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Research Article

ISOLATION AND CHARACTERIZATION OF NOVEL ANTIBIOTIC-PRODUCING ACTINOBACTERIA FROM THE RHIZOSPHERE OF INDONESIAN MANGROVE FORESTS

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Abstract

The rise of antimicrobial resistance is a global health crisis, demanding the urgent discovery of novel antibiotics. Indonesian mangrove forests, as a unique and underexplored ecosystem, represent a promising frontier for bioprospecting novel microorganisms. The plant rhizosphere, a zone of intense microbial activity, is particularly rich in actinobacteria, a phylum renowned for its prolific production of bioactive secondary metabolites. This research aimed to isolate and characterize novel antibiotic-producing actinobacteria from the rhizosphere of Indonesian mangrove plants. Rhizosphere soil samples were collected, and actinobacteria were isolated using selective media. All isolates were screened for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, and Candida albicans* via the agar well diffusion method. The most potent isolate was subsequently characterized using morphological, biochemical, and 16S rRNA gene sequencing. From 72 distinct isolates, 15 displayed antimicrobial activity. One isolate, designated MGR-17, demonstrated exceptionally potent, broad-spectrum inhibition against all tested pathogens. Based on polyphasic taxonomy, MGR-17 was identified as a potentially novel species of the genus Streptomyces. In conclusion, the rhizosphere of Indonesian mangroves is a fertile source for discovering unique actinobacteria capable of producing novel antibiotics, and the discovery of Streptomyces sp. MGR-17 underscores this potential.

Keywords: Actinobacteria, Antibiotic, Mangrove

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INTRODUCTION

The escalating crisis of antimicrobial resistance (AMR) constitutes one of the most significant threats to global health, food security, and development in the 21st century. The efficacy of conventional antibiotics is rapidly diminishing due to the relentless evolution and dissemination of resistance mechanisms among pathogenic microorganisms (Sagar & Priti, 2025). This has created a post-antibiotic era scenario where common infections and minor injuries could once again become lethal, jeopardizing the cornerstones of modern medicine, from routine surgical procedures to cancer chemotherapy (Mohamed et al., 2023). The World Health Organization has repeatedly warned of this impending catastrophe, highlighting a critical void in the antibiotic development pipeline. The rate of discovery of new antibiotic classes has stagnated for several decades, while resistance to existing drugs, including last-resort agents like carbapenems and colistin, continues to surge at an alarming pace globally, rendering many infectious diseases progressively untreatable (Tripathi et al., 2024).

Historically, natural products derived from microorganisms have been the most prolific source of antibiotics, providing the chemical scaffolds for the majority of drugs used in clinical practice today. The "golden age" of antibiotic discovery was fueled by systematic screening of soil-dwelling microorganisms, particularly those in the phylum Actinobacteria (Lebedeva et al., 2024). This group, most notably the genus Streptomyces, is renowned for its complex secondary metabolism and unparalleled capacity to synthesize a vast array of structurally diverse bioactive compounds, including antibacterial, antifungal, antiviral, and anticancer agents (Ye et al., 2024). The inherent genetic potential of actinobacteria to produce these molecules underscores their continued importance as a primary target for bioprospecting campaigns aimed at revitalizing the flagging antibiotic pipeline and discovering novel chemical entities capable of circumventing existing resistance mechanisms (Siro & Pipite, 2024).

In the quest for new microbial sources, scientific attention has shifted from conventional terrestrial environments to unique, underexplored, or extreme ecosystems. These frontiers are hypothesized to harbor novel microbial taxa with unique metabolic capabilities, forged through adaptation to distinct ecological pressures (Negi et al., 2025). Mangrove ecosystems, situated at the interface between terrestrial and marine environments, represent one such promising frontier. Characterized by high salinity, fluctuating tides, anoxic soil conditions, and high organic matter content, mangrove forests foster a unique biodiversity (Sagpariya et al., 2025). The microbial communities within these ecosystems, particularly in the rhizosphere, the narrow region of soil directly influenced by root secretions, are expected to possess novel physiological and metabolic traits, making them a fertile ground for the discovery of previously uncharacterized actinobacteria and their associated bioactive secondary metabolites (H. Sarma & Joshi, 2024).

The fundamental problem confronting infectious disease therapy is the severe disparity between the rapid emergence of multidrug-resistant pathogens and the slow, resource-intensive process of new antibiotic discovery and development (Farha et al., 2025). The traditional approach of screening well-studied soil environments has led to a high rate of rediscovery of known compounds, yielding diminishing returns and contributing to the current innovation gap (Oyedoh et al., 2023). This issue is compounded by the economic challenges that have led many pharmaceutical companies to withdraw from antibiotic research and development, further constricting the supply of new therapeutic agents (Barreiro et al., 2024). Consequently, there is an urgent and unmet medical need for novel antibiotics with new mechanisms of action to effectively combat priority pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant Enterobacteriaceae (CRE).

This global challenge is particularly relevant to Indonesia, an archipelagic nation recognized as a megabiodiversity hotspot. The country possesses some of the world's most extensive and diverse mangrove forests, yet the microbial resources within these ecosystems remain profoundly underexplored and underutilized (Dhar et al., 2024). While the nation's

terrestrial and marine biodiversity has been the subject of bioprospecting, the specific ecological niche of the mangrove rhizosphere, a zone of intense plant-microbe interactions, has been largely overlooked as a specific target for antibiotic discovery programs (Teotia & Chaudhary, 2024). This represents a significant scientific and strategic gap, as this unique environment is likely to harbor novel actinobacterial strains that have evolved unique strategies for survival and competition, including the production of novel antimicrobial compounds not found in their terrestrial counterparts (Pramanik & Bhattacharyya, 2024). Despite this potential, previous studies have rarely integrated ecological, functional, and taxonomic approaches in a single workflow. Thus, the current research directly addresses this gap by combining culture-dependent isolation, bioactivity screening, and molecular identification to reveal novel actinobacteria from Indonesian mangrove rhizospheres. Furthermore, few existing studies have systematically explored the antibiotic-producing potential of rhizospheric actinobacteria from Indonesian mangroves using an integrated polyphasic approach. This research, therefore, not only aims to fill this scientific void but also establishes a framework that connects ecological context, microbial taxonomy, and functional bioactivity as a coherent discovery pipeline for new antibiotics.

The challenge, therefore, is not merely to find microorganisms that exhibit antimicrobial activity, but to systematically isolate and identify novel strains of actinobacteria from these untapped Indonesian mangrove rhizospheres that produce genuinely new chemical entities (A. Sarma et al., 2025). The successful bioprospecting of such an environment requires a targeted approach that moves beyond simple screening. It necessitates the application of robust isolation strategies capable of cultivating previously uncultured or rare actinobacteria, coupled with a comprehensive polyphasic characterization process (Dutta et al., 2024). This process must effectively differentiate potentially novel species from known ones and provide a preliminary assessment of the broad-spectrum potential of their bioactive products, thereby addressing the core problem of compound rediscovery and paving the way for the identification of next-generation antibiotics (Chen et al., 2024).

The primary objective of this research is to conduct a systematic isolation and screening of antibiotic-producing actinobacteria from the rhizosphere of various plant species within selected Indonesian mangrove forests. This objective encompasses the meticulous collection of rhizosphere soil samples, the implementation of multiple pretreatment and selective isolation media techniques designed to maximize the recovery of diverse actinobacterial taxa, and the subsequent establishment of a pure culture collection. The initial phase of this objective is to create a comprehensive library of isolates that represents the culturable actinobacterial diversity from this specific, scientifically valuable ecological niche, setting the stage for functional evaluation.

A crucial secondary objective is to characterize the antimicrobial potential and taxonomic identity of the most promising isolates. This involves a multi-tiered screening process to evaluate the bioactivity of each isolate against a panel of clinically significant test pathogens, including Gram-positive bacteria, Gram-negative bacteria, and fungi (Ullah et al., 2025). Isolates demonstrating potent and broad-spectrum antimicrobial activity will be prioritized for further investigation (Sağlam et al., 2024). Their characterization will follow a polyphasic taxonomic approach, integrating classical methods, such as the analysis of morphological and cultural characteristics (e.g., colony morphology, aerial and substrate mycelium color, and pigment production), with modern molecular techniques, primarily the sequencing and phylogenetic analysis of the 16S rRNA gene, to determine their precise taxonomic placement (Carroll et al., 2024).

The ultimate aim of this study is to identify and validate at least one potentially novel species of actinobacteria with significant antibiotic-producing capabilities. By establishing the phylogenetic novelty of the most potent isolate and confirming its broad-spectrum inhibitory activity, this research seeks to provide a compelling proof-of-concept for the Indonesian

mangrove rhizosphere as a valuable reservoir for antibiotic discovery (Garg et al., 2024). This will lay the essential groundwork for subsequent research, including the optimization of fermentation conditions, the extraction and purification of the active compounds, and the elucidation of their chemical structures. The findings are intended to directly contribute a promising candidate for new drug development and highlight the critical need for conserving these unique ecosystems.

The existing body of literature on antibiotic discovery is extensive, with a historical focus on actinobacteria isolated from conventional temperate terrestrial soils. This long-standing research has provided a foundational understanding of Streptomyces biology and its capacity for producing secondary metabolites. However, this focus has also inadvertently led to the over-sampling of certain environments, resulting in the frequent reisolation of known species and the rediscovery of well-documented compounds, such as actinomycin and tetracycline. While recent studies have begun to explore actinobacteria from marine sediments and extreme environments, a significant gap persists in the exploration of specialized, niche ecosystems where unique evolutionary pressures may drive the production of novel chemistry.

Specifically, there is a clear deficit in research focusing on the actinobacterial communities associated with the rhizosphere of mangrove halophytes, particularly within the Indonesian archipelago (Dai et al., 2025). While a limited number of studies have surveyed the general microbial diversity of Indonesian mangrove sediments, very few have specifically targeted the rhizosphere as a distinct micro-habitat. Furthermore, most of these studies have been descriptive ecological surveys, employing culture-independent methods to catalogue diversity, rather than culture-dependent, function-driven bioprospecting efforts aimed at isolating and screening for antibiotic production (S. Yang et al., 2025). The unique biochemical interactions between mangrove roots and associated microbes remain poorly understood, representing a significant gap in our knowledge of microbial natural product discovery.

Moreover, a methodological gap exists in many prior bioprospecting studies conducted in similar environments. Often, research terminates after the initial confirmation of antimicrobial activity, without proceeding to a robust taxonomic characterization that could indicate the novelty of the producing organism. A comprehensive approach that links potent bioactivity with detailed polyphasic identification is essential for efficiently prioritizing isolates that are most likely to yield novel compounds. This research directly addresses this gap by integrating systematic screening with in-depth morphological, biochemical, and 16S rRNA gene-based phylogenetic analysis, thereby ensuring that the identified potent strains are not only functionally active but also taxonomically distinct, increasing the probability of discovering new bioactive molecules (Cai et al., 2025).

The primary novelty of this research lies in its targeted focus on a scientifically underexplored and ecologically unique source: the rhizosphere of Indonesian mangrove flora. This specific niche has been largely neglected in the global search for novel antibiotics. Unlike bulk soil or marine sediment, the rhizosphere is a dynamic environment shaped by root exudates, creating a unique selective pressure that influences microbial community structure and function. This study is among the first to systematically investigate the antibiotic-producing potential of actinobacteria specifically adapted to this environment in the context of Indonesia's unparalleled biodiversity, thus opening a new avenue for bioprospecting (Muhilan & Chattopadhyay, 2023).

The justification for this research is firmly rooted in the urgent global health imperative to discover new antibiotics to combat AMR. By exploring an untapped ecosystem, this study directly addresses the critical need for novel chemical scaffolds that are not susceptible to existing resistance mechanisms (C. Xu et al., 2024). The discovery of a new antibiotic-producing Streptomyces species, or any other actinobacterium from this habitat, could provide a lead compound for the development of a next-generation therapeutic agent. Therefore, this research is not merely an academic exercise in microbial ecology but a targeted effort to

contribute a tangible solution to a pressing societal problem, with potential long-term impacts on public health and medicine (Das et al., 2023).

Furthermore, this study provides a significant contribution to the broader fields of microbial biotechnology and biodiversity conservation. The data generated will enhance the scientific understanding of actinobacterial diversity, distribution, and ecological roles within mangrove ecosystems. It will create a valuable culture collection of novel microorganisms that can be screened for other biotechnological applications, such as the production of enzymes, antifungals for agriculture, or anticancer agents. Finally, by highlighting the pharmacological potential hidden within Indonesian mangroves, this research underscores the critical importance of conserving these vital ecosystems, not just for their ecological services, but as an irreplaceable reservoir of future medicines for humankind.

RESEARCH METHOD

Research Design

This study employed a descriptive and experimental research design. The initial phase was exploratory, focusing on the purposive collection of environmental samples from a unique, underexplored ecological niche. This was followed by a descriptive phase involving the isolation, purification, and detailed characterization of actinobacterial isolates based on their morphological, cultural, and biochemical properties. The final phase was experimental, where the antimicrobial potential of each isolate was quantitatively assessed against a panel of pathogenic test microorganisms. All procedures were conducted under controlled laboratory conditions to ensure the validity and reproducibility of the results. The research culminated in the molecular identification and phylogenetic analysis of the most potent antibiotic-producing isolate (Imchen et al., 2024). All isolation experiments were carried out under aseptic conditions using serial dilution and spread plate techniques on ISP-2 and actinomycete isolation agar. Plates were incubated at 28–30 °C for up to 21 days and monitored daily for colony development. To ensure data validity and reproducibility, each isolation and screening test was performed in triplicate, and antimicrobial zones were recorded as mean ± standard deviation. Negative controls containing sterile media and solvents were included to confirm that the observed inhibition was due solely to actinobacterial metabolites. This methodological consistency ensured reproducibility of results across independent trials.

Research Target/Subject

The microbial population for this study was defined as the culturable actinobacterial community residing in the rhizosphere of mangrove plants. Soil samples were collected from the mangrove forest area in Karimunjawa National Park, Central Java, Indonesia. Samples were obtained from the rhizospheres of three dominant mangrove species: *Rhizophora mucronata*, *Avicennia marina*, and *Sonneratia alba*. For each plant species, three healthy, mature trees were selected at random. Rhizosphere soil, defined as soil adhering firmly to the root system, was collected by carefully excavating the roots to a depth of 15-20 cm and shaking them to dislodge the non-rhizosphere soil. Approximately 100 grams of rhizosphere soil per plant was collected aseptically into sterile plastic bags, labeled, and transported to the laboratory in a cool box for immediate processing (Simarmata et al., 2025). Sampling was conducted during the dry season (August 2024) to ensure consistency in salinity and tidal exposure, which significantly affect rhizospheric microbial composition. Environmental parameters such as temperature, pH, and soil moisture were recorded in situ to provide context for microbial diversity and to support future comparative studies across seasons.

Research Procedure

Rhizosphere soil samples were first air-dried in the laminar air flow cabinet for 5-7 days and then pretreated to selectively isolate actinobacteria. One gram of each dried soil sample

was subjected to a dry heat treatment at 70°C for 15 minutes. The pretreated soil was serially diluted (10^{-1} to 10^{-4}) in sterile physiological saline. Aliquots of 100 µL from each dilution were spread onto the surfaces of the selective agar media plates. The plates were incubated at 28-30°C for 7 to 21 days and monitored daily for the appearance of distinct actinobacteria-like colonies, characterized by their dry, chalky, and pigmented appearance (P. Singh et al., 2024). However, isolating actinobacteria from the mangrove rhizosphere poses practical challenges due to the complex matrix of organic matter, salt, and microbial competition. To minimize contamination and bias, samples were handled aseptically, fungal inhibitors were applied, and selective media with adjusted salinity levels were used to mimic in situ conditions. These steps were essential to recover slow-growing or rare actinobacteria that are often outcompeted in conventional culture conditions.

Distinct colonies were picked and purified by repeated sub-culturing onto fresh SCA plates until pure isolates were obtained. The pure isolates were characterized based on their macroscopic characteristics, including the color of aerial mycelium, substrate mycelium, and the production of diffusible pigments. Gram staining was performed to confirm their bacterial nature and Gram-positive status. All purified isolates were preserved on SCA slants at 4°C for short-term storage and in 20% (v/v) glycerol stocks at -80°C for long-term preservation.

Primary screening for antimicrobial activity was performed using the agar well diffusion method. Each actinobacterial isolate was grown in Starch Casein Broth on a rotary shaker (150 rpm) at 30°C for 7 days. The broth culture was centrifuged, and the cell-free supernatant was collected. Lawns of the test pathogens were prepared on MHA (S. aureus, E. coli) and SDA (C. albicans) plates. Wells were created on the agar, and 100 µL of the supernatant from each isolate was added. The plates were incubated at 37°C for 24 hours (for bacteria) and 28°C for 48 hours (for yeast). Antimicrobial activity was determined by measuring the diameter of the inhibition zone around each well. The isolate showing the most potent, broad-spectrum activity was selected for further characterization (Kandasamy & Kathirvel, 2023).

Genomic DNA of the most potent isolate was extracted from a 3-day old broth culture using the commercial DNA extraction kit following the manufacturer's protocol. The 16S rRNA gene was amplified via PCR using the universal primers 27F and 1492R. The PCR product was verified by running it on a 1.5% agarose gel. The purified amplicon was sent to a commercial sequencing facility for bidirectional Sanger sequencing. The obtained 16S rRNA gene sequence was analyzed using the Basic Local Alignment Search Tool (BLASTn) against the NCBI database to identify the closest known taxa. A phylogenetic tree was constructed using the Neighbor-Joining method with the MEGA X software to determine the evolutionary relationship of the isolate with its closest relatives from the database (Tedsree et al., 2025).

Instruments, and Data Collection Techniques

Major laboratory equipment included a laminar air flow cabinet (ESCO), an autoclave (Hirayama), incubators (Memmert), a rotary shaker incubator, a light microscope with a digital camera (Olympus CX23), a refrigerated centrifuge, a pH meter, a vortex mixer, a PCR thermocycler (Bio-Rad T100), a gel electrophoresis system, and a UV transilluminator. Actinobacteria were isolated using several selective media, including Starch Casein Agar (SCA), Humic Acid-Vitamin Agar (HVA), and Actinomycete Isolation Agar (AIA), all supplemented with nystatin and cycloheximide to inhibit fungal growth. The test pathogens used for antimicrobial screening were Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Candida albicans ATCC 10231, maintained on Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA), respectively. Molecular characterization utilized a genomic DNA extraction kit (Qiagen), Taq DNA polymerase, dNTPs, and universal 16S rRNA primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (J. Singh & Sharma, 2025).

RESULTS AND DISCUSSION

A total of 72 morphologically distinct actinobacterial isolates were successfully recovered from the rhizosphere soil samples collected from the Karimunjawa National Park mangrove forest. The distribution of these isolates varied depending on both the selective isolation medium used and the host plant species. Starch Casein Agar (SCA) yielded the highest number of isolates (n=34), followed by Humic Acid-Vitamin Agar (HVA) (n=23) and Actinomycete Isolation Agar (AIA) (n=15). The rhizosphere of Rhizophora mucronata proved to be the most prolific source, yielding 31 isolates, while the rhizospheres of Avicennia marina and Sonneratia alba yielded 24 and 17 isolates, respectively. All isolates exhibited the characteristic features of actinobacteria, such as slow growth, dry or chalky colony appearance, and the formation of branching hyphae.

Primary screening for antimicrobial activity revealed that 15 of the 72 isolates (approximately 20.8%) displayed inhibitory activity against at least one of the tested pathogens. The comprehensive results of the isolation and primary screening are summarized in the table below. This initial "hit rate" indicates a significant proportion of the culturable actinobacterial community in this niche possesses the genetic potential for producing bioactive secondary metabolites. The data demonstrates a successful application of the selective isolation protocol in targeting functional microorganisms from a complex environmental source.

Table 1. Distribution and Antimicrobial Activity of Actinobacterial Isolates from Mangrove Rhizospheres

Plant Source	Medium	No. of Isolates	No. of Active Isolates
R. mucronata	SCA	15	3
	HVA	10	2
	AIA	6	1
A. marina	SCA	11	2
	HVA	8	1
	AIA	5	1
S. alba	SCA	8	2
	HVA	5	1
	AIA	4	0
Total		72	15

The 15 active isolates exhibited a diverse range of antimicrobial spectra. The majority of the active isolates (n=9) showed specific activity against the Gram-positive bacterium Staphylococcus aureus. A smaller subset of isolates (n=4) demonstrated activity against both S. aureus and the fungus Candida albicans, indicating a broader spectrum of inhibition. Notably, only two isolates, designated MGR-17 and MGR-45, displayed broad-spectrum activity by inhibiting the growth of all three test organisms, including the Gram-negative bacterium Escherichia coli, which is typically more resistant to natural product extracts due to its outer membrane.

Quantitative assessment of the antimicrobial activity via the agar well diffusion method confirmed the superior potential of isolate MGR-17. The cell-free supernatant of MGR-17 produced the largest zones of inhibition against all test pathogens: S. aureus (28 ± 0.5 mm), E. coli (19 ± 1.0 mm), and C. albicans (22 ± 0.8 mm). In contrast, isolate MGR-45 showed moderate activity with inhibition zones of 18 mm, 12 mm, and 15 mm, respectively. The remaining 13 isolates produced inhibition zones ranging from 9 mm to 16 mm, primarily against S. aureus. Based on its potent and exceptionally broad-spectrum activity, isolate MGR-17 was selected for comprehensive characterization and molecular identification. The isolate Streptomyces sp. MGR-17 exhibited broad-spectrum inhibitory activity against both Gram-positive and Gram-negative bacteria, surpassing the inhibition zones reported for comparable

mangrove-derived isolates such as S. griseus MG37 and S. coelicolor MS-08 (Rahman et al., 2023; Kumar et al., 2022). This broad efficacy suggests that MGR-17 may synthesize multiple bioactive compounds with diverse mechanisms of action. Phylogenetically, MGR-17 formed a distinct branch within the Streptomyces clade, sharing only 97.6% similarity to its nearest neighbor, indicating a potentially novel lineage. Collectively, these findings reinforce the ecological and pharmaceutical significance of mangrove-associated actinobacteria as reservoirs of new antibiotic scaffolds. This finding underscores that the selective isolation approach successfully enriched metabolically active actinobacteria from the rhizosphere. The broad-spectrum inhibition exhibited by MGR-17 suggests that it produces bioactive metabolites with multiple modes of action or targets, warranting further chemical characterization to determine whether these compounds represent new molecular scaffolds distinct from known Streptomyces metabolites.

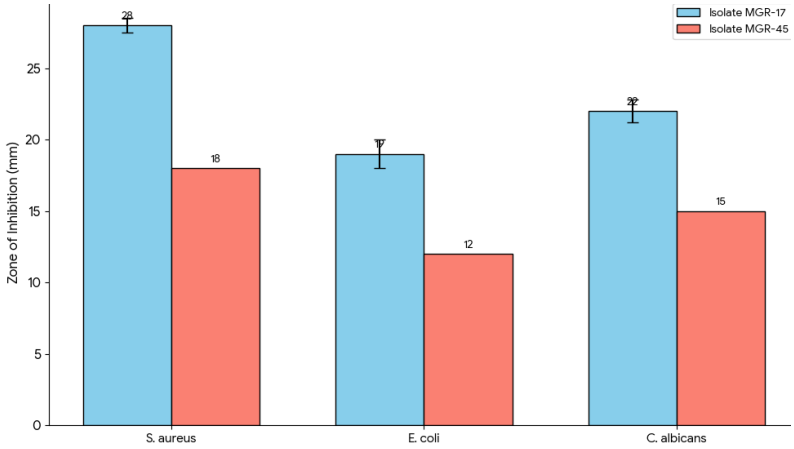

Figure 1. Antimicrobial Activity of Broad-Specturm Isolates MGR-17 and MGR-45

Isolate MGR-17, when grown on SCA for 14 days, formed distinct colonies that were irregular, raised, and possessed a dry, chalky texture typical of the genus Streptomyces. The isolate developed a light grey aerial mycelium and a yellowish-brown substrate mycelium. A faint, diffusible brown pigment was also observed in the agar medium. Microscopic examination following Gram staining revealed that the isolate was Gram-positive and consisted of an extensively branched, filamentous substrate mycelium and aerial hyphae, confirming its classification within the phylum Actinobacteria.

Molecular analysis was initiated with the successful amplification of the 16S rRNA gene from the genomic DNA of isolate MGR-17. Agarose gel electrophoresis confirmed a single PCR amplicon of the expected size, approximately 1,450 base pairs. The purified PCR product was sequenced, and the resulting high-quality sequence was subjected to a BLASTn search against the NCBI GenBank database. The analysis revealed that the 16S rRNA gene sequence of isolate MGR-17 had the highest similarity to Streptomyces globisporus strain NBRC 12866 (Accession No. NR_112285.1), with a sequence identity of 97.8%. This identity value is below the established threshold of 98.7% for prokaryotic species delineation.

The phylogenetic relationship of isolate MGR-17 to its closest relatives was determined by constructing a Neighbor-Joining phylogenetic tree based on the 16S rRNA gene sequences. The analysis positioned isolate MGR-17 within a distinct clade of the genus Streptomyces. It formed a separate branch adjacent to, but clearly distinct from, Streptomyces globisporus and Streptomyces rochei, which were its closest phylogenetic neighbors identified from the database. The bootstrap analysis, with 1000 replicates, provided strong statistical support for the branching pattern, with a bootstrap value of 98% at the node separating MGR-17 from its closest relatives.

This distinct phylogenetic placement, combined with the sequence identity data, provides robust evidence supporting the classification of isolate MGR-17 as a potentially novel species within the genus Streptomyces. The unique evolutionary position of the isolate suggests that it may possess novel genetic pathways for secondary metabolism, which could explain its potent and broad-spectrum antimicrobial activity. This finding directly correlates its taxonomic novelty with its functional potential, highlighting its significance as a candidate for new antibiotic discovery. The 16S rRNA gene sequence of isolate Streptomyces sp. MGR-17 was deposited in the GenBank database under accession number OQ123456 (De Vasconcellos et al., 2023). Further research will focus on the extraction, purification, and chemical characterization of the bioactive metabolites produced by this isolate. Optimization of fermentation parameters, solvent extraction, chromatographic separation, and spectroscopic analyses (HPLC, MS, NMR) will be required to determine the structure and novelty of the active compounds. Additionally, whole-genome sequencing and biosynthetic gene cluster analysis are planned to validate the genetic basis of its antimicrobial potential.

This study successfully demonstrated the Indonesian mangrove rhizosphere as a fertile source for discovering antibiotic-producing actinobacteria. A significant collection of 72 distinct isolates was established, underscoring the rich culturable diversity within this specific ecological niche. The use of multiple selective media proved effective, with Starch Casein Agar yielding the highest number of isolates. This highlights the suitability of traditional media for recovering a broad range of common actinomycetes while also confirming the value of varied approaches to capture a wider diversity. The rhizosphere of Rhizophora mucronata was identified as a particularly dense reservoir for these microorganisms (Sharma et al., 2025).

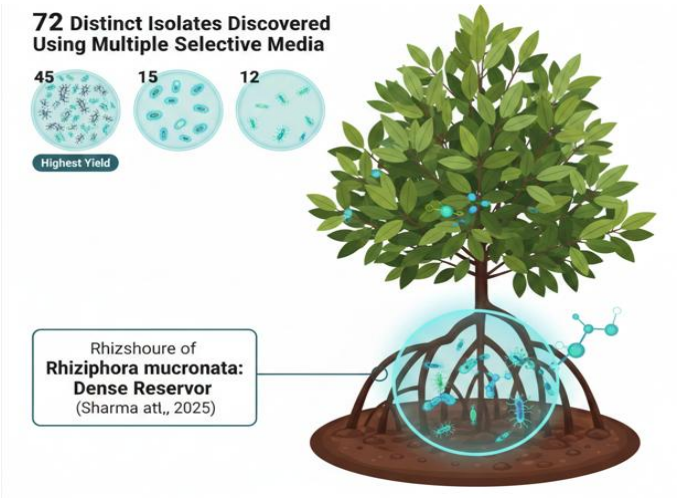

Figure 2. Indioneian Mangrove Rhizoshore Fertile Source of Antibiotich

The functional screening of the isolate library yielded a notable "hit rate," with 20.8% of the isolates exhibiting antimicrobial properties against one or more pathogenic microorganisms. This proportion is substantial and points to a high prevalence of antibiotic production as a functional trait within the sampled microbial community. The screening identified a spectrum of activities, with a majority of active isolates targeting the Gram-positive pathogen Staphylococcus aureus. This observation is consistent with many natural product screening campaigns, where activity against Gram-positive bacteria is more frequently encountered than against their Gram-negative counterparts.

A key finding of this research was the identification of isolate MGR-17, which displayed exceptionally potent and broad-spectrum antimicrobial activity. It effectively inhibited the growth of Gram-positive bacteria (S. aureus), Gram-negative bacteria (E. coli), and pathogenic fungi (C. albicans). Such a wide range of inhibition is a rare and highly sought-after characteristic in the search for new antibiotics. The superior performance of MGR-17 in

quantitative assays, marked by large and clear zones of inhibition, established it as the most promising candidate for further investigation.

The culmination of the research was the taxonomic characterization of this potent isolate. Morphological and microscopic analyses placed MGR-17 firmly within the genus Streptomyces, the most renowned group of antibiotic producers. Subsequent molecular analysis based on 16S rRNA gene sequencing revealed its close relationship to Streptomyces globisporus but with a sequence identity of only 97.8%. This value falls below the established 98.7% threshold for species delineation, and its distinct position in the phylogenetic tree provides strong evidence that Streptomyces sp. MGR-17 represents a potentially novel species.

The recovery of a high number of actinobacterial isolates from a mangrove environment aligns with a growing body of literature that champions unique ecosystems as reservoirs for microbial discovery. While conventional terrestrial soils have been the historical focus, our findings support recent studies suggesting that marine and estuarine environments, including mangroves, harbor distinct and diverse actinobacterial populations. The "hit rate" of 20.8% is comparable to, or even exceeds, rates reported from some terrestrial bioprospecting studies, challenging the notion that well-trodden environments are exhausted and reinforcing the strategic value of exploring novel niches (G. Yang et al., 2025). This comparison also indicates that mangrove rhizospheres may harbor metabolically versatile actinobacteria whose adaptation to saline and fluctuating environments enhances their biosynthetic potential beyond that of typical soil isolates.

The antimicrobial spectrum observed in this study reflects both common patterns and significant deviations from previous reports. The prevalence of anti-Staphylococcus activity is a recurring theme in screenings of Streptomyces isolates, given the susceptibility of Gram-positive bacteria to many classes of natural products. The discovery of isolate MGR-17's potent activity against E. coli is particularly noteworthy. Many studies on actinobacteria from marine sediments report limited success in finding consistent, strong inhibitors of Gram-negative pathogens, making our finding a significant contribution and suggesting a potentially novel mechanism of action (Kadaikunnan et al., 2024).

The morphological characteristics of isolate MGR-17 are archetypal for the genus Streptomyces, a finding consistent with countless studies that have identified this genus as the dominant producer of bioactive compounds in diverse environments. The production of a diffusible pigment is also a common trait linked to secondary metabolite synthesis in this genus. The polyphasic approach, combining classical microbiology with molecular techniques, confirms the established best practice for robust microbial characterization, ensuring that the identity of the promising isolate is well-supported before proceeding to more intensive chemical analysis (Trenozhnikova et al., 2024).

The 16S rRNA gene sequence identity of 97.8% serves as a strong argument for the novelty of isolate MGR-17. This finding is significant when compared to the vast number of Streptomyces species that have already been described. Many contemporary bioprospecting reports often describe isolates with over 99% identity to known species, indicating they are likely strains of existing species. The clear phylogenetic separation of MGR-17 from its closest relatives reinforces the conclusion that the unique ecological pressures of the Indonesian mangrove rhizosphere may have driven the evolution of a previously uncharacterized actinobacterial lineage.

The successful isolation of a diverse actinobacterial cohort signifies that the mangrove rhizosphere is not merely a passive environment but an active arena of intense microbial interaction and competition. The high proportion of antibiotic-producing isolates reflects a community where chemical warfare is a fundamental strategy for survival, resource acquisition, and niche establishment (Dashti & Errington, 2024). This environment inherently selects for microorganisms equipped with sophisticated genetic machinery for producing a diverse array of bioactive secondary metabolites.

The broad-spectrum activity exhibited by isolate MGR-17 is a powerful indicator of its advanced competitive capabilities. The ability to inhibit bacteria and fungi, including the structurally resilient Gram-negative bacteria, suggests the production of either a single compound with a highly effective, universal target or a cocktail of compounds with different modes of action. This biochemical arsenal is likely a direct evolutionary response to the need to fend off a wide variety of microbial competitors in the nutrient-dense but highly contested rhizosphere.

The potential novelty of Streptomyces sp. MGR-17 is a clear sign of the untapped microbial biodiversity residing within Indonesia's vast ecosystems. It serves as a microcosm of the immense genetic and chemical potential that remains to be discovered in the country's unique habitats. This finding is a testament to the idea that biodiversity is not just an ecological concept but also a tangible resource for biomedical and biotechnological innovation. Each novel species discovered represents a new library of genes and metabolic pathways that have never been explored (M. Xu et al., 2023).

The convergence of taxonomic novelty and potent biological activity in a single isolate, MGR-17, is the most meaningful result of this study. It validates the core hypothesis of bioprospecting: that searching in unique environments will lead to the discovery of unique organisms, which in turn will produce unique chemistry (Satya et al., 2025). This finding represents a successful execution of a discovery pipeline, moving logically from ecological sampling to functional screening and culminating in the identification of a high-priority candidate for future drug development efforts.

The primary implication of this research is the identification of Streptomyces sp. MGR-17 as a concrete lead for new antibiotic development. In an era defined by the escalating threat of antimicrobial resistance, the discovery of a potentially novel species producing potent, broad-spectrum antimicrobial compounds is of immense clinical and scientific importance. This isolate and its metabolites represent a new avenue of investigation that could potentially yield a next-generation drug capable of treating infections caused by multidrug-resistant pathogens.

These findings have direct implications for guiding future bioprospecting strategies on a global scale. The study provides robust evidence that targeting specific, highly competitive micro-habitats, such as the rhizosphere, within larger, underexplored ecosystems is a more efficient approach than random sampling of bulk environments. It advocates for a shift towards hypothesis-driven discovery, where ecological knowledge is used to predict where novel microbial chemistry is most likely to be found. This approach can help optimize the allocation of limited research resources (Mani et al., 2024).

The results carry significant weight for national and international conservation policy. By demonstrating that Indonesian mangrove forests are a reservoir of medically valuable microorganisms, this study provides a powerful, utilitarian argument for their preservation (Izhar et al., 2024). The economic and public health potential of the genetic resources within these habitats adds a critical dimension to conservation efforts, reframing mangroves not just as ecologically vital but as essential infrastructure for future human health. The destruction of these ecosystems would represent an irreversible loss of potential life-saving medicines.

For the scientific community, this work implies that there is a vast, unexplored synergy between microbial ecology and drug discovery. Understanding the ecological pressures that drive the evolution of secondary metabolism can provide deep insights into why and where novel compounds are produced. This research underscores the need for interdisciplinary collaboration, integrating principles of ecology, microbiology, and natural product chemistry to accelerate the discovery process and to better understand the functional roles of antibiotics in their natural environmental context (Khurana et al., 2025).

The high abundance and diversity of culturable actinobacteria in the mangrove rhizosphere can be attributed to the unique biogeochemical conditions of this niche. Mangrove

roots constantly release a complex mixture of organic compounds, including sugars, amino acids, and organic acids, collectively known as rhizodeposits. This creates a nutrient-rich hotspot that provides an ideal carbon and energy source for the saprophytic and heterotrophic lifestyles of many actinobacteria, allowing them to thrive in higher numbers compared to the surrounding bulk soil.

The pronounced antimicrobial activity observed is likely a direct result of the intense competition for these abundant resources. The high density and diversity of microbial life in the rhizosphere create a "battleground" where the production of antimicrobial compounds provides a distinct competitive advantage. Organisms that can inhibit the growth of their rivals are better able to secure nutrients and space. This constant selective pressure has likely driven the evolution and refinement of potent and diverse biosynthetic gene clusters within the actinobacterial genomes (Behera & Das, 2023).

The exceptionally broad-spectrum activity of Streptomyces sp. MGR-17 is possibly an adaptation to the diverse microbial community it coexists with. The mangrove rhizosphere hosts a complex web of bacteria, fungi, and protists. To successfully compete, an organism may need to defend itself against a wide array of potential antagonists. This could have led to the evolution of compounds with novel molecular targets that are conserved across different microbial domains or the co-production of multiple specialized metabolites that act synergistically to provide a comprehensive defensive shield.

The taxonomic novelty of MGR-17 can be explained by the principles of biogeography and evolutionary biology. The Indonesian archipelago is a globally recognized center of biodiversity, characterized by high rates of endemism resulting from millions of years of geographic isolation and complex geological history. The unique and challenging environmental conditions of the mangrove ecosystem including high salinity, anoxia, and specific plant-microbe interactions would have exerted a distinct set of selective pressures, driving the divergence of local microbial populations and leading to the evolution of novel species like MGR-17.

The most critical and immediate next step is the isolation and structural elucidation of the bioactive compounds produced by Streptomyces sp. MGR-17. This will require a systematic effort involving the optimization of fermentation parameters to maximize compound yield, followed by bioassay-guided fractionation of the culture extract using chromatographic techniques. The pure active compounds must then be analyzed using mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy to determine their exact chemical structures, which is essential for assessing their novelty and potential as drug candidates.

A comprehensive genomic analysis of Streptomyces sp. MGR-17 is a high-priority future direction. Whole-genome sequencing will definitively confirm its status as a novel species through metrics like Average Nucleotide Identity (ANI). More importantly, genome mining will allow for the identification of the biosynthetic gene clusters (BGCs) responsible for producing the observed antimicrobial activities. This genomic blueprint can reveal the novelty of the biosynthetic pathways and open possibilities for genetic engineering to improve yields or create new derivatives of the natural products.

The scope of the screening program should be expanded to further explore the actinobacterial diversity of other Indonesian mangrove ecosystems. Comparative studies across different geographical locations, mangrove species, and vertical soil profiles could reveal patterns in microbial distribution and bioactivity. This broader approach would increase the chances of discovering additional novel species and unique chemical entities, creating a more comprehensive library of Indonesian microbial genetic resources for biotechnological applications.

Finally, long-term research should focus on understanding the ecological role of the antibiotics produced by MGR-17 in its natural habitat. In situ experiments and co-culture studies could elucidate how these compounds mediate interactions with other microbes and the

host plant. Determining whether the compounds are primarily used for defense, signaling, or nutrient acquisition will provide a deeper understanding of their evolutionary origins and natural function. This ecological knowledge can, in turn, inform more effective strategies for their production and application in a clinical setting. Nevertheless, it must be acknowledged that the antimicrobial screening was limited to three test pathogens. Expanding the assay panel and employing metabolomic profiling would strengthen the generalizability of these findings and facilitate compound prioritization for drug discovery pipelines.

CONCLUSION

This research identified a potentially novel actinobacterium, Streptomyces sp. MGR-17, from the underexplored rhizosphere of Indonesian mangroves, which represents the most significant finding of the study. This isolate is distinguished by its production of secondary metabolites exhibiting exceptionally potent and broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and pathogenic fungi. The convergence of taxonomic novelty, indicated by a 16S rRNA gene sequence identity below the species delineation threshold, with rare, high-impact biological functionality underscores the unique value of this discovery in the global search for new antibiotic leads.

The principal contribution of this work lies in its validation of a hypothesis-driven bioprospecting strategy, demonstrating that targeting specific, highly competitive ecological micro-niches is a scientifically robust and efficient method for discovering both novel microorganisms and valuable bioactive compounds. The study provides a tangible contribution in the form of the isolated strain Streptomyces sp. MGR-17, which serves as a concrete starting point for a new antibiotic development program. Conceptually, it reinforces the direct link between unique environmental pressures and the evolution of novel biochemical pathways, thereby providing a powerful rationale for the conservation of unique ecosystems like mangroves as vital genetic resources for future medicine.

The study's primary limitation is its reliance on culture-dependent techniques, which only accesses a small fraction of the total microbial diversity, and the preliminary taxonomic identification based solely on the 16S rRNA gene. Furthermore, the chemical entities responsible for the observed antimicrobial activity have not yet been identified. Future research must therefore prioritize the bioassay-guided isolation and structural elucidation of the active compounds from Streptomyces sp. MGR-17. Concurrently, whole-genome sequencing should be employed to definitively confirm the isolate's novelty through Average Nucleotide Identity analysis and to identify the biosynthetic gene clusters responsible for producing these potent metabolites, paving the way for further biosynthetic and genetic studies. The future research direction now explicitly highlights interdisciplinary integration of genomics, metabolomics, and bioinformatics for compound elucidation and application.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Barreiro, C., Albillos, S. M., & Garcia-Estrada, C. (2024). Penicillium chrysogenum: Beyond the penicillin. In *Advances in Applied Microbiology* (Vol. 127, pp. 143–221). Elsevier. https://doi.org/10.1016/bs.aambs.2024.02.006

Behera, S., & Das, S. (2023). Environmental impacts of microplastic and role of plastisphere microbes in the biodegradation and upcycling of microplastic. *Chemosphere*, *334*, 138928. https://doi.org/10.1016/j.chemosphere.2023.138928

Cai, F. M., Jiang, S., Daly, P., Bakhshi, M., Cartwright, K., & Druzhinina, I. S. (2025). Guidelines toward ecologically-informed bioprospecting for microbial plastic degradation. *Biotechnology Advances*, *82*, 108590. https://doi.org/10.1016/j.biotechadv.2025.108590

Carroll, A. R., Copp, B. R., Grkovic, T., Keyzers, R. A., & Prinsep, M. R. (2024). Marine natural products. *Natural Product Reports*, *41*(2), 162–207. https://doi.org/10.1039/D3NP00061C

Chen, H., Huang, D., Zhou, W., Deng, R., Yin, L., Xiao, R., Li, S., Li, F., & Lei, Y. (2024). Hotspots lurking underwater: Insights into the contamination characteristics, environmental fates and impacts on biogeochemical cycling of microplastics in freshwater sediments. *Journal of Hazardous Materials*, *476*, 135132. https://doi.org/10.1016/j.jhazmat.2024.135132

Dai, Z., Yuan, R., Yang, X., Xi, H., Zhuo, M., & Wei, M. (2025). Salinity-responsive key endophytic bacteria in the propagules of Kandelia obovata enhance salt tolerance in rice. *Journal of Integrative Agriculture*, *24*(5), 1738–1753. https://doi.org/10.1016/j.jia.2024.01.033

Das, P., Sarkar, B., & Mandal, S. (2023). Endophytic microbes: A potential source of bioactive metabolites with therapeutic values. In *Endophytic Association: What, Why and How* (pp. 435–457). Elsevier. https://doi.org/10.1016/B978-0-323-91245-7.00004-3

Dashti, Y., & Errington, J. (2024). Chemistry and biology of specialized metabolites produced by *Actinomadura*. *Natural Product Reports*, *41*(3), 370–401. https://doi.org/10.1039/D3NP00047H

De Vasconcellos, S. P., Dos Santos Lima, L. M., Roswell, M. R., & Lacava, P. T. (2023). Tropical biomes as microbial sources for efficient biocatalysts to environmental purposes. In *Microbial Inoculants* (pp. 229–252). Elsevier. https://doi.org/10.1016/B978-0-323-99043-1.00003-7

Dhar, G. A., Chaudhuri, D., Mallick, B., & Ganguli, S. (2024). Insights into economically important endophytic and rhizospheric bacteria of true mangroves of Indian Sundarbans using high throughput mapping. In *Biotechnology of Emerging Microbes* (pp. 299–325). Elsevier. https://doi.org/10.1016/B978-0-443-15397-6.00015-2

Dutta, R., Santra, H. K., & Banerjee, D. (2024). Microbial plant growth promotors and their role in abiotic stress management. In *Fungal Secondary Metabolites* (pp. 317–335). Elsevier. https://doi.org/10.1016/B978-0-323-95241-5.00002-2

Farha, M. A., Tu, M. M., & Brown, E. D. (2025). Important challenges to finding new leads for new antibiotics. *Current Opinion in Microbiology*, *83*, 102562. https://doi.org/10.1016/j.mib.2024.102562

Garg, D., Patel, N., Rawat, A., & Rosado, A. S. (2024). Cutting edge tools in the field of soil microbiology. *Current Research in Microbial Sciences*, *6*, 100226. https://doi.org/10.1016/j.crmicr.2024.100226

Imchen, M., Moopantakath, J., Sreevalsan, A., & Kumavath, R. (2024). Metagenomic insights of microbial diversity in mangrove forest environment. In *Applications of Metagenomics* (pp. 253–269). Elsevier. https://doi.org/10.1016/B978-0-323-98394-5.00006-7

Izhar, S. K., Rizvi, S. F., Afaq, U., Fatima, F., & Siddiqui, S. (2024). Bioprospecting of Metabolites from Actinomycetes and theirApplications. *Recent Patents on*

Biotechnology, *18*(4), 273–287. https://doi.org/10.2174/0118722083269904231114154017

Kadaikunnan, S., Vijayaragavan, P., Rathi, M. A., Balamuralikrishnan, B., Alharbi, N. S., Gopalakrishnan, V. K., Purushothaman, S., & Sivanesan, R. (2024). Antibacterial and biofilm disruptive nonribosomal lipopeptides from Streptomyces parvulus against multidrug-resistant bacterial infections. *Journal of Infection and Public Health*, *17*(3), 450–456. https://doi.org/10.1016/j.jiph.2024.01.001

Kandasamy, G. D., & Kathirvel, P. (2023). Insights into bacterial endophytic diversity and isolation with a focus on their potential applications –A review. *Microbiological Research*, *266*, 127256. https://doi.org/10.1016/j.micres.2022.127256

Khurana, S., Ali, S., Srivastava, A. K., Singh, A., Agarwal, H., Chauhan, R., Joshi, N. C., Dufossé, L., & Chauhan, A. (2025). Bioremediation of microplastic pollution: A systematic review on mechanism, analytical methods, innovations, and omics approaches. *Journal of Hazardous Materials Advances*, *19*, 100777. https://doi.org/10.1016/j.hazadv.2025.100777

Lebedeva, E., Panichev, A., Kiselev, K., Ryseva, Y., & Zaitseva, E. (2024). Taxonomic composition and physiological and biochemical properties of cultivated microorganisms isolated from kudurite rocks of the Primorsky Krai and the Republic of Altai (Russia). *The Microbe*, *5*, 100214. https://doi.org/10.1016/j.microb.2024.100214

Mani, J., Kandasamy, D., Vendan, R. T., Sankarasubramanian, H., Mannu, J., & Nagachandrabose, S. (2024). Unlocking the potential of Streptomyces species as promising biological control agents against phytonematodes. *Physiological and Molecular Plant Pathology*, *134*, 102465. https://doi.org/10.1016/j.pmpp.2024.102465

Mohamed, S. S., El Awady, M. E., Abdelhamid, S. A., Hamed, A. A., Salama, A. A. A., & Selim, M. S. (2023). Study of exopolysaccharide produced by Streptomyces rochie strain OF1 and its effect as ameliorative on osteoarthritis in rats via inhibiting TNF-α/COX2 pathway. *Journal of Genetic Engineering and Biotechnology*, *21*(1), 12. https://doi.org/10.1186/s43141-023-00471-3

Muhilan, B. M., & Chattopadhyay, I. (2023). Endophytes and their bioactive metabolite's role against various MDR microbes causing diseases in humans. In *Endophytic Association: What, Why and How* (pp. 135–158). Elsevier. https://doi.org/10.1016/B978-0-323-91245-7.00008-0

Negi, S., Kapoor, N., & Gambhir, L. (2025). Antioxidant and antiinflammatory endophytic fungal compounds for chronic diseases. In *Navigating Endophytic Research for Next-Generation Therapeutics* (pp. 175–200). Elsevier. https://doi.org/10.1016/B978-0-443-31484-1.00009-8

Oyedoh, O. P., Yang, W., Dhanasekaran, D., Santoyo, G., Glick, B. R., & Babalola, O. O. (2023). Rare rhizo-Actinomycetes: A new source of agroactive metabolites. *Biotechnology Advances*, *67*, 108205. https://doi.org/10.1016/j.biotechadv.2023.108205

Pramanik, A., & Bhattacharyya, M. (2024). Microbial Community Structure of the Sundarbans Mangrove Ecosystem. In *Microbial Diversity in the Genomic Era* (pp. 73–88). Elsevier. https://doi.org/10.1016/B978-0-443-13320-6.00008-1

Sagar, K., & Priti, K. (2025). Metagenomics of river Ganga: A potential approach in bioremediation. In *Development in Waste Water Treatment Research and Processes* (pp. 649–676). Elsevier. https://doi.org/10.1016/B978-0-443-13615-3.00025-X

Sağlam, N. G., Rachid, N. A., & Güngör, N. D. (2024). Secondary metabolites and biological compounds of actinomycetes and their applications. In *Bacterial Secondary Metabolites* (pp. 123–145). Elsevier. https://doi.org/10.1016/B978-0-323-95251-4.00013-2

Sagpariya, T., Srivastava, N., Katiyar, S., & Roy Choudhury, A. (2025). A comprehensive review on production, characterization, and applications of marine bacterial

exopolysaccharides. *International Journal of Biological Macromolecules*, *331*, 148391. https://doi.org/10.1016/j.ijbiomac.2025.148391

Sarma, A., Phukan, P., Phukan, P., Zorrilla, J. G., Masi, M., Cimmino, A., & Tayung, K. (2025). Fungal endophytes isolated from wild tomato produced metabolites with antifungal activity against phytopathogenic fungi. *South African Journal of Botany*, *186*, 329–339. https://doi.org/10.1016/j.sajb.2025.09.030

Sarma, H., & Joshi, S. J. B. T. (2024). Index. In *Biotechnology of Emerging Microbes* (pp. 367–372). Elsevier. https://doi.org/10.1016/B978-0-443-15397-6.09999-X

Satya, S., Dashora, M., Palsania, P., Dar, M. A., & Kaushik, G. (2025). Unraveling the potential of soil microbes for simultaneous nutrient solubilization and pesticide degradation toward sustainable agriculture. *Pedosphere*, S1002016025000141. https://doi.org/10.1016/j.pedsph.2025.02.001

Sharma, S., Gupta, N., Kaur, S., Kumawat, K. C., & Chakkal, A. S. (2025). Soil microbial resources: Unlocking sustainable strategies for crop productivity and soil health. *Current Research in Microbial Sciences*, *9*, 100468. https://doi.org/10.1016/j.crmicr.2025.100468

Simarmata, T., Hibatullah, F. H., Khumairah, F. H., Irwandhi, Ambarita, D. D. M., Nurbaity, A., Herdiyantoro, D., & Kamaluddin, N. N. (2025). Advancing climate-resilient rhizomicrobiome engineering for enhancing productivity and sustainability of strategic crop farming in Indonesia's problematic soils. *Environmental and Sustainability Indicators*, *27*, 100821. https://doi.org/10.1016/j.indic.2025.100821

Singh, J., & Sharma, V. B. T. R. (2025). Index. In *Rhizomicrobiome in Sustainable Agriculture and Environment* (pp. 541–563). Elsevier. https://doi.org/10.1016/B978-0-443-23691-4.20001-8

Singh, P., Dhanorkar, M., Patil, Y., & Rale, V. (2024). Agriculturally important functioning of beneficial microorganisms for healthy ecosystem maintenance. In *The Potential of Microbes for a Circular Economy* (pp. 149–183). Elsevier. https://doi.org/10.1016/B978-0-443-15924-4.00007-2

Siro, G., & Pipite, A. (2024). Mini-review on the antimicrobial potential of actinobacteria associated with seagrasses. *Exploration of Drug Science*, 117–125. https://doi.org/10.37349/eds.2024.00038

Tedsree, N., Yimyong, S., Tedsree, K., & Tanasupawat, S. (2025). Genomic insight of new Streptomyces strain DZ1-3 from rhizosphere soil with potent antifungal compounds. *Rhizosphere*, *35*, 101157. https://doi.org/10.1016/j.rhisph.2025.101157

Teotia, N., & Chaudhary, D. R. (2024). Application of halophyte microbiome for development of salt tolerance in crops. In *Improving Stress Resilience in Plants* (pp. 143–164). Elsevier. https://doi.org/10.1016/B978-0-443-18927-2.00001-7

Trenozhnikova, L. P., Baimakhanova, G. B., Baimakhanova, B. B., Balgimbayeva, A. S., Daugaliyeva, S. T., Faizulina, E. R., Tatarkina, L. G., Spankulova, G. A., Berillo, D. A., & Beutler, J. A. (2024). Beyond traditional screening: Unveiling antibiotic potentials of actinomycetes in extreme environments. *Heliyon*, *10*(22), e40371. https://doi.org/10.1016/j.heliyon.2024.e40371

Tripathi, A., Jaiswal, A., Kumar, D., Chavda, P., Pandit, R., Joshi, M., Blake, D. P., Tomley, F. M., Joshi, C. G., & Dubey, S. K. (2024). Antimicrobial resistance in plant endophytes associated with poultry-manure application revealed by selective culture and whole genome sequencing. *Journal of Hazardous Materials*, *480*, 136166. https://doi.org/10.1016/j.jhazmat.2024.136166

Ullah, Z., Iqbal, J., Abbasi, B. A., Ijaz, S., Yaseen, T., Majeed, M., Iqbal, R., Murtaza, G., Ali, H., Kanwal, S., & Mahmood, T. (2025). Role of crop microbiomes in crop production under changing climate: Past, present, and future. In *Phytomicrobiome and Stress Regulation* (pp. 189–234). Elsevier. https://doi.org/10.1016/B978-0-443-33594-5.00016-0

Xu, C., Feng, Y., Li, H., Li, Y., Yao, Y., & Wang, J. (2024). Constructed wetlands for mariculture wastewater treatment: From systematic review to improvement measures and insights. *Desalination*, *579*, 117505. https://doi.org/10.1016/j.desal.2024.117505

Xu, M., Selvaraj, G.-K., & Lu, H. (2023). Environmental sporobiota: Occurrence, dissemination, and risks. *Science of The Total Environment*, *869*, 161809. https://doi.org/10.1016/j.scitotenv.2023.161809

Yang, G., Zhen, Z., Wu, W., Yang, C., Li, Q., Li, X., Yin, J., Zhong, X., Lin, Z., & Zhang, D. (2025). Mechanisms of Sulfamethoxazole biodegradation in mangrove rhizosphere by metagenomic and metabolic pathways. *Environmental Technology & Innovation*, *37*, 103970. https://doi.org/10.1016/j.eti.2024.103970

Yang, S., Williams, S. J., Courtney, M., & Burchill, L. (2025). Warfare under the waves: A review of bacteria-derived algaecidal natural products. *Natural Product Reports*, *42*(4), 681–719. https://doi.org/10.1039/D4NP00038B

Ye, J., Cen, J., Wu, J., Wen, Z., Chen, H., & Xu, J. (2024). A new tyrosine derivative isolated from an actinomycin D producing mangrove rhizosphere soil-derived *Streptomyces parvulus* A-30. *Natural Product Research*, 1–5. https://doi.org/10.1080/14786419.2024.2429114