Advances in Application of Alginate Lyase And Its Enzymatic Hydrolysate

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Abstract. Alginate is a linear, water-soluble acidic seaweed polysaccharide composed of L-guluronic acid and its C5 epimer D-mannuronic acid, a general term for hydrophilic derivatives of alginic acid. It is widely found in various brown algae. Alginate lyase and its enzymatic hydrolysate have a variety of biological activities and become a new research hotspot. This paper reviews the source, classification, enzymatic mechanism and enzymatic properties of alginate lyase, and summarizes the function and application of alginate lyase and its enzymatic hydrolysate brown algal oligosaccharide, in order to provide a base for the development and application of alginate lyase and its products.

1. Source and classification of alginate lyase

1.1. Source of alginate lyase
Alginate lyase is mainly derived from marine bacteria, marine algae, marine mollusks and echinoderms that feed on alginate, etc., as well as terrestrial fungi and very few viruses. Among them, marine bacteria have the largest variety of alginate lyases, and are the most widely studied sources of alginate lyase, such as *Vibrio*, *Pseudomonas*, *Flavobacterium*, *Klebsiella*, Nitrogen-fixing bacteria, *Bacillus*, *Enterobacter*, *Streptomyces* and the like. In addition, the presence of alginate lyase was also found in the hepatopancreas of certain marine organisms [1].

1.2. Classification of alginate lyase
According to the specificity of alginate lyase degradation substrate, the alginic acid lyase can be divided into polymannose lyase (EC4.2.2.3) and poly-Glucuronide lyase (EC4.2.2.11). There are also bifunctional lyases in which both polysaccharides are cleavable, and such alginate lyases with dual degradation functions have higher degradation efficiencies.

According to the different ways of action, alginate lyase can be divided into exo-alginate lyase and endo-alginate lyase. The exo-alginate lyase can be used to excise the uronic acid monomer or dimer oligosaccharide at the end of alginic acid. The viscosity of the alginate solution decreases slower and the rate of reducing sugar formation is faster. The endo-alginate lyase acts randomly on the 1,4-O-glycosidic bond in the long chain of alginic acid, which can reduce the viscosity of the alginate solution faster, the rate of reducing sugar formation is slower. The final products are oligosaccharides
with different degrees of polymerization, including disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, etc. [2].

According to the molecular weight, alginate lyase protein can be divided into three categories [3]: the first type is a small enzyme with a molecular weight of 20–35 kDa; the second type is a medium-sized enzyme with a molecular weight of about 40 kDa; the third type is a large enzyme with a molecular weight greater than 60 kDa.

By analyzing the primary structure of the known alginate lyase, it can be divided into 7 major families according to their hydrophobic clusters, namely PL-5, PL-6, PL-7, PL-14, PL-15, PL-17 and PL-18 [4]. Most of these endo-alginate lyase belong to the PL-5 and PL-7 families. Most of the exo-alginate lyase are classified in the PL-15 and PL-17 families.

By studying the three-dimensional structure of alginate lyase, it mainly presents several special structures, including parallel β-helical structure, (α/α) barrel structure, and jam volume structure [5].

Compared with other enzymes found in many studies, the classification of alginate enzymes is not perfect, and there may be some alginate hydrolase or other enzymes.

2. The mode and mechanism of alginate lyase
Alginate lyase cleaves the 1→4 glycosidic bond of alginate by β-elimination reaction. At the same time as the depolymerization of alginate, an oligosaccharide uronic acid of 4-deoxy-L-erythro-hex-4-enopyranosyluronic with unsaturated double bond was formed between the non-reducing ends C4 and C5 of the product. The oligosaccharide has a strong absorption peak at 230–240 nm [6]. Both D-mannuronic acid and L-guluronic acid produce unsaturated derivatives [7], and the double bond reaction is unique to enzymatic degradation.

Gacesa [8] proposed a catalytic mechanism of alginate lyase, assuming that the catalytic mechanism of alginate lyase is a three-step reaction, which is: (1) the neutralization of the salt bridge is used to remove the negative charge on the carboxyl anion. (2) a matrix-catalyzed reaction that attracts protons on 5 carbon; (3) a carboxyl group provides electrons, undergoes transfer, and forms a double bond between C4 and C5, resulting in a β-elimination reaction occurring on the 4-O glycosidic bond; Thereby forming unsaturated mannuronic acid.

3. Substrate specificity of alginate lyase
Alginate is a linear polymer polysaccharide composed of α-L-guluronic acid and its isomer β-D-mannuronic acid linked by 1,4 glycosidic bonds, and its arrangement is divided into three types. They are: poly-α 1,4-L-guluronate (pG), poly-β1,4-D-mannuronate (pM) and irregular hybrid fragments pMG. The substrate specificity of alginate lyase is defined by the difference in its degradable M or G segment. The alginate lyase is classified into pM lyase, pG lyase, and pMG lyase according to the manner of cleavage at alginate pM or pG.

Substrate specificity is related to the source environment of the lytic enzyme producing bacteria. According to the literature, the enzyme produced by Pseudomonas is a pM segment-specific lyase [9]; the enzyme produced by Klebsiella is a pG segment alginate lyase [10]. The enzyme produced by Bacillus also has the ability to degrade M and G segments.

4. Enzymatic properties of alginate lyase
Studies have shown that alginate lyases from different sources have differences in molecular size, substrate specificity, and degradation products. Its enzymatic properties are also unique.

Under normal circumstances, the optimum pH value of most alginate enzymes is 7.0–7.6, and a few of the most suitable pH are acidic; most alginate lyases are inducible, and a few are constitutive; their molecular weight is generally 24 k D–110 k D. Optimum temperature is generally between 30°C ~ 50°C, however, the optimum temperature of a small amount of alginate lyase is higher than 50°C. Because the temperature stability of most alginate lyase is compared poor, alginate lyase-related enzymatic reaction should not be carried out at high temperatures.
Like other enzymes, the activity of alginate lyase is also affected by some metal ions, metal ion chelators and denaturants. In most cases, Na⁺, K⁺, Ca²⁺, Mg²⁺ and lower concentration of sodium chloride (<0.3mol/L) are enzyme activators, which promote the enzyme activity; while SDS, EDTA, Fe²⁺, Fe³⁺, Cu²⁺, Mn²⁺, Hg²⁺, Ba²⁺, Zn²⁺ and higher sodium chloride concentration (>1.0mol/L) have different degrees of inhibition on enzyme activity.

5. Method for measuring enzyme activity of alginate lyase
The method for measuring the enzyme activity of alginate lyase is divided into two kinds of qualitative determination and quantitative determination. Qualitative assays include unique carbon source growth methods and plate identification methods. Quantitative determination includes Orcinol assay [7], Ultraviolet absorption assay [7], Thiobarbituric acid assay [7], Viscometry assay. In addition, there are reducing sugar methods and the like. In the actual enzyme activity measurement, the degradation characteristics of the enzyme are often reflected by the simultaneous measurement of viscosity, reducing sugar and absorbance values [11].

6. Application of alginate lyase
The application of alginate lyase includes degrading alginate, preparing alginate oligosaccharides, preparing protoplasts, assisting to extract DNA from seaweed, degrading seaweed waste and reusing [12], treating pulmonary cystic fibrosis and performing research on the micronization of alginate structure [13]. Alginate lyase can be combined with some antibiotics to degrade alginate produced by pathogenic bacteria in the lungs of patients with pulmonary cystic fibrosis, resulting in enhanced permeability of the cell wall of the pathogen, which is beneficial to the antibiotics to inhibit the growth and reproduction of pathogenic bacteria [14]. In addition, alginate lyase can also be used to propagate surface microbes enriched in macromolecular polysaccharide degrading bacteria.

7. Application of brown algae oligosaccharides
The brown algae oligosaccharide is a small molecular sugar chain fragment formed by degradation of alginic acid, and its polymerization degree is generally below 20. The preparation methods include acid hydrolysis, enzymatic hydrolysis and pyrolysis. In enzymatic hydrolytic preparation of brown algal oligosaccharides, alginic acid lyase is used to degrade macromolecular long-chain alginic acid into small molecules. Compared with the preparation of brown algal oligosaccharides by acid hydrolysis and pyrolysis, preparation of brown algal oligosaccharides by alginate lyase possesses the advantages of high reaction efficiency, mild reaction conditions, strong controllability, no pollution to the environment, and easy directional preparation. Therefore, it has received much attention. Algae oligosaccharides have a variety of functions and a wide range of applications.

In the food industry, brown algal oligosaccharides can be used as a food material or additive to enrich the taste of the product and increase nutrition.

In terms of agricultural production, brown algal oligosaccharides can promote plant growth, enhance plant resistance and inhibit the growth and reproduction of plant pathogens.

In the paper industry, water-soluble brown algal oligosaccharides can be used instead of part of the rosin to improve the gloss and smoothness of the paper and to improve the stagnation resistance of the paper.

In the field of medicine, as the brown algae oligosaccharide has the functions of intestinal conditioning, detoxification, blood sugar and lipids lowering, anti-inflammatory, antibacterial, immune regulation, it can be used to develop therapeutic foods for patients with diabetes, obesity, rectal colon cancer, and habitual constipation.

In the field of feedstaff, brown algae oligosaccharide as a feed additive overcomes the shortcomings of all conventional antibiotics, probiotics, enzyme preparations and other traditional additives. It is small in use, natural, no residue and strong stability. Brown algal oligosaccharides can change microorganism constituents in the animal digestive tract, enhance the disease resistance of the animal body, promote the growth of the animal, increase the daily weight gain and the nutrition of the
animal, and has the advantage of inhibiting the pathogenic bacteria such as Escherichia coli and Staphylococcus aureus [15]. Therefore, brown algal oligosaccharides are excellent feed additive.

In addition, brown algal oligosaccharides can also be widely used in many fields such as petroleum, metallurgy, and daily chemical industry.

8. Prospectives
In recent years, with the extensive research on alginate lyase, it has been found that alginate lyase has a wide application value. The research and production of alginate lyase has far-reaching significance. At present, more than 50 alginate enzymes have been isolated and identified from various microorganisms, animals and plants, and about 20 alginate lyase genes have been successfully cloned and sequenced. Although the current research on the mode of action and the enzyme digestion products of alginate lyase is very extensive, there is still a small amount of systematic research on a certain strain and its alginate lyase. Therefore, the extensive researches on alginate lyase will be the important future for targeted utilization of alginate lyase. In addition, as several shortages in the production process of alginate lyase, such as lower enzyme activity, difficult to separate the enzyme from substrate due to their tightly bounded, and pathogenicity of the enzyme-producing strain, it is difficult to realize the industrial production of alginic acid lyase. Up to now, there has not been a high-yield and low-cost commercial enzyme preparation, and its practical application is limited. Therefore, genetic engineering and cell engineering should be used to explore new enzyme production technology. The immobilization technology could also be used to modify the enzyme, improve enzyme production, and innovate the enzyme application mode in the future. The commercial preparation of alginate lyase is the necessary way to overcome its limitation in application.

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