



Exploring the Antimicrobial Potential of Marine-Derived Actinobacteria: Metabolomics Analysis and Identification of Novel Bioactive Metabolites

¹-Esam Abdullah Mohammed Alaamri,²-Mustafa Ahmed Edrees Hamzi,³-Hafid Ahmed Mohammed Baydi,⁴-Yasir Ibrahim Yahya Kariri,⁵-Hassan Mohammed Ghabesh Ayashi,⁶-Aqdi Ahmed Saleh Y,⁷-Waseem Mohammed Hadidi Majari,⁸-Yasir Afandi Hamed Hakami,⁹-Majid Ahmed Abduh Refaei,¹⁰-Mohammed Ali Qasem Otayfi,¹¹-Ahmad Mohammad Tyhan Hazzazi,¹²-Hussain Ali Halawi,¹³-Hassan Abdu Ahmed Dohali,¹⁴- Fuad Ali Abdu Jabbari,¹⁵-Ali Ibrahim Alabdali

1. KSA, Ministry Of Health, Abu Arish General Hospital
2. KSA, Ministry Of Health, Abu Arish General Hospital
3. KSA, Ministry Of Health, ABU ARISH GENERAL HOSPITAL
4. KSA, Ministry Of Health, Abu Arish General Hospital
5. KSA, Ministry Of Health, ABU ARISH GENERAL HOSPITAL
6. KSA, Ministry Of Health, JAZAN GENRAL HOSPITAL
7. KSA, Ministry Of Health, Abu Arish General Hospital
8. KSA, Ministry Of Health, Abu Arish General Hospital
9. KSA, Ministry Of Health, Blood Bank Center Jizan
10. KSA, Ministry Of Health, Sabia General Hospital
11. KSA, Ministry Of Health, Abu Arish General Hospital
12. KSA, Ministry Of Health, Central Laboratory And Blood Bank
13. KSA, Ministry Of Health, Abu Arish General Hospital
14. KSA, Ministry Of Health, FIFA General Hospital
15. KSA, Ministry Of Health, Mental Health Hospital

Abstract

Although it cannot be prevented, antimicrobial resistance is a serious public health concern that contributed to around 5 million deaths in 2019. It can be managed by looking for new antibacterial substances. Because their secondary metabolites include a variety of biological actions, such as antibacterial, antifungal, antiviral, and anticancer properties, marine actinobacteria are a valuable resource for biotechnological study and the exploration of novel metabolites.

Aim:

The purpose of this work is to do a thorough metabolomics analysis of the bioactive metabolites of marine-derived Actinobacteria and assess their antibacterial efficacy. Finding new antimicrobial agents and clarifying the connection between certain metabolites and antimicrobial efficacy are the goals of the study.

Methods:

A study of previous research on Actinobacteria crude extract was carried out, and the agar well diffusion method was used to assess the crude extract's antibacterial activity. Gas chromatography-mass spectrometry (GC-MS) was used to identify the components of metabolites.

Results:

Marine Actinobacteria strains with strong antibacterial activity were found in the study, especially against bacteria that are resistant to multiple drugs. Bioactive metabolites that are strongly associated with antimicrobial properties were identified by metabolomics profiling.

conclusion:

marine Actinobacteria offer interesting metabolites for drug development; more characterisation of Streptomyces metabolites could result in the development of novel medications for a number of illnesses.

Keywords: Streptomyces, Actinobacteria, Antimicrobial, Gas chromatography-mass spectrometry (GC-MS), and Macroalgae

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Introduction:

Public health is threatened by antibiotic resistance worldwide, yet there aren't enough new antibacterial therapies. As a result, there's a growing need to find new antibiotic pharmaceuticals and bioactive substances to eradicate antibiotic-resistant pathogens. [1] Particularly in the genus Actinobacteria, microorganisms represent a rich source of new components with a variety of therapeutic applications [2].

The prokaryotes known as actinobacteria, which are found in both aquatic and terrestrial environments, are the most economically and pharmaceutically valuable microorganisms. The genus Streptomyces in particular produces a variety of metabolites, which are a limitless and vital source of bioactive components [3]. Over 70% of the current antibiotics are produced by the Streptomyces genus [4]. Approximately 10,000 of the almost 23,000 metabolites that microbes make are members of the genus Actinobacteria, accounting for 45% of all metabolites produced by microorganisms [5]. These metabolites have biological properties include anticancer, antifungal, and antibacterial properties [6].

Researchers are concentrating on extreme environments and unique habitats, like marine environments, because microbes in these settings evolve adaptive pathways to synthesize novel biological components and develop a unique defense mechanism against the stress of their harsh habitats. Unfortunately, it is challenging to explore novel antibiotics from Actinobacteria isolated from soil environments [7]. When the diversity of Actinobacteria is compared to terrestrial environments, it is discovered that marine Actinobacteria produce distinct types of bioactive components, which have better qualities than those of terrestrial Actinobacteria, making the marine environment a perfect source of novel metabolites [8].

Classification and Characteristics of Actinobacteria

One of the largest bacterial phyla, Actinobacteria is a varied group of Gram-positive bacteria that is distinguished by having a high GC (guanine-cytosine) content in its DNA. Although there are many genera in this phylum, the most prominent ones are Streptomyces, Nocardia, Mycobacterium, and Corynebacterium because of their significance in both medicine and the economy. Numerous physical and physiological characteristics are displayed by members of the Actinobacteria phylum. Their intricate secondary metabolic processes, which enable them to generate a range of bioactive substances such as pigments, enzymes, and antibiotics, are what make them most famous. Perhaps the most well-known species in the phylum is Streptomyces, which produces a wide range of clinically significant antibiotics, including erythromycin, tetracycline, and streptomycin [9, 10].

Similar to mushrooms, actinobacteria are distinguished by their filamentous, branching shape. During their vegetative growth phase, when they produce large hyphae that can break apart into smaller rods or spores, this characteristic is very noticeable. Actinobacteria's ecological success is largely due to their metabolic diversity, which enables them to colonize a variety of habitats. These microbes flourish in a variety of

biological niches, from freshwater and soil to harsher settings like deserts and marine ecosystems. Actinobacteria are ecologically resilient due to their complex shape and capacity to produce spores, which are extremely resilient to environmental stressors such as desiccation, UV light, and high salinity [11].

Marine Actinobacteria versus Terrestrial Actinobacteria

Due to their unique metabolic profiles and the generation of novel secondary metabolites with strong antibacterial action, marine actinobacteria—which are isolated from marine environments such as sediments, sponges, and macroalgae—have attracted a lot of attention lately. Marine actinobacteria are frequently found in environments with particular ecological stresses, such as increased salinity, pressure, and the presence of a wide variety of marine animals, in contrast to terrestrial actinobacteria, which have been thoroughly investigated for their capacity to produce antibiotics. These circumstances encourage the synthesis of specific substances that their terrestrial counterparts would not be able to produce. Additionally, actinobacteria have evolved to create chemicals that function as antibiotics, antifouling agents, anti-cancer agents, and anti-inflammatory substances due to the particular environmental constraints of the marine ecosystem [12, 13].

Most of the *Streptomyces* genus's commercially available antibiotics were first isolated from terrestrial sources. The main source of these bacteria's isolations is soil, where they coexist with a wide variety of other microbes. [14, 15].

Bioactive Compounds from Marine Actinobacteria

In recent years, marine actinobacteria have become a prospective source of new bioactive chemicals, especially antibacterial agents. The environmental circumstances in which these bacteria live are mostly responsible for the chemical variety seen in these substances. Numerous bioactive natural compounds found in the marine environment serve as chemical defense mechanisms against infections, competition, and predators. Numerous secondary metabolites, such as oleic acid, oxalic acid, benzoic acid, stearic acid, palmitic acid, glycerol, 2,3-butanediol, β -sitosterol, diphenyl (trimethylsilyl) phosphine, and bis (2-ethylhexyl) phthalate, have been discovered to be produced by marine actinobacteria. These compounds all have antimicrobial activity against a variety of pathogenic and multidrug-resistant microorganisms, which all show a great deal of biological activity. [16].

The antibiotic Lacrimtoxin, which was identified from *Streptomyces* sp. in the Pacific Ocean, is one of the most well-known bioactive substances obtained from marine actinobacteria. Strong activity against Gram-negative infections, such as the well-known antibiotic-resistant bacteria *Pseudomonas aeruginosa*, has been demonstrated by this chemical. Actinomycin D, which was extracted from *Streptomyces parvullus* from marine sediments, is another notable substance. It has shown action against a variety of Gram-positive bacteria and, because of its cytotoxic effects, may be used as an anticancer agent [17]. Furthermore, it has been discovered that marine actinobacteria generate special sulfur-containing substances called thiopeptide antibiotics, which have potent antimicrobial qualities against a range of pathogens, including strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis* that are resistant to multiple drugs [18].

Additionally, recent research has discovered new classes of bioactive substances from marine actinobacteria, including *Brevibacillus* species, which produce unique antimicrobial peptides that may be utilized to treat illnesses brought on by resistant strains. An understudied source of novel natural compounds that could be crucial in tackling the growing worldwide problem of antibiotic resistance is marine-derived actinobacteria [19, 20]. The investigation of these substances and the application of cutting-edge methods such as metabolomics show promise for the development of even more varied and potent antibacterial drugs.

Types of Antimicrobial Metabolites

Actinobacteria produce antimicrobial chemicals that fall into a number of general types, such as polyketides, non-ribosomal peptides, alkaloids, terpenoids, and other secondary metabolites. The polyketides and non-ribosomal peptides, which have broad-spectrum efficacy against a range of pathogens,

including bacteria, fungus, and viruses, are among the most researched. For instance, it has been demonstrated that *Streptomyces parvullus*, which produces the polyketide antibiotic Actinomycin D from marine sediments, possesses strong antibacterial qualities against a variety of Gram-positive and Gram-negative bacteria. Some of these substances have antitumor, anticancer, and anti-inflammatory properties in addition to their antimicrobial ones, underscoring their multipurpose potential [21, 22].

Another type of bioactive substances are non-ribosomal peptides (NRPs), which are produced by modular enzyme complexes that enable the creation of structurally varied molecules with important pharmacological characteristics. Lacrimtoxin, a peptide antibiotic that is generated from a marine *Streptomyces* species, is among the most well-known instances. Strong antibiotic action against multidrug-resistant bacteria, such as *Pseudomonas aeruginosa*, a well-known pathogen linked to hospital-acquired infections, has been demonstrated for this chemical. NRPs are useful in the fight against resistant infections because of their capacity to target particular microbial cell structures, such as membrane integrity, protein synthesis, and cell wall formation [23, 24].

Actinobacteria also produce another family of compounds called alkaloids, which have shown strong antibacterial activity. These substances are frequently engaged in preventing bacterial cell division or metabolic processes, and they usually contain nitrogen atoms in their chemical structures. It has been discovered that certain alkaloids produced by marine-derived actinobacteria, like those in the genus *Micromonospora*, have anti-*Mycobacterium tuberculosis* and anti-other resistant strains of both Gram-positive and Gram-negative bacteria. It has been discovered that the alkaloid *Micromonospora* sp. breaks down the membranes of bacteria, which adds to its antibacterial properties [25, 26].

Mechanisms of Antimicrobial Activity

There are several ways to explain the antibacterial activity of actinobacteria metabolites. One of the most prevalent is the suppression of cell wall formation, which is essential for the development and survival of bacteria (figure 1). A key component of the effectiveness of many antibiotics is the way polyketides, like erythromycin and tetracycline, attach to bacterial ribosomes and stop protein production. Furthermore, by breaking down lipid bilayers, certain actinobacterial metabolites, such as the previously mentioned lacrimtoxin, attack the integrity of bacterial membranes, causing intracellular contents to leak out and cell death [27]

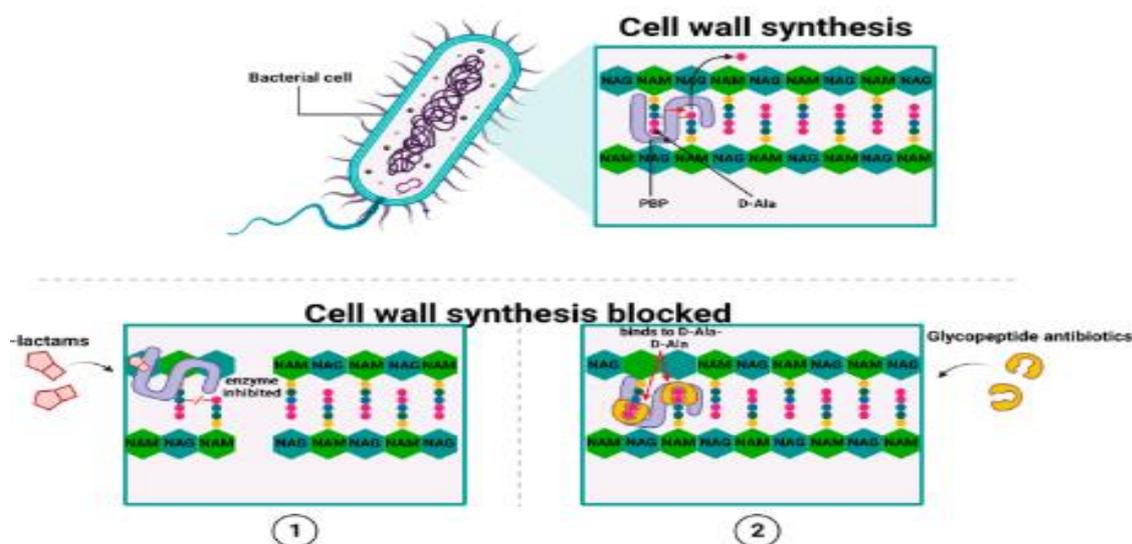


Figure 1 inhibition of cell wall synthesis

The suppression of nucleic acid synthesis is another significant mechanism (figure 2). By binding to the bacterial RNA polymerase, antibiotics such as Rifamycin and Actinomycin D efficiently stop bacterial reproduction by preventing transcription. It has been demonstrated that additional actinobacterial metabolites suppress DNA replication and repair, hence halting bacterial growth. Additionally, several

most well-known and often used. Erythromycin, which is naturally produced by *Streptomyces erythraeus*, is useful against a variety of bacterial illnesses, including those brought on by respiratory pathogens, intracellular bacteria, and Gram-positive cocci. Macrolides, including azithromycin and clarithromycin, are useful tools in therapeutic settings since they have been changed to address problems like stability and resistance. Inhibiting protein synthesis and stopping bacterial growth, macrolides work by attaching to the bacterial ribosome, more especially the 50S subunit [33, 34]. Recent research has also looked into how macrolides affect the immune system, and there is evidence that they may be used to treat inflammatory conditions such as cystic fibrosis [35].

Tetracyclines

Many *Streptomyces* species produce tetracyclines, which are among the most commonly used antibiotics in veterinary and human medicine. A wide range of microorganisms, including those resistant to previous therapies, can be effectively combated by these antibiotics. Common examples of medications that have been used to treat infections such as respiratory infections, acne, and sexually transmitted illnesses include tetracycline, doxycycline, and minocycline. Tetracyclines work by attaching to the 30S ribosomal subunit, which prevents aminoacyl-tRNA from attaching to the ribosome and preventing the production of proteins. In addition to their anti-inflammatory qualities, tetracyclines have been used to treat Lyme disease and malaria [36]. Tetracycline resistance has become a major problem despite their effectiveness, which has led to continuous research into finding new analogs and ways to combine them with other antimicrobial agents [37].

Aminoglycosides

Streptomyces species like *Streptomyces griseus*, which makes streptomycin, and *Streptomyces kanamyceticus*, which produces kanamycin, are the source of aminoglycosides, another class of antibiotics made by actinobacteria. These medicines work very well against mycobacteria and Gram-negative bacteria. For instance, kanamycin is used to treat multidrug-resistant tuberculosis (MDR-TB), whereas streptomycin is utilized as a first-line treatment for tuberculosis. By attaching itself to the 30S ribosomal subunit, aminoglycosides misinterpret mRNA and stop protein synthesis early. Although aminoglycosides are very effective, toxicity issues, especially nephrotoxicity and ototoxicity, limit their usage and have prompted attempts to create less toxic derivatives [38, 39].

Polyketides

Streptomyces species produce polyketides, a structurally varied and pharmacologically important class of antibiotics, using a modular enzyme system. Among these substances are common antibiotics like vancomycin, rifamycin, and erythromycin. Vancomycin is a last-resort antibiotic for infections brought on by resistant Gram-positive bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), whereas rifamycins are used to treat leprosy and tuberculosis. Vancomycin prevents Gram-positive bacteria from synthesizing their cell walls, whereas rifamycin works by inhibiting bacterial RNA polymerase. Research into novel rifamycin and vancomycin derivative generations is still ongoing as a result of the emergence of antibiotic resistance [40, 41].

Chloramphenicol

Streptomyces venezuelae also produces the antibiotic chloramphenicol. Typhoid fever and bacterial meningitis are among the bacterial diseases that have been treated with this broad-spectrum antibiotic. Chloramphenicol works by attaching itself to the 50S ribosomal subunit and inhibiting the synthesis of proteins. However, it is typically only used for severe infections or in situations where other antibiotics are ineffective because of the serious side effects it can cause, such as bone marrow suppression. There is still research being done to create compounds of chloramphenicol with better safety profiles [42, 43].

Current Advances in Antibiotics Derived from Actinobacteria

Actinobacteria's capacity to produce antibiotics has drawn considerable attention in recent years due to the growing issue of antimicrobial resistance (AMR). Novel antibiotics from previously unstudied

Streptomyces strains have been discovered thanks to developments in genomes, metabolomics, and high-throughput screening technologies. Zhang et al. (2023), for example, developed a novel family of antibiotics from marine Streptomyces species that had strong action against *Klebsiella pneumoniae* and multidrug-resistant *Escherichia coli* [44]. Additionally, Streptomyces antibiotic production has been optimized through the application of genetic engineering and synthetic biology, which has increased yields and raised the possibility of developing new, stronger antibiotics [45, 46].

Advances in Metabolomics Profiling of Marine Actinobacteria

Metabolomics, the extensive analysis of metabolites within biological systems, has recently emerged as an effective method for identifying novel bioactive chemicals from marine actinobacteria. The amalgamation of sophisticated high-throughput technologies and bioinformatics has facilitated the swift identification and characterisation of a wide spectrum of metabolites generated by these microbes. Metabolomics profiling enables scientists to identify distinctive chemical structures that may be obscured by conventional culture-based techniques. This method has enabled the identification of potential novel antibiotics, including several molecules with strong efficacy against multidrug-resistant bacteria [47].

Marine actinobacteria generate a diverse array of secondary metabolites, such as polyketides, non-ribosomal peptides, alkaloids, and terpenoids, many of which remain inadequately investigated. Metabolites such as actinomycin D and lacrimotoxin, derived from marine Streptomyces strains, exhibit significant antibacterial and anticancer effects, highlighting the necessity of employing metabolomics to fully exploit the therapeutic potential of these microorganisms [48]. Furthermore, the intricacy of marine microbial ecosystems, influenced by their different environmental stresses, yields a plethora of novel chemicals that are structurally distinctive compared to those from terrestrial organisms, presenting new opportunities for drug discovery [49].

Advancements in marine actinobacteria research, coupled with metabolomics and genetic data, yield enhanced understanding of the metabolic pathways responsible for the generation of bioactive chemicals. This knowledge enables researchers to investigate the genetic and enzymatic systems responsible for metabolite synthesis and to create novel strains with improved antibiotic manufacturing capability. Recent studies have shown that metabolomics profiling can assist in elucidating the unique functions of various metabolites in treating bacterial infections, hence enhancing the overall comprehension of their modes of action [50].

Role of Marine Actinobacteria in Combating Multidrug-Resistant Pathogens

The emergence of multidrug-resistant (MDR) bacteria is a significant public health challenge of the 21st century, exacerbated by the scarcity of new medicines entering the market to combat this escalating menace. Marine actinobacteria have emerged as a notably potential source of new antimicrobial drugs effective against resistant strains. These bacteria, which flourish in conditions of great ecological stress, including elevated salinity, pressure, and nutrient competition, have developed distinctive metabolic pathways to synthesize a wide variety of antimicrobial compounds [51].

A primary rationale for investigating marine actinobacteria in the quest for new antibiotics is their capacity to produce chemicals that address innovative mechanisms of bacterial resistance. Numerous sulfur-containing compounds, including thiopeptides, have been extracted from marine actinobacteria and demonstrated significant efficacy against resistant strains of *Staphylococcus aureus* and *Mycobacterium TB* [52]. These chemicals are thought to operate by reducing protein synthesis and compromising cell membrane integrity, rendering them effective against bacteria that have acquired resistance to traditional antibiotics [53].

The existence of intricate secondary metabolites such as polyketides and non-ribosomal peptides in marine actinobacteria presents opportunities for the creation of pharmaceuticals aimed at various bacterial illnesses. Recent studies have shown that these metabolites possess broad-spectrum efficacy against both Gram-positive bacteria and Gram-negative infections, which are notoriously challenging to treat with current antibiotics [54]. The identification of novel bioactive chemicals from marine actinobacteria is a key

advancement in combating antibiotic resistance, offering alternative therapeutic options for infections induced by multidrug-resistant bacteria.

Impact of Synthetic Biology and Genetic Engineering on Antibiotic Production

The utilization of synthetic biology and genetic engineering methods on marine actinobacteria has transformed antibiotic discovery. Progress in these domains enables researchers to alter the genetic composition of actinobacterial strains, improving their capacity to generate high-yield and innovative antibiotics. Through the manipulation of the biosynthetic pathways of actinobacteria, researchers can enhance the production of bioactive metabolites and incorporate novel enzyme capabilities that facilitate the synthesis of previously inaccessible substances [55].

A prominent instance is the enhancement of *Streptomyces* species for the synthesis of polyketide antibiotics. Utilizing genetic engineering, researchers have augmented the expression of essential biosynthetic genes, resulting in elevated yields of antibiotics including erythromycin and rifamycin [56]. Furthermore, synthetic biology tools like CRISPR-Cas9 have enabled the accurate modification of microbial genomes, resulting in genetically altered strains with improved antimicrobial properties or resilience to environmental stressors commonly found in marine ecosystems [57].

The amalgamation of these technologies has facilitated the discovery of new families of antibiotics by stimulating dormant biosynthetic pathways in actinobacteria. Numerous marine actinobacterial genomes have cryptic gene clusters that remain unexpressed under conventional laboratory settings. By employing targeted genetic alterations, researchers can activate dormant pathways, resulting in the identification of novel antibiotics with distinct structures and modes of action [58]. This methodology has resulted in the identification of multiple viable therapeutic candidates, underscoring the potential of synthetic biology in combating antibiotic resistance.

Challenges and Future Directions in Marine Actinobacteria Research

Notwithstanding the great promise of marine actinobacteria in drug development, numerous hurdles persist in completely using their capabilities. A significant challenge is the difficulty of extracting and cultivating these microbes, especially from deep-sea habitats, where conventional culturing techniques sometimes prove inadequate. To address this constraint, researchers are progressively utilizing metagenomics and other culture-independent methodologies, which facilitate the extraction and study of genetic material directly from environmental samples [59]. These techniques have led to the identification of numerous novel actinobacterial strains, many of which produce chemicals with antibacterial properties that remain to be thoroughly investigated

.A further problem pertains to the scalability of antibiotic manufacture. Although marine actinobacteria can generate useful bioactive chemicals, increasing their production to satisfy commercial requirements is a challenging endeavor. Improvements in bioreactor design and the optimization of fermentation processes will be essential for the commercial viability of marine-derived antibiotic manufacturing. Furthermore, ongoing investigation into the biosynthetic processes of marine actinobacteria will be crucial for improving their yield and rendering the manufacture of new antibiotics more economically viable [60].

The future of marine actinobacteria research is anticipated to be significantly influenced by the integration of systems biology, artificial intelligence, and machine learning. These techniques can detect novel patterns in microbial metabolism and forecast the antibiotic potential of uncharacterized substances by examining extensive datasets derived from metabolomics and genomic research. This will expedite the identification of novel antibiotics and allow researchers to remain proactive against emerging drug-resistant bacteria [61].

Conclusion

Actinobacteria, especially those originating from marine ecosystems, are a progressively significant source of innovative and powerful antimicrobial agents. These microbes have developed the ability to synthesize a diverse range of bioactive compounds, including polyketides, non-ribosomal peptides, alkaloids, and

terpenoids, all of which demonstrate significant antimicrobial activity. Numerous chemicals exhibit efficacy against diverse pathogens, including bacteria, viruses, and fungi, some of which demonstrate resistance to numerous current antibiotics. Marine actinobacteria, flourishing in distinctive and frequently severe ecological environments, exhibit a variety of metabolic pathways enabling them to produce new compounds absent in terrestrial animals. These distinctive chemicals offer potential solutions for the escalating global challenge of antimicrobial resistance (AMR). By harnessing the medicinal potential of marine actinobacteria, researchers may discover novel medicines to enhance existing antibiotic therapies or maybe establish totally new classes of antibiotics. Furthermore, the marine environment, frequently underexamined relative to terrestrial ecosystems, constitutes a substantial and predominantly unexploited reservoir of bioactive chemicals. Due to the pressing need to address antimicrobial resistance (AMR), the investigation of marine actinobacteria represents a vital research domain, providing potential for novel therapeutics to tackle resistant infections and advance the future of medicine.

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استكشاف الإمكانيات المضادة للميكروبات للبكتيريا المقرونة البحرية: تحليل الميتابولومات وتحديد المركبات الحيوية الجديدة المضادة للبكتيريا

الملخص

يعد مقاومة المضادات الحيوية تهديدًا كبيرًا للصحة العامة، حيث تسببت في حوالي 5 ملايين وفاة في عام 2019. وعلى الرغم من أنها ليست قابلة للوقاية، إلا أنه يمكن السيطرة عليها من خلال البحث عن مركبات مضادة للبكتيريا جديدة. تُعتبر البكتيريا العنقودية البحرية (Marine Actinobacteria) مصدرًا مهمًا للبحث البيوتكنولوجي واكتشاف المستقبلات الجديدة، حيث تمتلك مستخلصاتها الثانوية العديد من الأنشطة البيولوجية بما في ذلك النشاط المضاد للبكتيريا والفطريات والفيروسات والأورام.

الهدف: يهدف هذا البحث إلى تقييم النشاط المضاد للبكتيريا للبكتيريا العنقودية البحرية واستخلاص ملف ميتابولومي شامل للمستقلبات البيولوجية النشطة. تسعى الدراسة إلى تحديد المركبات المضادة للبكتيريا الجديدة وتوضيح العلاقة بين المستقلبات المحددة وفعالية النشاط المضاد للبكتيريا

المنهجية: تم إجراء مراجعة للأدبيات الموجودة، شملت الدراسات حول مستخلصات البكتيريا العقديّة البحرية، وتم تقييم النشاط المضاد للبكتيريا للمستخلصات الخام باستخدام طريقة الانتشار في الأجار ضد البكتيريا. تم تحديد مركبات المستقلبات باستخدام تقنية كروماتوغرافيا الغاز-الطيف الكتلي.(GC-MS)

النتائج: تم تحديد سلالات من البكتيريا العقديّة البحرية التي أظهرت نشاطاً مضاداً للبكتيريا قوياً، لا سيما ضد البكتيريا المقاومة للأدوية المتعددة. كشف تحليل الميتابولوم عن مستقلبات بيولوجية نشطة، كانت مرتبطة بشكل قوي بالآثار المضادة للبكتيريا.

الخاتمة: بشكل عام، توفر البكتيريا العقديّة البحرية مستقلبات واعدة لتطوير الأدوية، وقد يؤدي التعرف بشكل أعمق على مستقلبات *Streptomyces* إلى اكتشاف أدوية جديدة للعديد من الأمراض.

الكلمات المفتاحية: بكتيريا عقديّة، مضاد للبكتيريا، مضاد للبكتيريا، *Streptomyces*، الطحالب البحرية