Research progress of alginate lyases on function and application

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Abstract. Alginate lyases are useful tools to degradate alginate, alginate lyase enzymes have been widely applied in various fields such as food additions, medical care and agricultural industries. Alginate oligosaccharides produced by alginate lyases show activities on antitumor, antioxidant and bateriostasis. Those enzymes will increase the progress of bioethanol production using alginate, which is a new way to solve the energy crisis. This review has collected the major sources of these lyases, characteristics, structure and application of them.

1.Introduction

Alginate is formed by the 1,4-glycosiconsists of two uronic acids, β-D-mannuronic acid (M) and its C5 epimer α-L-guluronic acid (G) [1]. These units form 3 kinds of different blocks, including poly β-D-mannuronate (polyM), poly α-L-guluronate (polyG) and the heteropolymer (polyMG), which are linked by a 1-4 glycosidic bond [2]. In brown algae, alginate comprises up to 40% of dry weight, which can be degraded by alginate lyases via a lyase reaction (Fig 1) [3]. With the discovery of more and more alginate lyase enzymes, researchers have paid more attention on them because they can be used in food and medical treatment. Alginate oligosaccharides are produced from alginate, which have lots of functions such as plant-growth promotion, anticancer, antioxidant and bacteriostasis.

With the depletion of fossil energy, microalgae as the third generation biofuel, which can be used to produce the bioethanol. The alginate has obtained increased attention due to its production of bioethanol using the alginate lyases. Yoshiyuki Ueno has reported that dark fermentation in the marine green algae *Chlorococcum littorale* can produce 450 μmol ethanol g^{-1} at 30 ℃[4]. And Adam J. Wargacki *et al.* engineered a platform that can achieve a titer of 4.7 % volume/volume and a yield of 0.281 weight ethanol/weight dry microalgae. In this paper, sources, characteristics, structure of alginate and their application are reviewed.

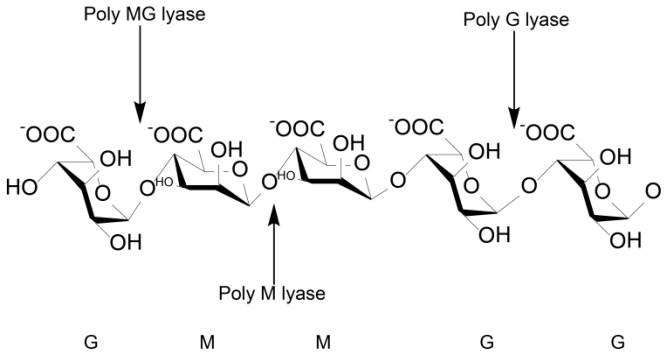

Figure 1. The substrate specificity of alginate lyase. M, β-D-mannuronic acid; G, α-L-guluronic acid.

2.Alginate lyases

2.1 Sources of alginate lyase

Alginate lyase enzymes (EC 4.2.2.3) with kinds of substrates specificities have been cloned and characterized from many microorganisms. They are mostly produced by marine bacteria and fungi. Up to now, there are some enzymes that have been identified (Table 1). These enzymes can be divided into polyG lyase (EC4.2.2.11) and polyM lyase (EC4.2.2.3).

Table 1 Characteristics of alginate lyases from different microorganisms

Source	Substrate Specificity	Molecular Mass (kDa)	Optimum Temperature	Optimum pH	Reference(s)
Azotobacter vinelandii	PloyG	25.9	N.D.	7.8	[11]
		26.6	N.D.	6.8	
		49.4	N.D.	4.2	
Azotobacter vinelandii	PolyM, PolyMG	39	N.D.	5.1	[12]
Agarivorans sp.	PloyG	31	30 ℃	10	[13]
Pseudoalteromonas elyakovii	PloyG, PloyM	32	30 ℃	7.0	[14]
Pseudoalteromonas sp. CY24	PloyG, PloyM	57.4	40 ℃	7.0	[15]
Pseudomonas sp. QD03	PloyG	42.8	37 ℃	7.5	[16]
Pseudomonas sp. Os-ALG-9	PloyM	79	30 ℃	7.0	[17]
Pseudomonas sp.	PloyG	27	55 ℃	7.0	[18]
Vibrio sp. 510-64	PloyG	34.6	35 ℃	7.5	[19]
Vibrio sp. QY101	PloyG, PloyM	39	30 ℃	7.5	[20]
Vibrio sp. YWA	PloyM	62.5	25 ℃	7.0	[21]
Vibrio sp. Ykw-34	PloyG, PloyM	60	40 ℃	7.0	[22]
Alteromonas sp. No. 272	PloyG, PloyM	33.9	30 ℃	7.5-8.0	[23]
Streptomyces sp. A5	PloyG	32	37 ℃	7.5	[24]
Streptomyces sp. ALG-5	PloyG	28.2	30 ℃	8	[25]
Corynebacterium sp.	PloyG	27	55 ℃	7.0	[26]
Klebsiella aerogenes Type25	PloyG	31.6	37 ℃	7.0	[27]

N.D., not detected

2.2 Structures of alginate lyase

Alginate lyases are grouped into seven polysaccharide lyase families, PL-5, -6, -7, -14, -15, -17, and -18 according to amino acid sequence and structural features. Many endolytic bacteria enzymes are belong to PL-5, PL-7 [6-10].

Alginate lyases are grouped into 3 families based on the 3-dimensinal structures, containing parallel β-helix family, $(\alpha/\alpha)_6$ barrel family and jelly-roll family(Fig 2). These 3-dimensinal structures make it possible to research the relationship between structure and function. The parallel β-helix family include PA1167 from *Pseudomonas aeruginosa*, AlyA1 from *Zobellia galactanivorans* and AlyA from *Klebsiella pneumonia,* which belong to PL-7 family, while $(\alpha/\alpha)_6$ barrel family include A1-III from *Sphingomonas* sp. A1 and jelly-roll family include AlyGC from PL-6 family.

They can also be separated into 3 types according to molecular masses. Three types are small lyases, medium-sized and large lyases whose molecular mass are 20-35 kDa, around 40 kDa and above 60 kDa, respectively.

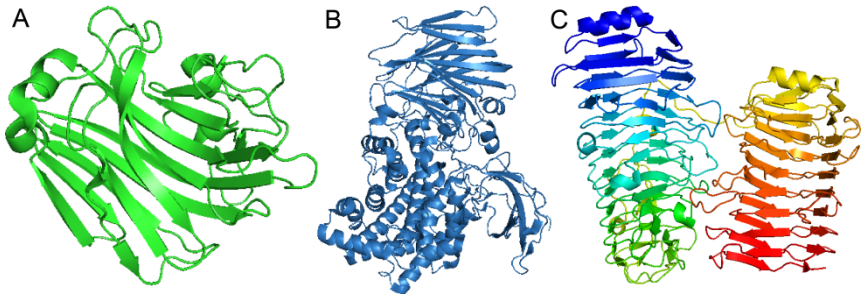

Figure 2. The overall structure of alginate lyases from different family, A: A1-II (PDB:2CWS)from jelly-roll family; B: Atu3025 (PDB:3A0O) from $(\alpha/\alpha)_6$ barrel family; C: AlyGC (PDB:5GKD) from parallel β-helix family

2.3 Characteristics of alginate lyase

There are some characteristics of alginate lyases produced from different microorganisms that have been shown in Table 1. Most of alginate lyases showed maximum activity between pH 7.0 and 8.0, while the optimal temperature at 25 ℃ -50 ℃. Most of alginate lyases were actived by adding the metal ions of Ca^{2+}, Na^{+} and Mg^{2+}, and inhibited by the metal ions of Ba^{2+} and Hg^{2+}. AlyPI from *Pseudoalteromonas* sp. CY24 showed optimal enzyme activity at 40 ℃ and pH 7.0 with the 100 mmol/L NaCl, and showed a better stability between pH 6.0 and 10.6. Song *etc*. reported AlySY08 from *Vibrio* sp. QY101 showed the optimal enzyme activity at 40 ℃ and pH 7.6 in phosphate buffer. Moreover the activity of the enzyme was enhanced by the addition of 500 mmol/L NaCl and 1.0 mmol/L Ca^{2+}, and was inhibited in the presence of 5.0 mmol/L Ni^{2+}, 1.0 mmol/L Fe^{2+} and 1.0 mmol/L EDTA. In 2006, this group reported another alginate lyase named ALgL, which exhibited maximal activity at pH 7.5 and 37 ℃ and was enhanced by the Na^{+}, K^{+} and Ca^{2+}.

2.4 Substrate specificity

Alginate is a liner block copolymer of two uronic acids, such as PolyM, PloyG, PolyGM, PloyMG. The environment where organisms exist has a great influence on substrate specificity. Most of the lyases are M specific, such as the lyase from ATCC43367 , while some G specific lyases and MG specific lyases have already been characterized. The G specific lyases include the enzyme from *mollusk Lambis* sp. However, there are several alginate lyases showing activities on both of them, such as the lyases from *Vibrio* sp. QY101, *Vibrio* sp. Ykw-34 and *Alteromonas* sp. No. 272. The polyM lyase and the polyG lyase usually display low acitivity on the other homopolymer. Because the substrates used have poor quality and purity, which can be solved by using substrates of greater purity.

3.Function and application of alginate lyases

Alginate lyases were found in non-alginate- synthesizing and alginate-synthesizing organisms. And the

first ones can use alginate as a carbon source. They are very important in biosynthesis and biodegradation of alginate in the second ones. Alginate lyases control alginate polymer length and optimize the merization reaction in the biosynthesis of alginate.There is an alginate lyase named FlAlyA, can degrade all alginate blocks into unsaturated di-, tri-, tetra-, and pen-tasccharides, which helps it to extract DNA and RNA, from *Flavobacterium* sp.UMI-01 [28].

Alginate lyases are grouped into endo-type alginate lyases and exo-type alginate lyases. Endo-type alginate lyases can display polyM-, polyG- and polyMG-specific activity. The exo-type alginate lyase depolymerizes the alginate oligomers into unsaturated monosaccharide and it is nonenzymatically converted to 4-deoxy-L-erythro-hexoseulose uronic acid (DEH), and then DEH is reduced by DEH reductase into 2-keto-3-deoxy-gluconate (KDG), which is fed into the Enter-Doudoroff (ED) pathway [29].

Alginate lyases are key tools, which can be used to produce oligosaccharides from alginate with kinds of activities. Alginate oligosaccharides were able to promote plant growth and plant resistance, inhibition of fungi growth, and have the potential to be a kind of environment-friendly biofertilizer and biopesticide [30-33]. And it can also be applied in medical treatment. Alginate lyases are crucial to produce such useful oligomeric products.

With the fuel consuming, microalgae are the ideal sources to use to produce the bioethanol. Researchers have paid more attention on the production of bioethanol. There are some reports, Wargacki has engineered a microbial platform for bioethanol production, which is equivalent to ~80% of the theoretical value from the sugar composition in macroalgae. Therefore, alginate lyases are the dominant enzyme to study the production of biofuel.

4.Conclusion

There were various alginate lyases that have been identified, and the relationship between the structure and function among them was well studied. Some of them can produce the oligosaccharides with many activities and determine the alginate structure. And people can also use them to produce bioethanol to solve the problem of energy. Therefore, alginate lyases are another potential choice to solve the energy crisis.

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