ISSN-P: 2807-5987

"A review of microbial antibiotics extracted from marine fungi"

ISSN-E: 26632806

ISSN-P: 2807-5987

Abstract:

Because of the rising incidence of infectious diseases and the resulting rise in multidrug resistance among human pathogenic bacteria, there is an urgent need to find and develop new antimicrobial medicines with new mechanisms of action. Most of the antibiotics currently on the market were either produced from terrestrial organisms or semi synthetically from fermentation products. Antibiotic lead structures may be discovered through the isolation of microbes from hitherto unknown environments. As a result of their adaption to this severe environment, deep-sea microbes have the potential to develop new and potent secondary metabolites with powerful biological activity. This review focuses on new antibiotics derived from deep-sea microbes. Compounds are broken down into chemical classifications, bioactivities, and sources. They also discuss new developments in deep-sea microorganism extraction methods and strategies.

Keywords:

antibiotics; deep-sea; marine microorganisms; extreme habitat; marine sediments biodiscovery; marine bacteria; marine fungi; marine natural products.

الملخص:

بسبب ارتفاع معدل الإصابة بالأمراض المعدية وما ينتج عن ذلك من ارتفاع في مقاومة الأدوية المتعددة بين البكتيريا المسببة للأمراض البشرية، هناك حاجة ملحة لإيجاد وتطوير أدوية جديدة مضادة للميكروبات بآليات جديدة للعمل. تم إنتاج معظم المضادات الحيوية الموجودة حاليًا في السوق إما من كائنات أرضية أو شبه اصطناعية من منتجات التخمير. يمكن اكتشاف هياكل الرصاص للمضادات الحيوية من خلال عزل الميكروبات من بيئات غير معروفة حتى الآن. نتيجة لتكيفها مع هذه البيئة القاسية، تمتلك ميكروبات أعماق البحار القدرة على تطوير مستقبلات ثانوية جديدة وقوية ذات نشاط بيولوجي قوي. تركز هذه المراجعة على المضادات الحيوية الجديدة المشتقة من ميكروبات أعماق البحار. يتم تقسيم المركبات إلى تصنيفات كيميائية وأنشطة حيوية ومصادر. كما يناقشون التطورات الجديدة في أساليب واستراتيجيات استخراج الكائنات الحية الدقيقة في أعماق البحار.

1. Introduction:

When it comes to the most unexplored and extreme habitats on our planet, the deep-sea stands at the top of the list (Silber, J., Kramer, A., Labes, A., & Tasdemir, D. (2016). For example, the deep-sea is an extreme environment due to the fact that it experiences pressure increases of one atmosphere (atm) for every 10 meters (m) of water depth, so pressure ranges from 20 atm at the shelf-slope break to more than 1000 atm in the deepest trenches; (ii) the temperature drops with increasing depth, reaching values of around 2 °C; (iv) the oxygen concentration in the bottom waters can be much lower than the surrounding waters (Habbu, P., Warad, V., Shastri, R., Madagundi, S., & Kulkarni, V. H. (2016). The term "deep-sea environment" is still open to interpretation. A depth of greater than or equal to 1000 meters is now generally accepted as the norm for describing the deep sea, despite older classifications describing it as exceeding 200 meters in depth. Studies of deep-sea life have been hampered for decades by the difficulty of getting to the ocean's floor. More recently, deep ocean settings have become more accessible due to improved acoustic technology and greater access by Remotely Operated Vehicles (ROV) and submersibles, revealing the presence of biological activity (Hasan, S., Ansari, M. I., Ahmad, A., & Mishra, M. (2015). Microbial diversity was unexpectedly found in the deep-sea organic material accessed by the use of culture-dependent and culture-independent methods (Tortorella, E., et al (2018). Because of the extreme conditions, microorganisms that live in these places have evolved specialized techniques for surviving (Wiese, J., & Imhoff, J. F. (2019). As it turns out, a large number of these microorganisms are both piezo tolerant and pedophilic microorganisms but the lack of proper apparatus makes it difficult to cultivate these strains. There are changes in gene regulation, primary/secondary metabolic pathways, and natural product expression as a result of their adaptation to biochemical and physiological processes (NPs).

Some 2 percent of the more than 30 thousand products present in the ocean have been extracted from deep-sea animals in the last 50 years (Wu, B., et al. (2016). Especially for biotechnological and pharmacological purposes, antibiotics rank high among microbial NPs. For the fight against the spread of multidrug-resistant (MDR) bacteria, new antibiotics are needed (Ueda, K., & Beppu, T. (2017). Exploiting deep-sea bacteria and fungi, along with the compounds they produce, may lead to the identification of new antibiotics.

Antibiotic-resistant microorganisms pose a major challenge to researchers. As many as 25,000 people are killed each year in 29 European nations because of antibiotic-resistant germs. Staphylococcus aureus methicillin-resistant isolates (MRSA) over 20% are found in at least 11 European countries participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) with a rising trend (Auckloo, B. N., et al. (2017). The World Health Organization recently published a list of bacterial infections for which new antibiotics are urgently needed on a worldwide scale in order to help prioritise the research and development of new and effective antibiotic therapies. Carbapenem-resistant Acinetobacter baumannii, Pseudomonas aeruginoses, and members of the Enterobacteriaceae family, including Klebsiella pneumoniae, E. coli, Serratia spp., Proteus spp., Providencia spp., and Morganella spp., comprise Priority I (critical). For example, Enterococcus faecalis and Staphylococcus aureus, which are members of Priority II (high), are resistant to vancomycin. Streptococcus pneumoniae, which is not penicillin-resistant, is among the Priority III bacteria (medium) (Liu, M., et al. (2019).

In order to treat serious microbial infections, there is unquestionably a high demand for the creation of novel antibiotics that are more effective, have lower toxicity, or have a reduced chance of resistance. Many novel medicines can be found in nature. Between 1981 and 2014, over 80 antibacterial medications were approved that were either natural ingredients or directly derived from them. Recent years have seen an increase in interest in marine organisms and their natural products, which are seen as a vital and underappreciated source of raw materials for many industries. It is estimated that about 32,000 different compounds have been logged into the database for marine natural products. The marine pharmaceutical clinical pipeline, which consists of seven FDA-approved medications and 22 drug candidates in Phase III, II, or I development, contains about 30 of these. They are spread out across the globe (Netzker, T., et al. (2018).

Biodiversity and chemical variety are abundant in marine microorganisms. Metabolites produced by these microorganisms exhibit an array of biological effects as well as pharmaceutical implications. We should take advantage of this high potential for the exploration of promising compounds for the development of new drugs to treat infections. From unique marine habitats such as deep sea sediment and algae, sponges, bryozoans and coral we searched for new antibiotic-producing bacteria in order to overcome this challenge and find new compounds with antibacterial properties. Results from our lab from 2008–2017 are highlighted to show the huge potential of marine microorganisms as manufacturers of antibiotics. According to the initial surveys for antibiotic activity, approximately 50% of the bacterial isolates showed the ability to inhibit Gram-positive (Bacillus subtilis, Staphylococcal lentus) and Gram-negative (Escherichia coli) pathogenic and clinically relevant drug-resistant strains (Wu, C., et al. (2015).

so Antibacterial and antifungals are among the most commonly used drugs. Recently, as the resistance of bacterial and fungal pathogens has become increasingly serious, there is a growing demand for new antibacterial and antifungal compounds. Natural products from fungi are considered an important source for novel antibacterial and antifungal compounds because of their abundant fungal species diversity, their rich secondary metabolites and the improvements in their genetic breeding and fermentation processes. The antimicrobial activities of an increasing number of fungi living in distinctive environments are being investigated for the discovery of new antibacterial and antifungal compounds, such as endophytic fungi from wild plants and marine fungi. In the last decade, many novel bioactive natural products from marine fungi have been discovered that possess cytotoxic, anticancer, antiviral, antibacterial or antifungal activities. The antibacterial and antifungal compounds from marine fungi have quickly increased since 2010, and marine fungi have been an important source of antibacterial and antifungal compounds. This paper A review of microbial antibiotics extracted from marine fungi (Wu, B., et al. (2015).

the study Problem:

Natural products and their derivatives are of great importance in the discovery of new medicines, for example, for the treatment of cancer, diabetes, inflammatory diseases and infectious diseases caused by bacteria, fungi, viruses or parasites, but there are many difficulties and challenges facing the development of medicines and microbial antibiotics extracted from marine fungi and the line Large natural marine product tubes, and here was the research

problem that seeks to try to discover solutions to it so that the antibiotics that come out of these fungi are taken advantage of.

Study hypotheses:

- We assume that there is a significant relationship between natural products and their derivatives and the discovery of several new antibiotics and drugs.
- There is a significant relationship between the growing interest in marine microorganisms and the increased discovery of marine microbial resources for the discovery of new antibiotics.
- There is a relationship between the discovery of many new antibiotics by lake fungi and the development of treatment methods for various diseases.

Objectives of the study:

An increasing number of marine fungi are sources of new and potentially life-saving bioactive secondary metabolites. Given this great importance, this research aims to discuss some of these microbial antibiotics and new antibacterial, antiviral and antibacterial compounds isolated from fungi derived from the sea because of their important role in eliminating many diseases.

the importance of studying:

The importance of this study is as follows:

- Proving that marine fungi have a significant role in the production of many antibiotics.
- Discovering and developing new antimicrobial drugs with new modes of action.
- Demonstrate that most antibiotics currently on the market have been obtained from terrestrial organisms or derived semi-synthetically from fermentation products.
- Raising interest about marine fungi that have the ability to produce new chemical scaffolds that lead to potential drugs.
- The study also aims to stimulate discussion of farming strategies that can be used to produce new biologically active metabolites or increase their production.

The limits of the study:

- Objective limits: This paper seeks to provide a review of microbial antibiotics extracted from marine fungi.
- Time limits: This study is being conducted in the year 2021-2022.
- Spatial boundaries: This study is carried out within the borders of the Kingdom of Saudi Arabia.

study terms:

- definition of antibiotics: Bacterial infections can be treated with antibiotics, which are prescribed to both humans and animals. They either kill the bacteria or make it extremely difficult for them to grow and reproduce (Nweze, J. Aet al. (2020).
- Define microbial antibiotics: It is used to treat bacterial infections and kill or inhibit the development of bacteria with microbial antibiotics. Soil bacteria and fungi produce them naturally. There is less competition for limited resources like food and water when an antibiotic is used because the other organisms are wiped out (Wu, C., et al. (2015).
- marine fungi definition: A type of fungus known as a marine fungus is one that thrives in marine or estuarine conditions. They don't belong to a specific taxonomic family, but they do share a habitat. Species of marine fungi that can only be found in watery environments are known as obligatory marine fungi. They normally live in terrestrial or freshwater environments, but facultative marine fungi have the ability to thrive and even produce spores in marine environments (Imhoff, J. F. (2016).

Theoretical framework and previous studies:

The previous studies:

- **study of Auckloo, B. N., Pan, C., Akhter, N., Wu, B., Wu, X., & He, S. (2017). Stressdriven discovery of novel cryptic antibiotics from a marine fungus Penicillium sp.**

It has been shown that marine fungi are a huge source of new and biologically active secondary metabolites, which is reflected in the increasing number of published articles dealing with compounds from this group of fungi. To date, more than one hundred secondary metabolites from marine fungal organisms have been identified. The mycobiota of the coastal water in and around Mahabalipuram beach was collected from five different locations. Eight genera of filamentous fungi were discovered. It was found that Mahabalipuram beach had a higher density of populations and a wider variety of fungal genera and species than any of the other locations. Aspergillus (23.72 percent, 6 species) and Cephalosporium acremonium (37.6 percent, 8 species) were the most prevalent genera, followed by Penicillium (23.7 percent, 6 species) (21.28 percent , 16 spp.). The most common species was P. chrysogenum, followed by P. citrinum, A. niger, A. flavus, A.fumigatus, Cephalosporium acremonium, and Cladosporium sp. Cephalosporium acremonium and Cladosporium sp. Many more genera and species were discovered, but they were all extremely rare. When marine fungal strains Cephalosporium acremonium and P. citrinum were examined for secondary metabolic content, a wide range of activities were discovered, as were partial chemical structures, thanks to IR and NMR research. The secondary metabolites found in this study came from a variety of natural product structures. That marine fungi have the ability to synthesise a wide variety of new structures is encouraging.

- **Study of Blunt, J. W., Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., & Prinsep, M. R. (2018). Marine natural products. Natural Product Reports.**

The discovery of new pharmaceuticals, such as those to treat cancer, diabetes, inflammatory diseases, and infections caused by bacteria, fungi, viruses, or parasites, relies heavily on natural products and their derivatives. When it comes to chemical diversity, marine microorganisms offer a wealth of biologically active compounds because they are so diverse. Increasing attention has been paid to sustainable exploration of marine microbial resources for the discovery of new antibiotics because marine microorganisms are being seen as promising sources for the production of new compounds with potential medical applications, as highlighted in this article. The large marine natural product pipeline bottlenecks in drug development are also discussed.

- **Study of Gärtner, A., Wiese, J., & Imhoff, J. F. (2016). Diversity of Micromonospora strains from the deep Mediterranean Sea and their potential to produce bioactive compounds.**

Antibacterial agents derived from sponge-derived fungi are capable of producing a wide range of bioactive metabolites. The marine sponge Acanthostrongylophora ingens yielded eight derived-fungi constituents in a previous study. Vibrio cholerae Inaba, Enterococcus faecalis ATCC 29252, Multidrug-resistant Pseudomonsa (MDR-PA) and ATCC 27853, Streptococcus mutans ATCC 25175, Methicillin-resistant Staphylococcus aureu (MRSA) and Mycobacterium tuberculosis H37Rv were all tested for their antibacterial properties in this study. Only one of the isolated fungi, IB141, showed significant ability to inhibit the growth of all but S. mutans ATCC 25175. Using the agar diffusion method of Sabouraud Dextrose Agar (SDA) medium, we tested the antibacterial activity of the ethyl acetate extract of the fungus IB141 against the above bacteria and found that it inhibited the growth of S. aureus ATCC 25923 and M. tuberculosis H37Rv at concentrations of 0.025, 0.05, and 0.1 percent significantly. With increasing amounts of MeOH in CH2Cl2, this unprocessed crude ethyl acetate extract was column chromatographed on silica gel, and the resulting fractions were checked by Thin Layer Chromatography before being combined to yield four fractions. There were seven subfractions in the second fraction (F2) after chromatographic separation on silica gel with CH2Cl2-MeOH (0.3:4.7) elution. Analysis of the major peaks of the HPLC (reverse phase, C18) eluted with MeOH was performed on the three subfractions F22, F23, and F26. Palitantin, citreodrimene A, septicine, and ochratoxin A were found to make up the majority of subfraction F22, with retention times of 38.91, 36.98, and 13.04 minutes, respectively, for the three major components.

- **Study of Imhoff, J. F. (2016). Natural products from marine fungi – Still an underrepresented resource.**

Roselle, rosemary, clove, and thyme extracts were tested for their antimicrobial properties on a variety of food pathogens and spoilage microorganisms, including Listeria monocytogenes, Salmonella typhimurium, and Clostridium perfringens. Antimicrobial activity and minimum inhibitory concentrations (MIC) of plant extracts against Gram-positive bacteria (Bacillus cereus, Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli, Salmonella enteritidis, Vibrio parahaemolyticus, and Pseudomonas aeruginosa) have been determined using the agar well diffusion method (Candida albicans). The extracts were found to have antibacterial and antifungal properties when tested on a variety of different microorganisms and bacteria. There was no effect on Candida albicans (CA) on the antibacterial activity of Ethanolic Roselle Extract (P 0.05) against all of the tested bacteria. Thyme (ethanolic extract) had an inhibition zone of 15.8 mm and 25.2 mm, while clove (ethanolic extract) did not. For the most sensitive strain, Bacillus cereus (BC), clove extract appears to be 0.315 percent effective. Staphylococcus aureus (SA) and Escherichia coli (EC) cells were exposed to plant extracts to measure changes in internal pH (pHhint) and membrane potential in order to better understand the antimicrobial activity mechanism. In terms of pHint and hyperpolarization of the cell membrane, the results showed that the plant extracts had a significant impact on the cell membrane of both Gram-positive and Gram-negative bacteria. Finally, plant extracts can be used safely as food preservatives because of their antimicrobial properties.

- **Study of Conlon, B. P., … Lewis, K. (2015). A new antibiotic kills pathogens without detectable resistance.**

More and more marine fungi are producing bioactive secondary metabolites that have the potential to save people's lives. Several of the novel antibacterial, viral, and parasitic compounds isolated from marine-derived fungi have been discussed here, as have their potential roles in the eradication of diseases. Metabolic engineering and post-genomics approaches to drug development have been discussed as potential future commercial uses for these compounds.

- **Study of Mayer, A. M. S.. (2018). Marine pharmaceuticals: The clinical pipeline.**

Because of the rising incidence of infectious diseases and the resulting rise in multidrug resistance among human pathogenic bacteria, there is an urgent need to discover and develop new antimicrobial drugs with new mechanisms of action. The majority of antibiotics on the market today were derived either semi-synthetically from fermentation products or obtained from terrestrial organisms. Antibiotic-active lead structures may be discovered through the isolation of microorganisms from previously unknown habitats. These microorganisms, adapted to the extreme conditions of the deep sea, have the potential to produce novel secondary metabolites with potent biological activity because of their adaptation to this extreme environment. Antibiotics derived from deep-sea microorganisms are the focus of this review. The bioactivities, chemical classes, and origins of the compounds are all described. A new generation of deep-sea microorganism harvesting methods and strategies is also discussed by the authors.

- **Study of Mayer, A. M. S., Rodríguez, A. D., Taglialatela-Scafati, O., & Fusetani, N. (2017). Marine pharmacology in 2012-2013: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action.**

Fungi in marine environments are understudied, but they produce a wide range of natural products due to unique environmental pressures and nutrients. Because bacteria are the most studied marine microorganisms, it's easy to overlook the abundance of bioactive metabolites produced by marine fungi. Marine fungi, like their terrestrial counterparts, have the potential to produce new chemical scaffolds that could lead to new pharmaceuticals, like camptothecin, the penicillins, and cyclosporin A. Many silent biosynthetic gene clusters are involved in the production of bioactive compounds in the genomes of fungi, which are more phylogenetically diverse than bacteria. However, standard laboratory conditions have only been used to cultivate about 5% of the world's known fungi. There has been a steady rise in the number of natural products from marine fungi, but the number is still significantly lower than the number from their bacterial counterparts. There are many fungal metabolites found in extreme marine environments, including symbiotic associations and extreme pressures, temperatures, saltiness, and light. We discuss these metabolites in this article. We'll also talk about how to grow plants to make more bioactive metabolites or to make more of the ones we already have. A large number of reported structures are included in this review, but at times only a few of a large number of related structures are displayed.

- **Study of Newman, D. J., & Cragg, G. M. (2016a). Natural products as sources of new drugs from 1981 to 2014.**

Because the marine environment exposes marine organisms to such a wide range of chemical and physical conditions, the bioactive compounds they produce have a wide range of diversity. Bioactive compounds derived from marine bacteria and fungi have become a major focus of marine biotechnology research. To put it another way, marine microbes and fungi are ranked among all organisms because they produce a wide range of bioactive secondary metabolites with potential pharmaceutical uses. As a result, they hold great promise for the development of new drugs to combat diseases like cancer, a wide range of viral infections, malaria, and inflammation. Bioactive compounds obtained from marine bacteria and fungi, as well as some of the most promising pharmaceutical substances extracted and isolated from these sources, have received a great deal of attention over the last decade.

- **Study of Newman, D. J., & Cragg, G. M. (2016b). Drugs and drug candidates from marine sources: An assessment of the current "state of play".**

Only the broad-spectrum cephalosporin C can be traced back as a marine fungal-derived drug, despite an increase in novel marine natural products from fungi in the last three decades. Cephalosporins were discovered in Sardinia in the 1940s after a strain of Acremonium chrysogenum was found in sewage water collected from the Sardinian coast. Many antibacterial metabolites have been discovered through bioprospection of marine fungi since then, with some of these metabolites showing promise against drug-resistant strains. There is also evidence that some of these metabolites may be useful in combating pathogens listed as a priority by the World Health Organization. It is obvious that marine fungi, like their terrestrial counterparts, could be a source of new prototypes to combat antibiotic-resistant bacteria, given their success in discovering and developing antibiotics currently used in the clinical setting. Preclinical development of these compounds will be guided not only by their antibacterial potency but also by their targets, mechanisms of action, and efficacy against priority pathogens.

- **Study of Pham, T. M., Wiese, J., Erhard, A., & Imhoff, J. F. (2016). Diversity and antimicrobial potential of bacterial isolates associated with the soft coral Alcyonium digitatum from the Baltic Sea.**

Biologically active marine bacteria and fungi have a significant impact on the development of new products. In a cold, dark, and high-pressure environment, some of these marine species must cope. A surprising number of species with a high degree of diversity are able to thrive in these conditions, and their natural products are both fascinating and structurally complex. Even though only a small number of microorganisms have been studied for bioactive metabolites, a large number of active substances have been isolated, some of which have unique structural skeletons. This review focuses on the chemical potential of marine microorganisms, as well as their bioactive products and mechanisms of action, in the recent years (2007–09).

Theoretical framework:

2. microbial antibiotics extracted from marine fungi:

It has been widely accepted since the 1948 discovery of the antibiotic cephalosporin that marine fungi contain bioactive compounds. Marine fungi have the ability to colonise a wide range of marine habitats, including the most extreme ones, such as deep-sea environments, due to their exceptional adaptability. Fungi are abundant and diverse in these habitats, but it is expected that many more will be discovered in the future. It is possible to discover new bioactive compounds useful for drug discovery by studying the deep-sea fungal biodiversity (Wiese, J., et al. (2018). Gliotoxin, an antimicrobial compound first isolated from a deep-sea Aspergillus sp. strain, has been shown to inhibit the growth of Gram-positive bacteria such as Staphylococcus aureus and Bacillus subtilis. Seto Inland Sea mud in Japan was used to isolate the fungus. Antibiotic molecules from deep sea fungi were discovered after this discovery, but they still make up less than 10% of the molecules found in marine fungi isolated from surface waters. Deep-sea sediments in the South China Sea yielded 13 new species of fungal species (including several new types of phylotypes), some which were able to produce antimicrobial compounds against pathogenic bacteria and fungi like Micrococcus luteus, Pseudoaltermonas (World Health Organization. (2017).

Natural secondary metabolites known as prenylxanthones are an important source of biological and pharmacological activity. The South China Sea fungus Emericella sp. SCSIO 05240 yielded four new antifungal and antibacterial prenylxanthones, dubbed emerixanthones A–D. (3258 m). Bacteria such as Escherichia coli, Klebsiella pneumoniae and S. aureus can be killed by these molecules, as well as Aeromonas hydrophila and Aeromonas fluorescens (ATCC 7966). An additional compound in the xanthone family, Engyodontiumone H, was purified from a deep-sea fungus found in the South China Sea at a depth of 3739 metres. E. coli and B. subtilis were both inhibited by this molecule, with MIC values of 64 and 32 g/mL, respectively. proved antibiotic activity of xanthone derivatives by

ISSN-P: 2807-5987

inhibiting the bacterial phosphoenolpyruvate-dependent phosphotransferase system's enzyme I (EI) (PTS) (Wu, B., Ohlendorf, B., et al. (2015).

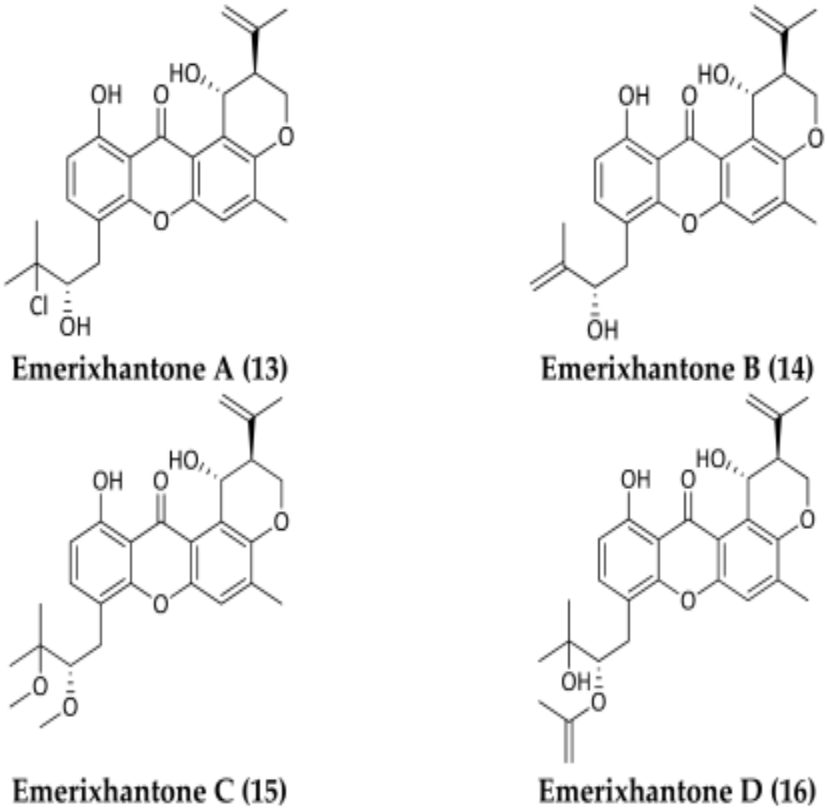
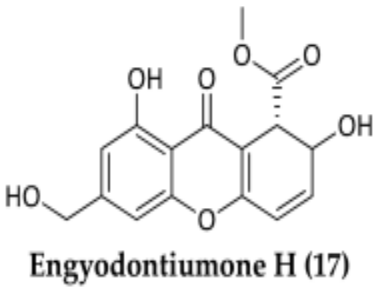


Figure 5. Structures of Emerixanthones A–D (**13–16**).



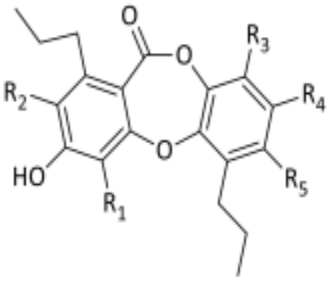
Structure of Engyontiumone H

In the South Atlantic Ocean, researchers found an unidentified species of Spiromastix sp. fungus that produced 15 new depsisone-based analogues known as spiromastixones A–O. MIC values for S. aureus, B. thuringiensis, and B. subtilis ranged from 0.125 to 8.0 micrograms per litre for these compounds. It was found that some of them had inhibitory effects on MRSA and MRSE methicillin-resistant strains of S. aureus as well as vancomycin-resistant E. faecalis and VRE strains of E. faecium and E. faecium (VRE) (Wu, B., et al. (2015).

Chaetomium sp. strain NA-S01-R1 was isolated at a depth of 4050 m in the West Pacific Ocean and recently identified new chlorinated azaphilone pigments with antibacterial and cytotoxic activities (Wu, B., Wiese, J., et al. (2016).

ISSN-E: 26632806

ISSN-P: 2807-5987



Spiromastixones A–H (Type A)

(18) A: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = OH$

(19) B: $R_2 = Cl$, $R_1 = R_3 = R_5 = H$, $R_4 = OH$

(20) C: $R_1 = Cl$, $R_2 = R_3 = R_5 = H$, $R_4 = OH$

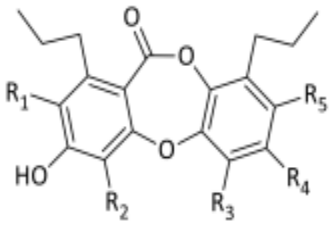
(21) D: $R_2 = R_3 = H$, $R_1 = R_5 = Cl$, $R_4 = OH$

(22) E: $R_3 = R_5 = H$, $R_1 = R2 = Cl$, $R_4 = OH$

(23) F: $R_1 = R_2 = R_5 = Cl$, $R_4 = OH$, $R_3 = H$

(24) G: $R_1 = R_2 = R_5 = Cl$, $R_3 = H$, $R_4 = OMe$

(25) H: $R_1 = R_2 = R_3 = Cl$, $R_5 = H$, $R_4 = OH$



Spiromastixones K–O (Type B)

(28) K: $R_1 = R_2 = R_5 = Cl$, $R_3 = H$, $R_4 = OMe$

(29) L: $R_1 = R_2 = R_3 = R_5 = Cl$, $R_4 = OMe$

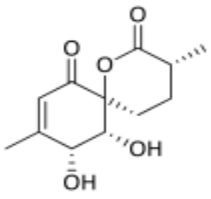
(30) M: $R_1 = R_2 = Cl$, $R_3 = R_5 = H$, $R_4 = OH$

(31) N: $R_1 = R_2 = R_5 = Cl$, $R_3 = H$, $R_4 = OH$

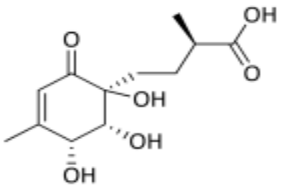
(32) O: $R_1 = R_2 = R_3 = R_5 = Cl$, $R_4 = OH$

Structures of Spiromastixones A–O

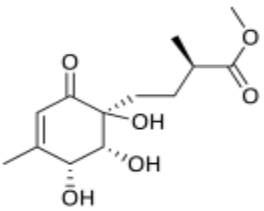
There are many more predicted biosynthetic genes in fungi than there have been so far in the discovery of new molecules, according to several analyses of fungus genomes. Using the OSMAC (one strain many compounds) approach could lead to the discovery of new antibiotics, as demonstrated in this study. Deep-sea-derived Penicillium F23-2 from Jiaozhou Bay in China produced five new ambuic acid analogues, penicyclones AE, which had antibacterial activity against S. aureus with MIC values ranging from 0.3 to 1.0 g/mL, according to this research. When grown on rice-based medium instead of PYG and PD media, this fungus produced different metabolites. An important weapon in the fight against multidrug resistant bacteria could be improved isolation and cultivation methods for deep sea fungi (Overy, D. P., Rämä, T., Oosterhuis, R., Walker, A. K., & Pang, K. L. (2019).



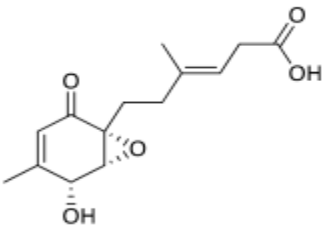
Penicylone A (33)



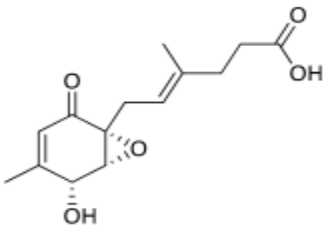
Penicyclone B (34)



Penicyclone C (35)



Penicyclone D (36)



Penicyclone E (37)

Structures of Penicyclones A–E

ISSN-E: 26632806

3. Improving the Biodiscovery Pipeline for Deep-Sea Antibiotics from marine fungi:

Antimicrobial compounds have been found in deep-sea environments, as shown in Section 2. It's possible we've only scratched the surface of a much larger pool of molecular scaffolds, however. Biodiscovery campaigns for antimicrobials and other bioactive compounds from this extreme ecosystem face a number of challenges, including the discovery of novel biological resources (microorganisms and/or their genetic material) (s). Innovations aimed at exploiting deep-sea microorganisms are needed to improve the current situation. Here, we're going to take a look at the limitations of deep-sea microbial research, as well as the most recent discoveries and advancements in the field.

3.1. Sampling Techniques:

More than 58 percent of the seafloor is at or above 4000 m, with pressures of up to 40 MPa on the marine sediments below. A major drawback of deep-sea sampling for bacteria isolation is that most of the microorganisms found there are obligate piezophiles, unable to grow at atmospheric pressure but particularly sensitive to changes in pressure. Subseafloor sediments must be brought to the surface via pressurized transport and treatment, which is essential for the preservation of microbes' viability and diversity. For geological and microbiological research, the German Project OMEGA developed a Multiple Autoclave Corer (MAC) in 2002 that can collect four 0.6 m cores while maintaining in situ conditions (e.g., pressure and temperature). After two months of storage at a depth of 776 metres below the surface of the North Pacific Ocean, the cores were still under pressure. A Dynamic Autoclave Piston Corer (DAPC) with a maximum depth of 2000 m was developed as a result of further efforts. "Hydrate autoclaving equipment," "pressurized core sampling and extrusion system," and "pressurized chambers for prokaryotic enrichment and isolation" were developed by Parkes' group in collaboration with colleagues (DeepIsoBUG). Using a subcoring and slicing system (Aminah I., Putra A. E., Arbain D., Handayani D., (2019), a sample subcore (20 mm) can be obtained from the seafloor core after it has been brought on board the vessel without depressurization (up to 25 MPa). To create an inoculum, the slice is placed in a low-pressure vessel (maximum 25 MPa) and shaken to produce a slurry. After that, the slurry is transferred to high-pressure culture vessels containing enrichment media at various pressures (up to 100 Mpa). A motor-driven chain connects the isolation chamber's 12 agar plates, allowing researchers to pick and choose which ones they want to work with. The samples' integrity and value are preserved because sediments are never depressurized. The use of "Soft robotic grippers," a new development in deep-sea sampling, involves robots. For the delicate handling of marine microorganisms, Galloway et al. created a remotely operated vehicle (ROV) with a robotic hand that can be operated remotely (Artasasta M. A.et al., (2019). The Deep Reef ROV and soft robotic grippers were brought to the Gulf of Eilat in the northern Red Sea for a pilot study. The device was tested at depths ranging from 100 to 170 metres and proved to be able to grab soft specimens without damaging them. As a result, this device could play a significant role in the exploration of the deep sea, despite its current limitations (it can only work with macroorganisms). In the future, as predicted by Galloway, robotic hands and underwater experiments could be added, and RNA preservers (e.g., RNAlater) could be used to facilitate transcriptomic experiments. A variety of marine fungi that produce microbial antibiotics can be discovered by using this method to study marine symbionts, known producers of metabolites, and fungi from the ocean floor (Al-Hashimi, A. G. (2012).

3.2. Isolation and Cultivation Techniques:

In the late 1950s, two pioneers in the field of deep-sea marine fungi isolation and cultivation, Zobell and Morita, developed titanium vessels that could withstand pressures of up to 100 MPa. No obligate piezophiles were found, but rather piezotolerant strains (Altemimi, A., et al. (2016).

It took until 1979 before scientists were able to isolate spirillium CNPT-3 from an amphipod collected in Philippine trenches with a pressure-retaining trap and use it to create the first obligately piezophilic strains.

Many piezophiles were discovered over the following years, but most of them only represented a small subset of the bacterial taxonomic diversity found in the deep sea (Alzoreky, N. S., and Nakahara, K. (2003).

Enrichment steps and nutrient-rich media are typically required for the isolation of fast-growing taxa in classical isolation methods. Techniques like dilution-to-extinction cultivation and natural seawater medium have been used to isolate novel strains, such as a novel member of the Roseobacter clade within alphaproteobacteria. When grown under optimal conditions (80 MPa and 10 C), the strain PRT1 is the slowest-growing and lowest-density piezophile yet, with a minimum doubling time of 36 hours (Aono, R., Ito, M., and Horikoshi, K. (1997).

As a result, it was even more difficult to grow the hyperthermophilic and piezophilic bacterial strains found in hydrothermal vents deep in the ocean, where they are more commonly chemolithoautotrophs. By using a piezophilic cultivation technique, scientists were able to grow deep-sea methanogenic bacteria as well as other chemolithoautotrophs. It was built using a combination of a syringe and a piston and could reach temperatures of up to 122 °C at 20 million pounds of pressure. Methanopyrus kandleri strain 116, a new hyperthermophilic methanogenic strain, was successfully isolated using this method. Sulfur- and/or H2-oxidizing chemolithoautotrophs from a thermal vent chimney were cultivated using this system in subsequent studies. There were several new strains of the previously uncultivated Thioprofundum lithotrophica that were isolated through their efforts. Piezobacter thermophilus, a new genus in the Rhodobacteraceae family, was found to be associated with the second bacterium (Azziz-Baumgartner, E., et al. (2005).

Methanogenic piezophilic strains were successfully isolated using specialised bioreactors. Microorganisms from 2533-meter-deep sea sediments were successfully cultured in an Aoki and coworker's bioreactor with polyurethane sponges, known as the "down-flow hanging sponge (DHS) bioreactor." Continuous operation of the bioreactor was used as a source of carbon. Anaerobic oxidation of methane (AOM) was confirmed in the bioreactor using 13C-labeled methane experiments. Archeal anaerobic methanotroph groups were confirmed to be the most prevalent microbial components after an extended incubation period of 2013 days. More recently, the cultivation of deep hyperthermophilic communities was done using anaerobic methods Immobilized bacteria are used in this process. An assortment of microorganisms, including Immobilization of the Mid Atlantic Ridge's active Rainbow Field thermal vent (2275 m depth). at higher temperatures (above 50°C) into beads and used to inoculate a bioreactor in continuous mode. Keeping the culture alive for 45 days proved to be a successful method for cultivating these organisms' microorganisms (Caleja, C., et al. (2016).

3.3. Metagenomics:

Most deep-sea microbes and marine fungi remain difficult to cultivate in the laboratory despite advances in the cultivation of these microbes. Also, our ability to obtain antibiotics from deep-sea microorganisms via traditional methods is limited. A new generation of powerful tools for DNA amplification, sequencing, and analysis has made significant strides in recent years. Metagenomics is a cultivation-free technique that can be used to study and exploit extreme environments. As a result of this approach being successfully used in deep-sea environments, new information about the microbial diversity and phylogeny of these organisms has emerged in the last few decades (Cheng, C., et al. (2015).

In the last few years, metagenomics has also been used to discover new natural products. We were able to clone and express a biosynthetic gene cluster from East China Sea sediments using a functional approach (1000 metre below sea surface). A library of 60,000 E. coli clones with an average insert size of 35 kbp was created. This library was then put through its paces using the chrome azurol metal ion indicator assay in search of compounds that could bind metal ions. Bisucarberin, the bioactive siderophore, was discovered in this manner. Analysis of gene clusters indicated that it came from an uncultured bacterium (Clarke, D., et al. (2017).

Two metagenomic libraries were derived from subsurface sediments collected at a depth of 3006 m in the Indian Ocean using the same method. screened libraries for analgesic and cytotoxic activity, and then selected clones for further investigation. Three promising indole alkaloids were identified by the researchers. Recombinant expression is the main drawback of this metagenomics approach. Deep-sea-derived gene clusters can be difficult to express in E. coli systems based on heterologous production. In order to improve metagenomics' chances of success, developing expression platforms based on piezophile microorganisms may be essential. Some progress has already been made in this regard. Pseudoalteromonas SM9913, a strain isolated from deep-sea sediment at 1855 m depth near the Okinawa Trough, has had a conjugal transfer and knockout system constructed and validated. In the past few months, we have developed a low-temperature-inducible protein expression vector (pSW2) based on a Shewanella piezotolerans WP3 filamentous phage (SW1). Bacteria in the genus Shewanella can be successfully transformed using this vector (Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A., et al. (2015).

The metagenomic sequence-based approach to drug discovery is becoming more straightforward and effective as new sequencing platforms and bioinformatics tools are developed. Sequencing of genomes and metagenomic samples is becoming increasingly affordable, allowing for studies at all scales, including large-scale ones. The work of Borchet and colleagues (Dhanani, T., Shah, S., Gajbhiye, N. A., and Kumar, S. (2017), who used a 454 pyrosequencing to examine the secondary metabolomic potential of the microbiomes of three different deep-sea sponge species collected from depths of 760 to 2900 m below sea level, demonstrated the utility of this approach.

Subunits of both the PKS (polyketide synthase) subunit as well as the NRPS subunit were specifically targeted by the researchers (nonribosomal peptide synthase). NRPS and PKS from microorganisms were found in the samples, indicating the presence of a large reservoir of these secondary metabolites (Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., and Kadri, A. (2017).

Many bioinformatics tools, such as PRISM, antiSMASH, and BAGEL, can quickly predict the presence of specific genes thanks to the study of secondary metabolites biosynthetic pathways. Thirteen Streptomyces strains, derived from shallow and deep sea sponges, were sequenced and their genomes were mined with antiSMASH 3.0. 485 clusters were identified, with the majority showing little or no similarity to previously reported secondary biosynthetic clusters, such as terpenes, NRPS, and bacteriocins (Grassino, A. N., Brncic, M., Vikic-Topic, D., Roca, S., Dent, M., and Brncic, S. R. (2016).

Also, don't forget that some "marine" natural products are also produced by terrestrial endophytic fungi, some of which are more amenable to optimising yields of the products. It was first found in a marine mushroom called Zopfiella marina SANK21274, but it was later found in the fermentation broth of Xylaria sp. Acra L38 from the Thai plant Aquilaria crassina, which has antifungal properties. Zofimarin production was increased eightfold when Xylaria sp. Acra L38 was grown in sucrose, maltose, glucose, and sodium nitrate, rather than Czapek yeast extract. These marine fungi are the source of all of the microbial antibiotics found in nature (Gurnani, N., Gupta, M., Shrivastava, R., Mehta, D., and Mehta, B. (2016).

4. Conclusions:

The discovery of new natural bioactive compounds in deep-sea habitats is now possible thanks to the advancement of technologies over the last decade. Natural compounds derived from deep-sea microorganisms may provide a new source for the development of drugs, according to recent studies. Antibiotics for microorganisms have been discovered.

A polyphasic taxonomy study of new strains should be the first step in the discovery of new compounds. To mimic the low nutrient content of deep sea environments, low nutrient media can be used for initial isolation. Although not all BGCs will be expressed under laboratory conditions, it is possible that some of them will be. As a result, the activation of cryptic gene clusters is an important research area. Natural product based drug discovery programmes were accelerated and more reliable through the use of advanced techniques such as MS and NMR. The rapid discovery and analysis of BGCs is made possible by next-generation DNA sequencing (NGS) and advanced bioinformatics tools such as AntiSMASH, BAGEL, SBSPKS, and SMURF. In combination with new heterologous expression and pathway engineering techniques, this approach could pave the way for the production of novel metabolites and eventually lead compounds. New compounds discovered in the deep sea have sparked a burgeoning market for research into their mechanisms of action. Combinatorial biosynthesis will be able to increase structural diversity by identifying and manipulating deep-sea gene clusters.

Through academic and biotechnological collaboration, the development of new natural product microbial antibiotics can be accelerated. In its infancy, the commercialization of deep sea-derived natural products and derivatives is approaching clinical trials. In order to discover new drug candidates for treating disease, it is critical to collect and catalogue microorganisms from deep-sea habitats.

Reference:

- Silber, J., Kramer, A., Labes, A., & Tasdemir, D. (2016). From discovery to production: biotechnology of marine fungi for the production of new antibiotics. Marine drugs, 14(7), 137.
- Habbu, P., Warad, V., Shastri, R., Madagundi, S., & Kulkarni, V. H. (2016). Antimicrobial metabolites from marine microorganisms. Chinese journal of natural medicines, 14(2), 101-116.
- Hasan, S., Ansari, M. I., Ahmad, A., & Mishra, M. (2015). Major bioactive metabolites from marine fungi: A Review. Bioinformation, 11(4), 176.
- Tortorella, E., Tedesco, P., Palma Esposito, F., January, G. G., Fani, R., Jaspars, M., & De Pascale, D. (2018). Antibiotics from deep-sea microorganisms: current discoveries and perspectives. Marine drugs, 16(10), 355.
- Wiese, J., & Imhoff, J. F. (2019). Marine bacteria and fungi as promising source for new antibiotics. Drug Development Research, 80(1), 24-27.

- Wu, B., Wiese, J., Wenzel-Storjohann, A., Malien, S., Schmaljohann, R., & Imhoff, J. F. (2016). Engyodontochones, antibiotic polyketides from the marine fungus Engyodontium album strain LF069. Chemistry–A European Journal, 22(22), 7452-7462.
- Ueda, K., & Beppu, T. (2017). Antibiotics in microbial coculture. The Journal of Antibiotics, 70(4), 361-365.
- Auckloo, B. N., Pan, C., Akhter, N., Wu, B., Wu, X., & He, S. (2017). Stress-driven discovery of novel cryptic antibiotics from a marine fungus Penicillium sp. BB1122. Frontiers in microbiology, 8, 1450.
- Liu, M., El-Hossary, E. M., Oelschlaeger, T. A., Donia, M. S., Quinn, R. J., & Abdelmohsen, U. R. (2019). Potential of marine natural products against drug-resistant bacterial infections. The Lancet Infectious Diseases, 19(7), e237-e245.
- Netzker, T., Flak, M., Krespach, M. K., Stroe, M. C., Weber, J., Schroeckh, V., & Brakhage, A. A. (2018). Microbial interactions trigger the production of antibiotics. Current Opinion in Microbiology, 45, 117-123.
- Wu, C., Kim, H. K., Van Wezel, G. P., & Choi, Y. H. (2015). Metabolomics in the natural products field–a gateway to novel antibiotics. Drug Discovery Today: Technologies, 13, 11-17.
- Wu, B., Wiese, J., Labes, A., Kramer, A., Schmaljohann, R., & Imhoff, J. F. (2015). Lindgomycin, an unusual antibiotic polyketide from a marine fungus of the Lindgomycetaceae. Marine drugs, 13(8), 4617-4632.
- Nweze, J. A., Mbaoji, F. N., Huang, G., Li, Y., Yang, L., Zhang, Y., ... & Yang, D. (2020). Antibiotics development and the potentials of marine-derived compounds to stem the tide of multidrug-resistant pathogenic bacteria, fungi, and protozoa. Marine drugs, 18(3), 145.
- Wu, C., Kim, H. K., Van Wezel, G. P., & Choi, Y. H. (2015). Metabolomics in the natural products field–a gateway to novel antibiotics. Drug Discovery Today: Technologies, 13, 11-17.
- Imhoff, J. F. (2016). Natural products from marine fungi—Still an underrepresented resource. Marine drugs, 14(1), 19.
- Auckloo, B. N., Pan, C., Akhter, N., Wu, B., Wu, X., & He, S. (2017). Stressdriven discovery of novel cryptic antibiotics from a marine fungus Penicillium sp. BB1122. Frontiers in Microbiology, 8, 1450.
- Blunt, J. W., Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., &
- Prinsep, M. R. (2018). Marine natural products. Natural Product Reports, 35, 8–53.
- Gärtner, A., Wiese, J., & Imhoff, J. F. (2016). Diversity of Micromonospora strains from the deep Mediterranean Sea and their potential to produce bioactive compounds. AIMS Microbiology, 2, 205–221.
- Imhoff, J. F. (2016). Natural products from marine fungi – Still an underrepresented resource. Marine Drugs, 14, 1–19.
- Conlon, B. P., … Lewis, K. (2015). A new antibiotic kills pathogens without detectable resistance. Nature, 517, 455–459.
- Mayer, A. M. S.. (2018). Marine pharmaceuticals: The clinical pipeline. Retrieved from http://marinepharmacology.midwestern.edu/clinPipeline.htm.
- Mayer, A. M. S., Rodríguez, A. D., Taglialatela-Scafati, O., & Fusetani, N. (2017). Marine pharmacology in 2012-2013: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. Marine Drugs, 15, pii:E273.
- Newman, D. J., & Cragg, G. M. (2016a). Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products, 79, 629–66.
- Newman, D. J., & Cragg, G. M. (2016b). Drugs and drug candidates from marine sources: An assessment of the current “state of play”. Planta Medica, 82, 775–789.
- Pham, T. M., Wiese, J., Erhard, A., & Imhoff, J. F. (2016). Diversity and antimicrobial potential of bacterial isolates associated with the soft coral Alcyonium digitatum from the Baltic Sea. Antonie Van Leeuwenhoek, 109, 105–119.
- Wiese, J., Abdelmohsen, U. R., Motiei, A., Hentschel Humeida, U., &
- Imhoff, J. F. (2018). Bacicyclin, a new antibacterial cyclic hexapeptide from Bacillus sp. strain BC028 isolated from Mytilus edulis. Bioorganic & Medicinal Chemistry Letters, 28, 558–561.
- World Health Organization. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organization.
- Wu, B., Ohlendorf, B., Oesker, V., Wiese, J., Schmaljohann, R., Malien, S., & Imhoff, J. F. (2015). Acetylcholinesterase inhibitors from a marine fungus Talaromyces sp. strain LF458. Marine Biotechnology, 17, 110–119.

- Wu, B., Wiese, J., Labes, A., Kramer, A., Schmaljohann, R., & Imhoff, J. F. (2015). Lindgomycin, an unusual antibiotic polyketide from a marine fungus of the Lindgomycetaceae. Marine Drugs, 13, 4617–4632.
- Wu, B., Wiese, J., Wenzel-Storjohann, A., Malien, S., Schmaljohann, R., & Imhoff, J. F. (2016). Engyodontochones, new antibiotics from the marine fungus Engyodontium album strain LF069. Chemistry, 22, 7452–7462.
- Overy, D. P., Rämä, T., Oosterhuis, R., Walker, A. K., & Pang, K. L. (2019). The neglected marine fungi, sensu stricto, and their isolation for natural products' discovery. Marine Drugs, 17(1), 42.
- Saha, M., Sarkar, S., Sarkar, B., Sharma, B. K., Bhattacharjee, S., & Tribedi, P. (2016). Microbial siderophores and their potential applications: a review. Environmental Science and Pollution Research, 23(5), 3984-3999.
- Aminah I., Putra A. E., Arbain D., Handayani D., (2019). Screening of cytotoxic activities toward WiDr and Vero cell lines of ethyl acetate extracts of fungi-derived from the marine sponge Acanthostrongylophora ingens. Journal of Applied Pharmaceutical Science 9(1):1-5.
- Artasasta M. A., Yanwirasti, Taher M., Djamaan A., Handayani D., (2019). Cytotoxic and antibacterial activities of marine sponge-derived fungus Aspergillus nomius NC06. Rasayan Journal of Chemistry 12(3):1463-1469.
- Al-Hashimi, A. G. (2012). Antioxidant and antibacterial activities of Hibiscus sabdariffa L. extracts. Afr. J. Food Sci. 6, 506–511.
- Altemimi, A., Watson, D. G., Choudhary, R., Dasari, M. R., and Lightfoot, D. A. (2016). Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins. PLoS One 11:e0148758. doi: 10.1371/journal.pone.014 8758.
- Alzoreky, N. S., and Nakahara, K. (2003). Antibacterial activity of
- extracts from some edible plants commonly consumed in Asia.
- Int. J. Food Microbiol. 80, 223–230. doi: 10.1016/S0168-1605(02)
- 00169-1.
- Aono, R., Ito, M., and Horikoshi, K. (1997). Measurement of cytoplasmic pH of the alkaliphile Bacillus lentus C-125 with a fluorescent pH probe. Microbiology 143, 2531–2536. doi: 10.1099/00221287-143-8-2531.
- Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H. S., Kieszak, S., Njapau, H., et al. (2005). Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ. Health Perspect. 113, 1779–1783. doi: 10.1289/ehp. 8384.
- Caleja, C., Barros, L., Antonio, A. L., Carocho, M., Oliveira, M. B., and Ferreira, I. C. (2016). Fortification of yogurts with different antioxidant preservatives: a comparative study between natural and synthetic additives. Food Chem. 210, 262–268. doi: 10.1016/j.foodchem.2016.04.114.
- Cheng, C., Yang, Y., Dong, Z., Wang, X., Fang, C., Yang, M., et al. (2015). Listeria monocytogenes varies among strains to maintain intracellular pH homeostasis under stresses by different acids as analyzed by a high-throughput microplate-based fluorometry. Front. Microbiol. 6:15. doi: 10.3389/fmicb.2015. 00015.
- Clarke, D., Tyuftin, A. A., Cruz-Romero, M. C., Bolton, D., Fanning, S.,
- Pankaj, S. K., et al. (2017). Surface attachment of active antimicrobial coatings onto conventional plastic-based laminates and performance assessment of these materials on the storage life of vacuum-packaged beef sub-primals. Food Microbiol. 62, 196–201. doi: 10.1016/j.fm.2016. 10.022.
- Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A., et al. (2015). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two tunisian cultivars. Arab. J. Chem. (in press). doi: 10.1016/j.arabjc.2015.07.014.
- Dhanani, T., Shah, S., Gajbhiye, N. A., and Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arab. J. Chem. 10, 1193–1199. doi: 10.1016/j.arabjc.2013. 02.015.
- Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., and Kadri, A. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits. J. Food Sci. Technol. 37, 483–492. doi: 10.1590/1678-457x.23516.
- Grassino, A. N., Brncic, M., Vikic-Topic, D., Roca, S., Dent, M., and Brncic, S. R. (2016). Ultrasound assisted extraction and characterization of pectin from tomato waste. Food Chem. 198, 93–100. doi: 10.1016/j.foodchem.2015. 11.095.

- Gurnani, N., Gupta, M., Shrivastava, R., Mehta, D., and Mehta, B. (2016). Effect of extraction methods on yield, phytochemical constituents, antibacterial and antifungal activity of Capsicum frutescens L. Indian J. Nat. Prod. Resour. 7, 32–39.