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Natural Products from Marine Fungi: Progress in Extraction and Application

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Abstract:

Marine fungi are an important class of biological resources, and their natural products are rich in chemical structure and diverse biological activities, and thus have a wide range of applications in the fields of drugs, food, agriculture and environment. Current research on marine fungal natural products focuses on the discovery and abundance of the source, the optimisation and innovation of extraction techniques and the bioactive applications of marine fungal natural products in drug production and other areas. There is still a research gap on how to better explore and understand the mechanism of bioactivity in depth, especially to better preserve the activity of marine fungal natural products under the change of environment from ocean to land and to more effectively use marine fungal resources to develop new drugs. This paper analyses the species and characteristics of marine fungi and their natural products, focuses on the current extraction and purification methods of the products and the existing problems, and elaborates on the current status and prospects of the application of marine fungal natural products. It provides a reference for the existing challenges and difficulties for future research. At present, in the field of marine fungi natural products, there are still problems such as difficult to restore marine conditions, time-consuming and labour-intensive customised extraction and purification methods for each natural product, etc. Future research can focus on finding the common points between marine fungi and terrestrial fungi, and focus on the development of biotechnology and so on.

Keywords: Marine fungi; Natural products; Extraction; Application.

1. Introduction

With the exploitation of terrestrial resources, more and more people are turning their attention to the oceans, which account for approximately 70% of the planet and still leave a significant portion unexplored and unexploited. The oceans are very abundant in natural resources, and the diversity of their internal environments determines the diversity and potential of marine organisms [1]. The seas, places where all living beings were born in the first place, will probably empower the future development of humankind.

Marine organisms, especially deep-sea creatures, are able to produce products with special properties in their unique environment (high salt and pressure, low light, temperature and oxygen). Take marine fungi as an example, their natural products usually have a variety of bioactivity characteristics including antibacterial, antitumour, antioxidant, anti-inflammatory and so on. Very rich chemical structures containing polyenones, terpenes, peptides, excellent biodegradability and low toxicity are also included, so that the natural products of marine fungi have a wide range of research value and prospects for application in the fields of medicine, agriculture, environmental protection and other field. Thus the extraction and application of marine fungi have also become an important field of research in the development and utilisation of marine resources.

At present, people still underestimate the value and importance of marine fungi. Marine fungi and their natural products that have been fully studied and exploited by human beings only account for a very small part of the total species, and there are still a lot of products beneficial to human beings that have not been discovered and utilized. The mostly commonly used methods currently of extraction of natural products from marine fungi, including solvent extraction, chromatographic separation, solid-phase extraction, ultrasonic extraction, and fermentation extraction, are not suitable for the general suitability for all the strains, and the extraction of natural products is not suitable for all the species. If efficiently extraction under the premise of preserving the biological activity of the natural products is needed, it takes a long time to carry

out specificity research on the fungi. The large differences between the specific environments in which marine fungi live and the culture environments typically available in laboratories also continue to pose a significant challenge to the full activity culture and product extraction of marine fungi on land.

At present, the application value of marine fungi still needs to be further developed, and there are still many problems that need to be optimized from the extraction methods of natural products to product applications. This article will comprehensively discuss the extraction, application technology, active substance analysis, etc. of natural products from different types of marine fungi.

The discussion of these issues is of great significance to the future extraction and application of natural products from marine fungi.

2. Diversity of Marine Fungi and Their Natural Product Resources

2.1 Research Status of Marine Fungi

Marine fungi play a very important role in marine ecosystems. In recent years, research on marine fungi has gradually attracted extensive attention from the scientific community, mainly due to their potential bioactive compounds and ecological significance.

Marine fungi are considered to be a potential reservoir of biotechnological resources. The environment will affect the physiological characteristics and metabolic traits of organisms, which in turn will directly influence the characteristics, functions and biological activities of their natural products. The uniqueness of the marine environment with high salinity, low temperature and high pressure has shaped the rich and unique microbial communities and their genetic functional resources, which has resulted in bioactive compounds of marine fungi with a wide range of bioactivities such as anti-infective, anti-tumour and immunosuppressive.

The diversity and resource distribution of marine fungi were studied. Through the collection of marine samples, including seawater, marine sediments, and the surface of marine organisms, marine fungi are isolated and identified, and the characteristics of their natural products are summarised and researched. Analysing the effects of the macro- and micro-environments on the natural products of marine fungi will help us to identify the desired products and explore their potential applications.

Researches on biosynthetic pathways and genomics of marine fungal natural products are conducted. The process of using genes to regulate the production of microbial secondary metabolites is a systematic project controlled by a complex regulatory network at multiple levels, in which transcriptional regulation is the main link in the regulation of gene expression in filamentous fungi. Common gene regulatory elements mainly include promoters and enhancers, which usually have transcription factor binding roles. During biological processes, these elements are dynamically developed or switched off, allowing transcription factors to bind or not to bind, thus regulating downstream gene expression and thus controlling the synthesis of secondary metabolites. Using high-throughput technologies such as genomics and transcriptomics, the biosynthetic pathways and metabolic regulatory networks of marine fungal natural products can be investigated, thus providing the necessary basis for product synthesis.

Drug development applications targeting marine fungal natural products are pushed forward. A variety of biological activities of marine fungi make it an important source of drugs and drug precursors, which is a relatively wide range of applications for marine fungal natural products. Its anti-tumour activity can inhibit the growth and spread of tumour cells, antioxidant and anti-inflammatory activity can be used for the prevention and treatment of chronic inflammatory diseases, and may affect cell growth, differentiation and apoptosis of bioregulatory activity can be used for the treatment of metabolic and immune-related diseases, can be used to combat the microbial activity of the characteristics of the drug-resistant strains of bacteria may be used in the treatment.

2.2 Types of Natural Products of Marine Fungi

There are many ways to classify the natural products of marine fungi, including classifying them according to the species of fungi, the sea area from which they originate, the biological activity of the products, etc.. Following classification is based on their chemical structure, so that the application of marine fungal natural products may be better further elaborated on.

1) Polycyclic polyketides: Most marine fungal products, including cycloenols, hydroxy acids, cyclic ketones, and polyketones, usually consist of multiple ring structures and are mostly complex compounds with large molecular weights. The more common biological activities include anti-microbial, anti-tumour, anti-oxidation, etc. They are usually produced in the secondary metabolic process of fungi, and the biosynthetic pathway is designed for polyketide synthesis.

2) Peptides: Amino acids including cyclic peptides, linear peptides and other products of marine fungi, with a large structural diversity. The more common biological activities include anti-microbial, anti-tumour, immunomodu-

latory, etc., which may be synthesised by fungi through non-ribosomal synthetic pathways.

3) Alkaloids: Marine fungal products containing basic nitrogen atoms, including indoles, quinolines, and others. Often have biological activities such as anti-microbial, neuromodulatory, anti-tumour, etc., and the process of biosynthesis involves the synthesis and modification of basic molecules.

4) Terpenoids: Marine fungal products consisting of isoprenoid units including sesquiterpenes and triterpenes. They mainly possess anti-microbial, anti-tumour and anti-inflammatory activities and may be produced through the synthetic pathway of intermediates such as formic acid and isoprene.

3. Extraction and Separation Method of Natural Products of Marine Fungi

3.1 Most Currently Used Methods

The main marine fungal natural product extraction methods commonly used today include:

Traditional solvent extraction method. Organic solvents of different polarities are used to extract natural products from fungal cultures. The salt concentration in the extraction solution is adjusted to be close to the salinity level of seawater to promote the solution and enrichment of saline compounds. For example, the current methods of extraction and purification of marine fungus-derived antioxidant molecules are mainly the more traditional methods chosen based on molecular class and culture type. Small molecule extraction is carried out by liquid-liquid extraction with organic solvents, or by adsorption resins that take up metabolites from the culture medium and then elute them with organic solvents [7]. The advantage of this method is that it is extremely convenient and applicable to a wide range of compound types, making it easier to use for larger scale extractions; however, the degree of extraction and purification is relatively low and depends on the nature of the product requiring the recombination and superimposition of different reagents and steps, making it unsuitable for more complex products.

A variety of chromatographic methods including gel column chromatography, silica gel column chromatography, and reverse column chromatography. High Performance Liquid Chromatography (HPLC) is a modified form of column chromatography. A mixed sample is passed through a column under high pressure, and the separation and purification of the target compounds is achieved by using the difference in partition coefficients of the components in the sample on a fixed packing material. Some structurally novel and biologically significant secondary metabolites are more commonly extracted and purified by such a method, such as the extract from coral- associated fungus chaetomium globosum C2F17 for the isolation of diversified chaetoglobosins[11]. This extraction method enables highly precise separations with high selectivity and controllability, but it is expensive and limited by solubility and sample handling.

Supercritical fluid extraction. using the special properties of supercritical fluid, the sample is extracted under supercritical conditions. in the case of slime molds In the study of secondary metabolites of Myxobacteria, SFE has demonstrated the powerful feature of being able to increase the concentration of metabolites in the resulting extract fraction to make new metabolites more readily available [2]. The significant advantages of this approach are its relatively low energy and liquid solvent consumption and its potential ability to maintain higher biological activity of the extracts. However, current related studies are underrepresented in the study of microbial natural products and the isolation may be relatively poor.

Take the research method used in the special issue paper of the 3rd International Conference on Marine Fungi Natural Products (MaFNaP) as an example. Neophenyl spirochetes from the sponge-associated fungus Stachybotrys chartarum MUT 3308 were studied using cornstarch agar seawater for sponge tissue cultivation, and extraction and isolation of the material were carried out by liquid chromatography, liquid-liquid extraction [3]. Fourier Transform Infrared Spectroscopy, Spinometry, Nuclear Magnetic Resonance Spectroscopy, High Resolution Mass Spectrometry, Solid Phase Extraction, and HPLC were used in Pyrenosetin D, a new pentacyclic decalcitrate tetradecanoic acid derivative derived from the algal fungus Pyrenochaetopsis sp. FVE-087 study [4]. Non-targeted metabolomics approach to discover environmentally relevant pyran-2-one chemical diversity in marine origin Penicillium spp. In the study of chemical diversity of isolated fungi, the isolated fungi were fermented and extracted using OSMAC method and subsequently analysed using HPLC-MS [5].

In the study to highlight the biotechnological potential of deep-sea crusting fungi through the prism of their antimicrobial activity, the fungal isolates were assessed using genetic marker identification and ecophysiological characterisation, amplification of secondary metabolite genes was also done using PCR; in the screening session, the agar plug diffusion assay was firstly used and the most promising fungal isolates were selected for the extraction of the secondary metabolites and analysed by HPLC-PDA-ELSD. This helped to evaluate the bioactive sub-

stances and potential drug candidates from marine fungi [5]. In the study of identification and characterisation of a novel type III polyketide synthase from marine yeast, total RNA of marine fungi was extracted, reverse transcription reaction was performed to synthesise cDNA, and then specific gene sequences were amplified by PCR, cloned into expression vectors and transformed into Escherichia coli cells for expression, and the recombinant proteins were purified by affinity purification using Ni-NTA; the extracts were also analysed during the experiment by using the LC-MS technique in order to identify the structure and composition of natural products produced by marine fungi [6].

3.2 Problems and future development direction

The current extraction methods for marine fungal natural products favour generality. However, in fact, in order to retain the activity of marine fungal natural products to a greater extent, there are high other conditions for their extraction methods to be limited, and face a number of difficulties [7]. Under the existing conditions, the specific difficulties are as follows:

1)Complex sample matrices can interfere with the extraction of target compounds. A large number of other microorganisms, organic and inorganic substances are present within the complex growth environment of marine fungi. This can affect the selection and effectiveness of extraction, increasing the complexity and difficulty of extraction.

2)Lack of specific extraction methods for different types of target compounds makes it difficult to achieve efficient enrichment and separation of target compounds.

3)Low extraction efficiency. Low content of target compounds, complex sample matrix, inadequate extraction conditions.

4)Difficulty in optimising extraction conditions. For different sea areas, different fungal strains have large differences, and it is difficult to simulate the real environment of the ocean, which requires a lot of testing and optimization [8].

Therefore, in order to optimise the extraction technology, it is necessary to consider the low-temperature extraction, appropriate ph value extraction, multi-stage extraction combined with different properties of solvents or extraction conditions, and bioactivity screening assisted extraction and so on.

In addition to this, if marine fungi can be activated so that more clusters of biosynthetic genes can be expressed under standard laboratory conditions, a large number of new biologically active secondary metabolites can be produced [9]. Epigenetic modulation, co-culture, precursor feeding, heterologous expression and altering fermentation parameters are all currently being investigated to activate silent biosynthetic gene clusters in marine fungi.

Another noteworthy issue is the fact that only 14.3% of the compounds isolated from marine microorganisms are unique and the rest are closely related to those isolated from terrestrial microorganisms [10]. Therefore, in the future, one needs to further investigate the way in which marine-targeted strains are isolated, and use technologies such as genome sequencing to direct attention to unique natural product diversity at an early stage, in order to better focus resources and manpower in the direction of digging for human needs in a purpose-driven manner.

4. Application of natural products

4.1 Application status and prospect

Although there are still large gaps in the much-needed research expertise and application markets for marine fungi and their natural products, marine fungal natural products are now widely used in a number of fields and show very promising prospects.

In the field of drug discovery and development, due to their rich chemical structures and biological activities, marine fungal natural products are widely used in drug discovery and development. In particular, their anticancer, antimicrobial and anti-inflammatory bioactivities have brought new possibilities to the medical market and disease treatment. Some compounds have also been used as candidates for anticancer drugs. Many compounds with antimicrobial activity in marine fungal natural products have also been used in antibiotic development.

In the field of nutraceuticals and cosmetics, some marine fungal natural products can be used to scavenge free radicals and slow down oxidative reactions due to their antioxidant properties [11].

In the field of biocontrol, some compounds with fungicidal activity produced by marine fungi can be used to develop biopesticides for disease and pest control. There are also compounds with surfactant properties that can be used in the preparation of products such as bio-detergents or bio-degreasers. With rational development, marine fungal natural products may be able to better serve issues such as the prevention and control of marine bio-pollution, in particular the great challenge this poses to the maintenance of marine instruments [12].

In terms of food, the unique properties of marine fungal natural products allow them to be used in the production of food additives, preservation and antiseptic; some of them have also been used as a source of colours to dimin-

ish the harmful effects of colouring agents on consumers and the environment [13].

4.2 Challenges and future direction

The current challenges in the application of marine fungal natural products are relatively focused. The first is the difficulty in obtaining resources. Due to the complexity and less predictable nature of the marine environment, the collection of samples and the isolation and purification of target compounds have become very complicated, especially in comparison with terrestrial fungi. The complex chemical structure nature of marine fungal natural products, such as large molecular weight and polycyclic structure, also brings certain challenges to their extraction, isolation and purification. In addition to this, to confirm their potential medicinal or other values, the natural products need to be verified and screened for activity, a tedious and complex process. On top of that, to ensure the stability of the active ingredients, it is necessary to ensure that the natural products of marine fungi are not overly affected by environmental changes during the development, research and use processes, or to create corresponding environments to activate the activities of marine fungi and their natural products to a greater extent. Undoubtedly, this whole process is time-consuming and costly.

In the future, the development and application of natural products from marine fungi will need to be optimised to meet these challenges. This will require the enhancement of biotechnology and analytical techniques, so that marine fungi and their natural products can be better screened and evaluated for bioactivity, and also better optimised to improve their pharmacological properties, etc., to better meet the application needs. In addition, the development of green production technology should not be ignored. In the development process of marine resources, environmental issues and sustainability issues must be taken into account. In the future, the natural products of marine fungi may be explored and strengthened in more different fields, and through interdisciplinary cooperation and comprehensive research, it is believed that the development of natural products of marine fungi will go to a higher peak in the future.

5. Conclusion

In this paper, the current research status and classification of marine fungi and their natural product diversity are described, focusing on the analysis of the current commonly used marine fungi natural product extraction and purification means and their problems, and put forward the future development direction of the research means. At the same time, the application of natural products is reviewed, and the future prospects of natural product application, possible challenges and problems to be overcome are analysed. Combined with the current research status, this paper provides a comprehensive overview of the current research status of marine fungi and their natural products, which provides valuable reference materials for researchers in related fields. The extraction methods, bioactivity evaluation and application prospects of marine fungal natural products are discussed in depth, providing theoretical and practical guidance for future related research. In addition, this paper reveals some limitations of marine fungi research, such as the difficulty of sample acquisition and the lack of efficient extraction methods, which points out the direction of improvement for future research. However, this paper does not fully explore all regions and types of marine fungi, and mainly focuses on existing extraction methods and application cases, and has not yet conducted in-depth research on new extraction techniques and application areas. If human beings can allow marine fungi to grow under their native environmental conditions and naturally metabolise the desired products, it will undoubtedly improve the activity and quality of natural products and simplify the extraction and purification steps.

In the future, the research system of marine fungi and their natural products needs to be further improved to develop more efficient and green extraction methods and explore a wider range of applications. Future research can focus on the discovery and development of deep-sea fungi, strengthen interdisciplinary cooperation, and use new technological means to explore the diversity and biological activity of marine fungi, so as to make greater contributions to the protection of marine ecosystems and the promotion of the development of human health industry.

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