

Research Progress and Development Strategy of Microalgae Polysaccharides

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Abstract. With the increasingly in-depth marine research, the high-value utilization of seafood has been a research trend, among which the research on microalgae polysaccharides is sufficient. At present, a large number of studies have shown that microalgae polysaccharides have a wide range of biological activities, but their structural analysis is not systematic. Thus, in the past, the research on microalgae polysaccharides mostly focused on a single dimension. In this paper, the structure, composition, analysis method, biological activity and application of microalgae polysaccharides are studied. Meanwhile, the current research status and achievements of microalgae polysaccharides are presented by integrating multiple indexes, and the defects of existing research are analyzed, which provides guiding suggestions for further research and development of microalgae polysaccharides.

Keywords: Microalgae Polysaccharide; Biological Activity of Microalgae Polysaccharides; Structural Analysis.

As unicellular microalgae widely distributed in land and sea, microalgae is rich in resources, numerous in varieties, short in growth cycle and high in photosynthesis efficiency. Besides, it has become a new resource being developed thanks to its advantages of high yield, low cost and large-scale cultivation, which has been widely used in food, medical treatment, cosmetics and other industries. Microalgae polysaccharide is one of the most vital active substances in microalgae. As an integral part of life substance, it can participate in many aspects of life process. In addition, abundant studies have manifested that its complex and changeable structure makes it show a wide range of biological activities, such as being antioxidant, anti-tumor, antibacterial, antiviral, lowering blood sugar and blood lipid and immunomodulatory. Hence, microalgae polysaccharides find desirable application prospects in the development of functional foods, the preparation of anti-aging cosmetics and the research and development of new drugs. At present, the research on microalgae polysaccharides has been attached by more significance. This paper reviews the composition, structure, biological activity and application progress of microalgae polysaccharide, which puts forth its current development defects and prospects, providing references for its further development and utilization.

1. Structure and Composition of Microalgae Polysaccharide

The total sugar content of microalgae ranges from 5% to 23%, and the relative molecular weight of microalgae is generally medium or high with a range of 10 to 1000kDa [1]. Most of them are composed of different monosaccharides (monosaccharides, disaccharides and oligosaccharides) linked and polymerized carbon chains through glycosidic bonds. The most common component in microalgae polysaccharides is d-glucose, which accounts for 21% ~ 87% of the total content. But d-fructose, d-galactose, d-mannose, l-arabinose and d-xylose are also found [2]. Galactose and mannose account for 1%-20% and 2%-46% respectively. The others are arabinose, fucose, rhamnose, ribose and xylose, accounting for 0%-17%. There are also some unusual methyl sugars, amino sugars (d-glucosamine and d-galactosamine), and their derivatives [3].

The structure of polysaccharides includes primary structure and secondary and higher structure. Its primary structure includes the composition of glycosyls, the linkage mode of glycosyls, the heterocarbon configuration of glycosidic bonds, the substitution of hydroxyl groups on each glycosyl residue, the position of branching points, the linkage order of each glycosyl residue, and the linkage points of sugar chain and non-sugar part. In addition to determining monosaccharide composition, the complete chemical characterization of complex polysaccharides includes their sequence and absolute configuration, the types of glycosidic bonds between units, the identification of substitution patterns and the localization of non-carbohydrate substituents [4]. As for the commonly used polysaccharide structure analysis methods, there are chemical analyses such as methylation analysis, Smith degradation, periodate oxidation, ethyl phthalolysis, etc. Physical methods include infrared spectroscopy, ultraviolet spectroscopy, mass spectrometry, nuclear magnetic resonance analysis, etc. As for biological methods, there is the application of tool enzymes, immunological methods, etc. The general method is to collect the information and data of polysaccharide structure from various perspectives through the existing technologies and methods, so as to finally synthesize them to infer the primary structure of polysaccharide. NMR and FAB-MS play a vital role in structural analysis. Some polysaccharides with simple structures can be determined by NMR alone [5].

Till now, marine microalgae polysaccharides can be divided into four categories according to their functions. They are structural and functional polysaccharides related to the cell wall, that is, cell wall polysaccharides; storage polysaccharide that provides energy reserve for cells; and exopolysaccharides usually excreted outside cells as part of normal physiological processes and under stress [2]. In these classifications, the structure of sulfated polysaccharides has been widely investigated because of its applicability in biomedical and food applications [3] [5]. Thus, it is discussed as a category of microalgae polysaccharides alone in this paper. The following four categories of microalgae polysaccharides are researched in detail to explore the structure and composition of microalgae polysaccharides.

1.1. Cell Wall Polysaccharide (CWP)

There are many kinds of polysaccharides in microalgae cell walls, which are widely distributed and varied. The biochemical structure of microalgae polysaccharides in cell walls increases with increasing species complexity [6]. In the cell wall, the main polysaccharides are β —(1→3)—glucan and β —(1→4)—glucan [6]. *Chlorella* with a rigid cell wall is mainly composed of glucosamine in chitin-like polysaccharide [7]. There is a main polysaccharide in the cell wall of diatom, which is composed of glucuronide and mannose [8]. The research progress of cell wall polysaccharides in red microalgae is limited [21]. The cell wall polysaccharide in microalgae is related to the salinity of the culture medium. For example, in a brown algae freshwater culture medium, its cell wall polysaccharide is composed of rhamnose, galactose and glucuronide. Besides, in seawater culture medium, the main monosaccharides are glucose, galactose and rhamnose. Complex polysaccharides containing branched α -l-rhamnose and xyfuranose are found in *parachlorella* HY1 [7]. In eukaryotic microalgae, cell walls composed of polysaccharides such as glucosamine, mannose and glucose can be found, so are cell walls rich in galactose, glucose and rhamnose [8].

1.2. Storage Polysaccharide (SP)

Different types of storage polysaccharides are found in microalgae and cyanobacteria [8]. Storage polysaccharides are usually composed of glucose monomers, with different glycosidic bonds connecting glucose molecules [6]. The glucose subunits of starch-type polysaccharides (starch, Florida starch and glycogen) are linked by α -1, 4 glycosidic bonds and α -1, 6 glycosidic bonds. *Laminaria* polysaccharides (proteins from *Laminaria japonica*, auramine and mafenide) contain glucose units linked together by β -1, 3 glycosidic bonds and β -1, 6 glycosidic bonds [9]. Different stored polysaccharides have various positions in microalgae cells [10]. β -1, 3-glucan is common in microalgae, mainly stored in vacuoles. The stored polysaccharides in *Chlamydomonas reinhardtii* are amylopectin, retrograde starch and amorphous gelatinized starch [11].

1.3. Exopolysaccharide (EPS)

In most cases, exopolysaccharides (EPS) from microalgae are considered the primary and secondary metabolites [12]. The main species of marine microalgae producing exopolysaccharides are porphyrin, *Dixioniella* and *Rhodella* [13]. The ability of microalgae to produce exopolysaccharides varies, and exopolysaccharide is usually synthesized by cyanobacteria and diatoms [5]. Not all forms of microalgae can synthesize exopolysaccharides. For example, *phaeodactylum tricornutum* can produce exopolysaccharide when it is oval, but that is not the case for spindle and triradial *phaeodactylum tricornutum* [14]. According to the related studies, exopolysaccharides are all heteropolysaccharides, and their composition changes greatly [15], which consist of up to 8-10 monosaccharides and several substituents, such as methyl, acetyl or sulfate groups [2]. Galactose is the main monosaccharide in the exopolysaccharides of *Tetraselmis rubens* (RCC133) and *Prasinococcus* sp. (RCC2684).

1.4. Sulfated Polysaccharides (SPs)

At present, the research focuses on the soluble polysaccharides in microalgae, most of which are acidic heteropolysaccharides with sulfation modification (sulfate radical has a significant impact on the activity of polysaccharides), similar to other marine seaweed polysaccharides. The sulfate content of sulfated polysaccharides (SPs) extracted from various microalgae strains is different [13]. The water-soluble polysaccharide extracted from green microalgae is composed of glucan and mannan linked by (β 1 \rightarrow 3, β 1 \rightarrow 4) and (α 1 \rightarrow 3)—(α 1 \rightarrow 4) respectively, and isogannan sulfated by anion [6].

2. Biological Activity of Microalgae Polysaccharide

The wide biological activity of microalgae polysaccharides is attributed to their structural variability and the existence of various functional groups. At present, the research on the activity of microalgae polysaccharides mainly focuses on its features of being antioxidant, anti-tumor, antibacterial, antiviral, lowering blood sugar and blood lipid, and immunomodulatory.

2.1. Antioxidant Activity

Oxygen free radicals are the main free radicals in the human body. Under normal circumstances, the generation and removal of free radicals in cells are in a dynamic equilibrium state. With the increase of age, this balance is destroyed, and excessive free radicals will induce an oxidation reaction of the body, damage the tissues and cells in the body, and then lead to aging. Oxidative stress is intertwined with many degenerative diseases such as cancer, diabetes and cardiovascular diseases [16]. Current research shows that polysaccharides from natural sources such as plants, animals and fungi have strong antioxidant activity [17]. Microalgae, as an algae with great research value, has also been proven that its polysaccharides have antioxidant activity in vivo and in vitro. There are many factors affecting the antioxidant activity of polysaccharides. Different extraction methods have various polysaccharide activities. Researchers use the following six methods, including repeated freeze-thaw method, microwave-assisted extraction method, ultrasonic method, alkali method, hot water method and cellulose method. According to the results, the antioxidant activity of polysaccharides obtained by the ultrasonic extraction method is better than that extracted by other methods [16]. The chemical structure and molecular weight of polysaccharides will also affect their activity. The higher the sulfate content in polysaccharides, the stronger the scavenging effect on superoxide radicals and the stronger the corresponding antioxidant activity [18]. The results of research on infant formula demonstrate that the composition and concentration of salt will affect the antioxidant activity of polysaccharides [19].

2.2. Anti-Tumor Activity

The World Health Organization (WHO) and other health institutions list cancer as one of the top ten causes of death in the world. Finding effective drugs to treat cancer is still a vital challenge for humans to overcome cancer. Zhang Jianzhi et al. studied the anti-tumor effects of three exopolysaccharides

(EPSs EPS-CP, EPS-SS and EPS-CS) on human colon cancer cells HCT116 and HCT8 in vitro. When the concentration of EPS-CP, EPS-SS and EPS-CS is 0.6mg/ml, the inhibitory effects on HCT116 and HCT8 cell lines are 17.2%, 19.2%, 18.7% and 35.9%, 38.6%, 22.9% respectively [20]. Some microalgae polysaccharides have no obvious anti-tumor effect by themselves, but polysaccharide derivatives or complexes obtained by structural modification or combination with other substances have significant anti-tumor effects. For example, alginate particles containing ovalbumin (OVA) peptide have a stronger inhibitory ability on tumor progression than alginate alone, which fights cancer by stimulating macrophages to secrete inflammatory and effector cytokines [21].

2.3. Antibacterial Activity

Silver nanoparticles (AgNPs) produced by exopolysaccharides of microalgae *B. braunii* and *C. pyrenoidosa* are effective antibacterial agents. When the concentration of AgNPs is 7.5µg/mL, it has strong toxicity to Gram-negative *Escherichia coli* without effect on healthy fibroblasts. Its antibacterial mechanism is related to ROS production and cell membrane damage [22]. In a study, the antibacterial activity of crude polysaccharides from ten microalgae was researched. According to its results, *Chlorella* seawater and *Anabaena* 7120 had better inhibitory effects on four tested strains, and the antibacterial effect of crude polysaccharides from *Chlorella* seawater was obviously better than that of other nine microalgae polysaccharides in the experiment. The water extracts of two microalgae, *Isochrysis galbana* (PEA) and *Nannochloropsis oculata* (PEB), mainly contain polysaccharides, so PEB and PEA can inhibit the growth of Gram-negative bacteria, Gram-positive bacteria and three *Candida albicans*. The sensitivity of Gram-negative bacteria to PEA is higher than that of Gram-positive bacteria [23]. In addition to microalgae polysaccharides, phenolic compounds contained in microalgae have also been proved to be related to antibacterial activity [24], with these compounds used as alternative antibacterial agents in the future.

2.4. Antiviral Activity

Among microalgae polysaccharides, sulfate polysaccharides and acid polysaccharides have the strongest antiviral activity. As for the antiviral mechanism of sulfated polysaccharide, it interacts with the positive charge domain of the viral glycoprotein envelope, occupying the attachment site of the virus and forming irreversible compounds. Typical microalgae rich in sulfate polysaccharides are *Spirulina platensis* and *Porphyria* algae [25]. Hayashi and Hayashi isolate a sulfated polysaccharide called Calcium *Spirulina* Polysaccharide (Ca-SP) by cellulose chromatography. Ca-SP interacts with the V3 ring region of the virus envelope containing gp120, and then prevents the formation of syncytium between infected cells and non-infected cells, proving its resistance to HIV1 virus [26]. Mayaravirus (MAYV) is an arbovirus, belonging to the *Togaviridae* family (envelope RNA virus) and *Alphavirus* genus. According to studies by Ribeiro et al., the inactivation of microalgae extract to Mayaravirus is better than that of the reference substance ribavirin, which shows that microalgae extract has great application potential in treating Mayaravirus. Hence, it is worth further exploring the inhibiting single-stranded RNA positive envelope virus infection [27]. Radonic et al. also found that polysaccharides released by *A. platensis* and *P. purpureum* into the culture medium had antiviral activity against two *Vaccinia* viruses and one *Ectromelia* virus in vitro and in vivo [28].

2.5. Lower Blood Sugar and Blood Lipid

Due to the characteristics of microalgae polysaccharide macromolecules, it is usually difficult to be directly absorbed by the intestinal tract and act on human cells. More studies have shown that polysaccharides can exert their biological activity by regulating intestinal microflora and producing microflora-derived metabolites. According to a study on the lipid-lowering activity of pyrophosphate polysaccharide (CPP) in rats induced by a high-fat diet, CPP can significantly reduce the body weight, serum total cholesterol, triglyceride and low-density lipoprotein cholesterol, and increase the serum high-density lipoprotein cholesterol, cecal total bile acid and SCFA. Meanwhile, its potential lipid-lowering mechanism is related to the regulation of intestinal microflora [29]. Water extracts and

ethanol extracts of microalgae *C. pyrenoidosa* and *S. platensis*, which are rich in polyunsaturated fatty acids, can reduce blood glucose in rats fed with high fat and sucrose. They can maintain the number of beneficial bacteria in the intestinal tract, such as *Austenius*, *paracoccus* and ruminants, while reducing the *Blautia* and *Turicibacter* [30]. *U.fasciata* is considered to be the source of polysaccharides with high sulfate content. Sulfated polysaccharide extracted from *U.fasciata* has inhibitory activity in the hypercholesterol rat model, which can effectively control cholesterol and blood lipid concentration [31].

2.6. Immunomodulatory Activity

Immunomodulation is one of the important biological activities of natural polysaccharides. Immunopolysaccharides have been emphasized by immunologists because of their wide sources, remarkable effects and low toxicity or non-toxicity. Polysaccharides in microalgae mainly regulate the immune function by promoting the proliferation and differentiation of lymphocytes, stimulating the phagocytosis of macrophages, and promoting the production of cytokines and antibodies. For example, *Chlorella* polysaccharide can significantly stimulate phagocyte index, activate T cells through delayed-type hypersensitivity, and improve antibody titer level [32]. Crude brown algae polysaccharide and its fraction can stimulate the proliferation of macrophages and promote the secretion of nitric oxide, prostaglandin E2 and pro-inflammatory cytokines [33]. In an anti-cancer cell study, a microalgae polysaccharide named *Thraustochytriidae* sp. GA affects B proliferation and cytokine secretion of T cells, which also shows immunomodulatory activity. There are many reasons that affect the immune activity of microalgae polysaccharides. Firstly, the immune activity of microalgae is quite different with various species. Secondly, the purified fraction has stronger immunomodulatory activity than crude polysaccharides with its dose-related effect [33]. In addition, the immunomodulatory activity of polysaccharides is affected by their molecular weight. Suárez et al. found that two larger arabinogalactan (1.88×10^5 and 1.02×10^6 Da) can stimulate the synthesis of nitric oxide, but galactofuran with molecular weight of 1.5×10^4 Da and arabinogalactan with molecular weight of 5×10^4 or 2.8×10^4 Da cannot promote the production of nitric oxide [34].

3. Application of Microalgae Polysaccharide

3.1. Application of Microalgae Polysaccharide in Food Industry

Microalgae polysaccharide is widely used in beverage, candy and other food processing because of its good viscosity, gelatinization, emulsifying stability and safety stability. Its application in food can not only improve the food taste, and increase the food functionality, but also improve the nutritional value of food.

Carrageenan is a high molecular polymer of sulfated polysaccharide extracted from algae, which has excellent characteristics of microalgae polysaccharide, such as excellent gel properties and rheological properties to interact with proteins. Microalgae polysaccharides such as carrageenan are often used as thickeners and setting agents for frozen foods, coagulants in the processing of jelly and soft sweets, water-retaining agents for meats, stabilizers and thickeners in condiments such as oyster sauce and soy sauce, and suspension stabilizers in drinks [35].

In addition to being used as a food additive, microalgae polysaccharides can be used for food preservation. Microalgae polysaccharides can be combined alone or with other non-polysaccharide compounds to produce coating, which can be used as a barrier to prevent gas and water exchange between food and the environment, providing new ideas for food preservation as antioxidant protection. Some studies have mentioned that it is beneficial to keep the fresh color of fruits and prolong the shelf life of fruits [36].

3.2. Application of Microalgae Polysaccharide in Cosmetics

Microalgae polysaccharides are helpful to protect skin structure and function when used in cosmetics. Microalgae polysaccharides can combine with cutin on the skin surface to form a layer of shrinking gel, which prevents water evaporation, thus having a good moisturizing effect. In addition, sulfate polysaccharides released by marine microalgae can prevent the accumulation and activity of free radicals and reactive chemicals [37], so they can serve as protective systems against these oxidative and free radical stressors. Therefore, microalgae polysaccharides can be used as antioxidant and anti-aging components in cosmetics. Studies have shown that zinc-added red microalgae sulfated polysaccharide-chitosan hydrogel has antibacterial potential [38]. Meanwhile, β -1, 3-glucan from microalgae can not only prevent external aging signs [6], but also have anti-inflammatory properties, so they can be developed as active ingredients of functional skin care products.

3.3. Application of Microalgae Polysaccharides in Medical Care

Microalgae polysaccharides have many beneficial qualities, such as biocompatibility, biodegradability, better stability and non-immunogenicity. As such, it is widely used as a drug transporter, bioactive material, building block and excipient to improve drug delivery systems [36]. Microalgae polysaccharide also has a wide range of pharmacological effects and various biological activities, such as being antiviral, anticoagulant, anti-tumor, antioxidant, etc. It can be used in drug therapy and also as an improvement for drugs.

Microalgae polysaccharides are often used in transdermal drug delivery for wound healing because they are biodegradable and harmless compared with traditional polymers. The transdermal patch based on polysaccharides can realize continuous drug release, and the patch has excellent flexibility and air permeability. In addition, because of its excellent biocompatibility, polysaccharide-based tissue scaffolds have unique bionic potential. Similarly, microalgae polysaccharide also shows some pharmacological effects. Some marine microalgae extracts have been proven to inhibit hyaluronidase activation, indicating that they include antiallergic and anti-inflammatory substances. Moreover, the extracts of diatoms and *Clostridium columnarum* are found to have antibacterial properties [39]. According to some studies, microalgae polysaccharides also have certain spasmolytic activity in non-vascular smooth muscle organs. Laguna et al. also mentioned their anticoagulant ability. The researchers found that tested marine microalgae extracts showed some analgesic and antioxidant activities [40].

4. Conclusion

In recent years, the research on microalgae polysaccharides has been increasing. In this paper, the structure, biological activity and application of microalgae polysaccharides are reviewed. Microalgae polysaccharide has a series of physiological and pharmacological activities, such as being antioxidant, anti-tumor, antibacterial, antiviral, lowering blood sugar and blood lipid, and immunomodulatory, which has broad application prospects in the food industry, cosmetics, medical care and other fields. Thus, it provides new raw materials for human health care and industrial production development.

Although microalgae polysaccharide has many advantages, there are still problems in its development and utilization. With the in-depth study of the structure of microalgae polysaccharides and the development of chemical analysis technology, great progress has been made in the study of its structure. However, due to the variety of microalgae polysaccharides, which have complex main chain and branched chain structures, the determination of their precise structure and complete sequence still faces great challenges. At present, a large number of studies have reported the pharmacological activities of microalgae polysaccharides, but their mechanism of action has not been deeply studied. Microalgae polysaccharides from natural microalgae may cause microbial contamination, viscosity reduction and hydration during storage, which leads to its limited application in medicine and biomaterials. At present, most studies on the activity of microalgae polysaccharides are in vitro, so it is necessary to study its toxic and side effects on the human body while developing

and utilizing microalgae resources. As the increasing global demands for energy with the destruction of the ecological environment, the demand for renewable energy skyrockets. Microalgae fix carbon dioxide through photosynthesis, which makes a critical contribution to the global carbon cycle as a good source of biofuel. However, microalgae polysaccharides have not been widely used in bioenergy and biofuel production, which needs further development. Nowadays, the research on microalgae polysaccharides is still in the primary stage, with many fields short of further study. The author proposes the following development strategies for microalgae polysaccharides:

- (1) Improve the extraction rate of microalgae polysaccharide and reduce the cost of extraction by using a combination of several techniques, such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and enzyme-assisted extraction (EAE);
- (2) In-depth study of microalgae polysaccharides for specific physiological activities of the research mechanism is needed. It is significant to study the specific composition, structure and molecular length of polysaccharide basic monomer for researching its specific biological activity;
- (3) Further study microalgae cellulose. Nano-cellulose has high mechanical strength, high surface area and crystallinity, and microalgae have broad prospects in the synthesis of nano-cellulose. *Chlorella* and nano-green algae contain 47-75% cellulose, which are good sources of nano-cellulose and can be used in food packaging, nano-composite materials and other fields.
- (4) In-depth study on microalgae sulfated polysaccharides is needed. Microalgae sulfated polysaccharide has a good effect on reducing blood lipid and blood sugar as a biological lubricant and drag reducer, but there are few studies. We can further study its toxicity and bioavailability to human subjects to apply it to human disease treatment. Based on its rheological and biochemical properties, it can further expand its applications in biomedical fields as a biological lubricant and in ship engineering fields as a drag reducer.
- (5) Use microalgae polysaccharides to develop biofuels. Bioethanol is the most widely used biofuel in the world at present, so it is necessary to further optimize the industrial production process and convert microalgae polysaccharides into bioethanol by pre-treatment and fermentation. At the same time, a variety of strategies can be comprehensively utilized to increase the content of polysaccharides in microalgae, such as genetic modification technology.

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