the isolate VR3 was 0.531 mg/ml for *Escherichia coli* and *Pseudomonas aeruginosa*, 1.062 mg/ml for *Staphylococcus aureus*, 0.265 mg/ml for *Proteus mirabilis*, and 2.125 mg/ml for *Klebsiella pneumonia*. The isolated *Streptomyces* strain can produce a lipopeptide with various industrial applications.

Author Contributions

Conceptualization, S.R. and J.R.; methodology, S.R.; investigation, writing—original draft preparation, S.R.; writing—review and editing, S.R.; supervision, J.R. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Meklat, A.; Sabaou, N.; Zitouni, A.; Mathieu, F.; Lebrihi, A. Isolation, Taxonomy, and Antagonistic Properties of Halophilic Actinomycetes in Saharan Soils of Algeria. *Appl. Environ. Microbiol.* **2011**, *77*, 6710-6714, <https://doi.org/10.1128/AEM.00326-11>.
2. Oskay, A.M.; Üsame, T.; Cem, A. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *Afr. J. Biotechnol.* **2004**, *3*, 441-446, <https://doi.org/10.5897/AJB2004.000-2087>.
3. Tang, S.L.; Nuttall, S.; Ngui, K.; Fisher, C.; Lopez, P.; Dyll-Smith, M. HF2: a double-stranded DNA tailed haloarchaeal virus with a mosaic genome. *Mol. Microbiol.* **2002**, *44*, 283-296, <https://doi.org/10.1046/j.1365-2958.2002.02890.x>.
4. Schneegurt, M.A. Media and Conditions for the Growth of Halophilic and Halotolerant Bacteria and Archaea. In *Advances in Understanding the Biology of Halophilic Microorganisms*, Vreeland, R.H., Ed.; Springer Netherlands: Dordrecht, **2012**; pp. 35-58, https://doi.org/10.1007/Correc978-94-007-5539-0_2.
5. Chaudhary, H.S.; Soni, B.; Shrivastava, A.R.; Shrivastava, S. Diversity and versatility of actinomycetes and its role in antibiotic production. *J. Appl. Pharm. Sci.* **2013**, *3*, S83-S94.
6. El Karkouri, A.; Assou, S.A.; El Hassouni, M. Isolation and screening of actinomycetes producing antimicrobial substances from an extreme Moroccan biotope. *Pan Afr. Med. J.* **2019**, *33*, 329, <https://doi.org/10.11604/pamj.2019.33.329.19018>.
7. Ravikumar, S.; Inbaneson, S.J.; Uthiraselvam, M.; Priya, S.R.; Ramu, A.; Banerjee, M.B. Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. *J. Pharm. Res.* **2011**, *4*, 294-296.
8. Ogunmwonyi, I.H.; Mazomba, N.; Mabinya, L.; Ngwenya, E.; Green, E.; Akinpelu, D.A.; Olaniran, A.O.; Bernard, K.; Okoh, A.I. Studies on the culturable marine actinomycetes isolated from the Nahoon beach in the Eastern Cape Province of South Africa. *Afr. J. Microbiol. Res.* **2010**, *4*, 2223-2230.
9. Atta, H.M.; Dabour, S.M.; Desoukey, S.G. Sparsomycin Antibiotic Production by *Streptomyces* Sp. AZ-NIOFD1: Taxonomy, Fermentation, Purification and Biological Activities. *J. Agric. Environ. Sci.* **2009**, *5*, 368-377.
10. Jagannathan, S.V.; Manemann, E.M.; Rowe, S.E.; Callender, M.C.; Soto, W. Marine Actinomycetes, New Sources of Biotechnological Products. *Marine Drugs* **2021**, *19*, 365, <https://doi.org/10.3390/md19070365>.
11. Helmke, E.; Weyland, H. *Rhodococcus marinonascens* sp. nov., an Actinomycete from the Sea. *Int. J. Syst. Evol. Microbiol.* **1984**, *34*, 127-138, <https://doi.org/10.1099/00207713-34-2-127>.
12. Colquhoun, J.A.; Mexson, J.; Goodfellow, M.; Ward, A.C.; Horikoshi, K.; Bull, A.T. Novel rhodococci and other mycolate actinomycetes from the deep sea. *Antonie van Leeuwenhoek* **1998**, *74*, 27-40, <https://doi.org/10.1023/A:1001743625912>.
13. Mincer Tracy, J.; Jensen Paul, R.; Kauffman Christopher, A.; Fenical, W. Widespread and Persistent Populations of a Major New Marine Actinomycete Taxon in Ocean Sediments. *Appl. Environ. Microbiol.* **2002**, *68*, 5005-5011, <https://doi.org/10.1128/AEM.68.10.5005-5011.2002>.
14. Yi, H.; Schumann, P.; Sohn, K.; Chun, J. *Serinicoccus marinus* gen. nov., sp. nov., a novel actinomycete with L-ornithine and L-serine in the peptidoglycan. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 1585-1589, <https://doi.org/10.1099/ijss.0.03036-0>.
15. Das, S.; Lyla, P.S.; Ajmal Khan, S. Distribution and generic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal. *Chin. J. Oceanol. Limno.* **2008**, *26*, 166-177, <https://doi.org/10.1007/s00343-008-0166-5>.
16. Freel, K.C.; Edlund, A.; Jensen, P.R. Microdiversity and evidence for high dispersal rates in the marine actinomycete '*Salinispora pacifica*'. *Environ. Microbiol.* **2012**, *14*, 480-493, <https://doi.org/10.1111/2Fj.1462-2920.2011.02641.x>.
17. Ghanem, N.B.; Sabry, S.A.; El-Sherif, Z.M.; Abu El-Ela, G.A. Isolation and enumeration of marine actinomycetes from seawater and sediments in Alexandria. *J. Gen. Appl. Microbiol.* **2000**, *46*, 105-111, <https://doi.org/10.2323/jgam.46.105>.
18. Solanki, R.; Khanna, M.; Lal, R. Bioactive compounds from marine actinomycetes. *Indian J. Microbiol.* **2008**, *48*, 410-431, <https://doi.org/10.1007/2Fs12088-008-0052-z>.
19. Thenmozhi, M.; Kannabiran, K. Anti-Aspergillus activity of *Streptomyces* sp. VITSTK7 isolated from Bay of Bengal coast of Puducherry, India. *J. Nat. Environ. Sci.* **2011**, *2*.

20. Valli, S.; Suvathi, S.S.; Aysha, O.S.; Nirmala, P.; Vinoth, K.P.; Reena, A. Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, 469-473, [https://doi.org/10.1016/S2221-1691\(12\)60078-1](https://doi.org/10.1016/S2221-1691(12)60078-1).
21. Bull, A.T.; Stach, J.E.M.; Ward, A.C.; Goodfellow, M. Marine actinobacteria: perspectives, challenges, future directions. *Antonie van Leeuwenhoek* **2005**, *87*, 65-79, <https://doi.org/10.1007/s10482-004-6562-8>.
22. Stach, J.E.; Maldonado, L.A.; Ward, A.C.; Goodfellow, M.; Bull, A.T. New primers for the class Actinobacteria: application to marine and terrestrial environments. *Environ. Microbiol.* **2003**, *5*, 828-841, <https://doi.org/10.1046/j.1462-2920.2003.00483.x>.
23. Hughes, C.C.; Prieto-Davo, A.; Jensen, P.R.; Fenical, W. The Marinopyrroles, Antibiotics of an Unprecedented Structure Class from a Marine *Streptomyces* sp. *Org. Lett.* **2008**, *10*, 629-631, <https://doi.org/10.1021/ol702952n>.
24. Ye, L.; Zhou, Q.; Liu, C.; Luo, X.; Na, G.; Xi, T. Identification and fermentation optimization of a marine-derived *Streptomyces Griseorubens* with anti-tumor activity. *Indian J. Mar. Sci.* **2009**, *38*, 14–21.
25. Abussaud, M.J.; Alanagreh, L.; Abu-Elteen, K. Isolation, characterization and antimicrobial activity of *Streptomyces* strains from hot spring areas in the northern part of Jordan. *Afr. J. Biotechnol.* **2013**, *12*, 7124-7132.
26. Parte, A.C. List of prokaryotic names with standing in nomenclature: *Int J Syst Evol Microbiol.* **2018** *68*(6), 1825-1829. <https://doi.org/10.1099/ijsem.0.002786>.
27. Rammali, S.; Hilali, L.; Dari, K.; Bencharki, B.; Rahim, A.; Timinouni, M.; Gaboune, F.; El Aalaoui, M.; khattabi, A. Antimicrobial and antioxidant activities of *Streptomyces* species from soils of three different cold sites in the Fez-Meknes region Morocco. *Sci. Rep.* **2022**, *12*, 17233, <https://doi.org/10.1038/s41598-022-21644-z>.
28. Madigan, M.T.; Martinko, J. Brock Biology of Microorganisms. 11th Edition, Prentice-Hall, U.S.A.: New Jersey, **2005**.
29. Sane, T.; Mulay, M.; Jha, V.; Chidrala, S.; Mange, A. Screening and Characterization of *Streptomyces* spp. Isolated from soil Producing a Potential Inhibitors of the *Proteus mirabilis*. *J. Biotechnol. Bioinforma. Res.* **2023**, *5*, 1-10.
30. Antido, J.W.A.; Climacosa, F.M.M. Enhanced Isolation of *Streptomyces* from Different Soil Habitats in Calamba City, Laguna, Philippines using a Modified Integrated Approach. *International Journal of Microbiology* **2022**, *2022*, 2598963, <https://doi.org/10.1155/2022/2598963>.
31. Ribeiro da Cunha, B.; Fonseca, L.P.; Calado, C.R.C. Antibiotic Discovery: Where Have We Come from, Where Do We Go?. *Antibiotics* **2019**, *8*, 45, <https://doi.org/10.3390/antibiotics8020045>.
32. Chater, K.F. *Streptomyces* inside-out: a new perspective on the bacteria that provide us with antibiotics. *Philos. Trans. R. Soc. B: Biol. Sci.* **2006**, *361*, 761-768, <https://doi.org/10.1098%2Frstb.2005.1758>.
33. Quinn, G.A.; Banat, A.M.; Abdelhameed, A.M.; Banat, I.M. *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. *J. Med. Microbiol.* **2020**, *69*, 1040-1048, <https://doi.org/10.1099%2Fjmm.0.001232>.
34. Maleki, H.; Dehnad, A.; Hanifian, S.; Khani, S. Isolation and Molecular Identification of *Streptomyces* spp. with Antibacterial Activity from Northwest of Iran. *BioImpacts : B.I.* **2013**, *3*, 129-134, <https://doi.org/10.5681%2Fbi.2013.017>.
35. Sahu, B.; Roymon, M.G. REVIEW ON CURRENT TECHNIQUES IN ISOLATION AND CHARACTERIZATION OF *Streptomyces* FROM SOIL. *Indian J. Sci. Res.* **2017**, *13*, 226–232.
36. Ogundare, A.O.; Ekundayo, F.O.; Banji-Onisile, F. Antimicrobial activities of *Streptomyces* species isolated from various soil samples in Federal University of Technology, Akure environment. *IOSR- J. Pharm. Biol. Sci.* **2015**, *10*, 22-30.
37. El-Hussein, A.A.; Alhasan, R.E.M.; Abdelwahab, S.A.; El-Siddig, M.A. Isolation and identification of *Streptomyces rochei* strain active against phytopathogenic fungi. *Br. Microbiol. Res. J.* **2014**, *4*, 1057–1068.
38. Laskaris, P.; Tolba, S.; Calvo-Bado, L.; Wellington, E.M. Coevolution of antibiotic production and counter-resistance in soil bacteria. *Environ. Microbiol.* **2010**, *12*, 783-796, <https://doi.org/10.1111/j.1462-2920.2009.02125.x>.
39. Lacey, H.J.; Rutledge, P.J. Recently Discovered Secondary Metabolites from *Streptomyces* Species. *Molecules* **2022**, *27*, 887, <https://doi.org/10.3390/molecules27030887>.
40. Jones, G.H. Actinomycin production persists in a strain of *Streptomyces antibioticus* lacking phenoxazinone synthase. *Antimicrob. Agents Chemother.* **2000**, *44*, 1322-1327, <https://doi.org/10.1128%2Faac.44.5.1322-1327.2000>.

41. Ait Assou, S.; Anissi, J.; Sendide, K.; El Hassouni, M. Diversity and Antimicrobial Activities of *Actinobacteria* Isolated from Mining Soils in Midelt Region, Morocco. *Sci. World J.* **2023**, *2023*, 6106673, <https://doi.org/10.1155/2023/6106673>.
42. Hamza, A.A.; Ali, H.A.; Clark, B.R.; Murphy, C.D.; Elobied, E.A. Optimization of fermentation conditions for actinomycin D production by a newly isolated *Streptomyces* sp. AH 11.4. *E3 J. Biotechnol. Pharm. Res* **2013**, *4*, 29-34.
43. Hamza, A.A.; Hassan, M.N.; Elyass, M.E. ISOLATION AND CHARACTERIZATION OF *Streptomyces* ISOLATES AS A SOURCE OF BIOACTIVE SECONDARY METABOLITES IN SUDAN. *J. Global Biosci.* **2015**, *4*, 2649–2661.
44. Ramakrishnan, J.; Shunmugasundaram, M.; Narayanan, M. *Streptomyces* sp. SCBT Isolated from Rhizosphere Soil of Medicinal Plants is Antagonistic to Pathogenic Bacteria. *Iran. J. Biotechnol.* **2009**, *7*, 75-81.
45. Sambamurthy, K.; Ellaiah, P. A new streptomycete producing neomycin (B&C) complex-*S. marinensis* (Part I). *Hindustan Antibiot. Bull.* **1974**, *17*, 24–28.
46. Mazumdar, R.; Dutta, P.P.; Saikia, J.; Borah, J.C.; Thakur, D. *Streptomyces* sp. strain PBR11, a forest-derived soil *Actinomycetia* with antimicrobial potential. *Microbiology Spectrum* **2023**, *11*, e03489-03422, <http://dx.doi.org/10.1128/spectrum.03489-22>.
47. Wilson, K. Preparation of genomic DNA from bacteria. In *Current protocols in Molecular Biology*, Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., Eds.; John Wiley & Sons: New York, **1990**; 241-245.
48. Weisburg, W.G.; Barns, S.M.; Pelletier, D.A.; Lane, D.J. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **1991**, *173*, 697-703, <https://doi.org/10.1128/jb.173.2.697-703.1991>.
49. Higgins, D.G.; Bleasby, A.J.; Fuchs, R. CLUSTAL V: improved software for multiple sequence alignment. *Bioinformatics* **1992**, *8*, 189-191, <https://doi.org/10.1093/bioinformatics/8.2.189>.
50. Al-Joubori, B.; Saadoun, I.; Hotchin, N.; Cunningham, D.; Alderwick, L. The isolation of novel terrestrial *Streptomyces* strains with antimicrobial and cytotoxic properties. *Arab J. Basic Appl. Sci.* **2023**, *30*, 285-298, .
51. Math, H.H.; Nayaka, S.; Rudrappa, M.; Kumar, R.S.; Almansour, A.I.; Perumal, K.; Kantli, G.B. Isolation, Characterization of Pyraclostrobin Derived from Soil Actinomycete *Streptomyces* sp. HSN-01 and Its Antimicrobial and Anticancer Activity. *Antibiotics* **2023**, *12*, 1211, <https://doi.org/10.3390/antibiotics12071211>.
52. Williams, S.T.; Goodfellow, M.; Alderson, G. Genus *Streptomyces* Waksman and Henrici 1943, 339AL. In *Bergey's Manual of Systematic Bacteriology*. Williams S.T., Sharpe, M.E. and Holt, J.G., Eds.; Williams and Wilkins, Baltimore, **1989**; Volume 4, 2452–2492.
53. Nayariseri, A.; Singh, P.; Singh, S.K. Screening, isolation and characterization of biosurfactant producing *Bacillus subtilis* strain ANSKLAB03. *Bioinformation* **2018**, *14*, 304-314, <https://doi.org/10.6026/97320630014304>.
54. Buchanan, R.E.; Gibbons, N.E. *Bergey's Manual of Determinative Bacteriology*. Eighth Edition, The Williams & Wilkins Co.: Baltimore, **1974**.
55. Lyons, A.J.; Pridham, T.G. Standard antimicrobial spectra as aids in characterization and Identification of actinomycetales. *Develop. Indus. Microbiol.* **1973**, *14*, 205-211.
56. Wang, X.; Huang, L.; Kang, Z.; Buchenauer, H.; Gao, X. Optimization of the Fermentation Process of Actinomycete Strain Hhs.015^T. *J. Biomed. Biotechnol.* **2010**, *2010*, 141876, <https://doi.org/10.1155/2010/141876>.
57. Djebbah, F.Z.; Belyagoubi, L.; Abdelouahid, D.E.; Kherbouche, F.; Al-Dhabi, N.A.; Arasu, M.V.; Ravindran, B. Isolation and characterization of novel *Streptomyces* strain from Algeria and its in-vitro antimicrobial properties against microbial pathogens. *J. Infect. Public Health* **2021**, *14*, 1671-1678, <https://doi.org/10.1016/j.jiph.2021.09.019>.
58. Egorov, N.S. *Antibiotics, A Scientific Approach*. Mir Publishers: Moscow, **1985**.
59. Schillinger, U.; Lücke, F.K. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* **1989**, *55*, 1901-1906, <https://doi.org/10.1128%2Ffaem.55.8.1901-1906.1989>.
60. Alam, K.; Mazumder, A.; Sikdar, S.; Zhao, Y.M.; Hao, J.; Song, C.; Wang, Y.; Sarkar, R.; Islam, S.; Zhang, Y.; Li, A. *Streptomyces*: The biofactory of secondary metabolites. *Front Microbiol* **2022**, *13*, 968053, <https://doi.org/10.3389%2Ffmich.2022.968053>.

61. Atta H. M. Biochemical studies on antibiotic production from *Streptomyces* sp.: taxonomy, fermentation, isolation and biological properties. *Journal of Saudi Chemical Society*. 2015;**19**(1):12–22. doi: 10.1016/j.jscs.2011.12.011.
62. Baron, J.E.; Peterson, R.L.; Finegold, M.S. Cultivation and isolation of viable pathogens. In *Diagnostic Microbiology*, 9th Edition, Mosby: London, **1994**; 79-96.
63. Donia, M.; Hamann, M.T. Marine natural products and their potential applications as anti-infective agents. *The Lancet. Infect. Dis.* **2003**, *3*, 338-348, [https://doi.org/10.1016/s1473-3099\(03\)00655-8](https://doi.org/10.1016/s1473-3099(03)00655-8).
64. Shirling E. B., Gottlieb D. Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*. 1966;**16**(3):313–340. doi: 10.1099/00207713-16-3-313.
65. Yoshida, A.; Seo, Y.; Suzuki, S.; Nishino, T.; Kobayashi, T.; Hamada-Sato, N.; Kogure, K.; Imada, C. Actinomycetal Community Structures in Seawater and Freshwater Examined by DGGE Analysis of 16S rRNA Gene Fragments. *Mar. Biotechnol.* **2008**, *10*, 554-563, <https://doi.org/10.1007/s10126-008-9092-y>.
66. Bull, A.T. *Microbial Diversity and Bioprospecting*. A.S.M. Press: Washington, **2004**.
67. Bérdy, J. Bioactive Microbial Metabolites. *The Journal of antibiotics* **2005**, *58*, 1-26, <https://doi.org/10.1038/ja.2005.1>.
68. Strohl, W.R. Antimicrobials. In *Microbial Diversity And Bioprospecting*, Bull, A.T., Ed.; A.S.M. Press: Washington, **2004**; 336–355.
69. Olano, C.; Mendez, C.; and Salas, J.A. Antitumour compounds from marine actinomycetes. *Mar. Drugs* **2009**, *7*, 210–248. doi: [10.3390/md7020210](https://doi.org/10.3390/md7020210)
70. Mann, J. Natural products as immunosuppressive agents. *Nat. Prod. Rep.* **2001**, *18*, 417-430, <https://doi.org/10.1039/B001720P>.
71. Pecznaska-Czoch, W.; Mordarski, M. Actinomycete enzymes. In *Actinomycetes in Biotechnology*, Goodfellow, M., Williams, S.T., Mordarski, M., Eds.; Academic Press: London, **1998**; 219–283, <https://doi.org/10.1016/B978-0-12-289673-6.50011-7>.
72. Oldfield, C.; Wood, N.T.; Gilbert, S.C.; Murray, F.D.; Faure, F.R. Desulphurisation of benzothiophene and dibenzothiophene by actinomycete organisms belonging to the genus *Rhodococcus*, and related taxa. *Antonie van Leeuwenhoek* **1998**, *74*, 119-132, <https://doi.org/10.1023/A:1001724516342>.
73. Fenical, W.; Baden, D.; Burg, M.; De Goyet, C.V.; Grimes, J.D.; Katz, M.; Marcus, N.H.; Pomponi, S.; Rhines, P.; Tester, P. Marine derived pharmaceuticals and related bioactive compounds. From Monsoons to Microbes: Understanding the Ocean's Role in Human Health. Washington, **1999**, 71-86.
74. Fenical, W.; Jensen, P.R. Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* **2006**, *2*, 666-673, <https://doi.org/10.1038/nchembio841>.
75. Stackebrandt, E.; Rainey, F.A.; Ward-Rainey, N.L. Proposal for a new hierarchic classification system, Actinobacteria classis nov. *Int. J. System. Evolut. Microbiol.* **1997**, *47*, 479-491, <https://doi.org/10.1099/00207713-47-2-479>.
76. Pathom-Aree, W.; Stach, J.E.; Ward, A.C.; Horikoshi, K.; Bull, A.T.; Goodfellow, M. Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. *Extremophiles* **2006**, *10*, 181-189, <https://doi.org/10.1007/s00792-005-0482-z>.
77. Elias, F.; Muddada, S.; Muleta, D.; Tefera, B. Purification and Characterization of Bioactive Metabolite from *Streptomyces monomycini* RVE129 Derived from the Rift Valley Soil of Hawassa, Ethiopia. *BioMed Res.Int.* **2022**, *2022*, 7141313, <https://doi.org/10.1155/2022/7141313>.
78. Anderson, A.S.; Wellington, E.M. The taxonomy of *Streptomyces* and related genera. *Int. J. System. Evolut. Microbiol.* **2001**, *51*, 797-814, <https://doi.org/10.1099/00207713-51-3-797>.
79. Moncheva, S.; Dontcheva, V.; Shtereva, G.; Kamburska, L.; Malej, A.; Gorinstein, S. Application of eutrophication indices for assessment of the Bulgarian Black Sea coastal ecosystem ecological quality. *Water Sci. Technol.* **2002**, *46*, 19-28, <http://dx.doi.org/10.2166/wst.2002.0136>.
80. Henry, C.M. Antibiotic Resistance. *Chem. Eng. News* **2000**, *78*, 41-58, <https://doi.org/10.1021/cen-v078n010.p041>.
81. Waksman, S.A. Species concept among the actinomycetes with special reference to the genus *Streptomyces*. *Bacteriol. Rev.* **1957**, *21*, 1-29, <https://doi.org/10.1128/br.21.1.1-29.1957>.
82. Waksman, S.A. *The Actinomycetes*. Vol. II. Classification, identification and descriptions of genera and species. **1961**.
83. Kuster, E. MORPHOLOGICAL AND PHYSIOLOGICAL ASPECTS OF THE TAXONOMY OF STREPTOMYCETES. *Microbiol. Esp.* **1963**, *16*, 193-202.

84. O'Donnell, A.G.; Embley, T.M.; Goodfellow, M. Future of Bacterial Systematics. In Handbook of New Bacterial Systematics, Academic Press: London, **1993**; 513-524.

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