Advances in the Mechanism of Action, Characteristics, and Applications of Alginate Lyases

Guanchu Ma[1,a], Chen Zhao[3,4,b], Kunlun Li[2,c], Le Su[1,d], Song Zhang[1,e], Qiulin Yue[1,f,†] and Lin Zhao[1,g,†]
[1]*State Key Laboratory of Biobased Material and Green Papermaking, Shandong Provincial Key Laboratory of Microbial Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, China*
[2]*Jinan Hangchen Biotechnology Co., Ltd., Jinan 250353, China*
[3]*National Engineering Laboratory of Crop Stress Resistance Breeding, Anhui Agricultural University, Hefei 230036, People's Republic of China*
[4]*Shandong Food Ferment Industry Research & Design Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, P.R. China*

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Abstract: Alginate is a natural polysaccharide. Alginate lyase could break the glycosidic bonds by β-elimination and form the unsaturated double bonds to complete the degradation of alginate. With the development of enzyme resources, screening and extracting valuable enzymes from various organisms and developing polysaccharide degradation products have become an important aspect of the development of biological resources. Combining various researches, this paper summarizes the research progress of alginate lyase in recent years from the mechanism of action mechanism, enzyme characterizations and enzyme application.

1 INTRODUCTION

Alginate is mainly a by-product of iodine and mannitol extracted from marine algae, which mainly exists in cell wall and intracellular substances. It is a copolymer composed of α-L- guluronic and its C5 isomer β-D-mannuronic in different proportions through β-1,4-glycosidic bond. At present, alginate oligosaccharides are used in various work and research, such as plant immune (Gurpilhares, 2019), food storage (Aitouguinane, 2020), biomedicine (Zhao, 2020), biofuel (Lu, 2019) and other fields. There are three main degradation methods of alginate: chemical method, physical method and enzymatic hydrolysis. In comparison, the advantage of enzymatic degradation is more obvious, its efficiency is high, the reaction conditions are mild, the process is easy to control and has little impact on the environment, so it is a better method to prepare alginate oligosaccharides. Therefore, in recent years, the research on alginate lyases, one of the seaweed tool enzymes and its degradation product, alginate oligosaccharides, has attracted increasing attention.

It is found that alginate lyases have important commercial application value for the development of bioengineering products. It can not only develop new physiologically active substances, but more importantly, it opens up a new field of industrial application. A more important application trend is to focus on the enzymatic production of algal oligosaccharides, providing new means for the research and development of biomedical production and food technology, especially the physiological effects of alginate oligosaccharides, an enzymatic hydrolysate called oligosaccharide, have been revealed, which has aroused more interest.

2 MECHANISM OF ACTION AND ENZYME CHARACTERIZATION

Alginate lyases catalyze the degradation of alginate through β-elimination, an unsaturated double bond was formed between C4 and C5, and a non-reducing end 4-deoxy-β-L-erythro-hex-4-enopyranosyluronicacid was generated, the conjugate of unsaturated oligosaccharides in double bonds and carboxyl groups results in a strong absorption peak at

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235nm for oligosaccharide products (Natsume, 1994). Gacesa et al. have hypothesized that the process of enzymatic hydrolysis of alginate by alginate lyase can be divided into three steps, namely: (a) eliminate the negative charge on the carboxyl ion by salt bridge neutralization reaction; (b) attract protons on the C5 position; (c) The unsaturated double bond between C4 and C5 is formed by β-elimination of the transfer of electrons from the carboxyl group (Gacesa, 1987).

Recently, a large number of scientific achievements of alginate lyase have been reported by researchers, and their enzymatic properties have also been studied through heterologous expression. It has filled the blank of alginate lyases research, and provided sufficient experimental verification for future commercial application (Table 1). The enzymatic properties of alginate lyases produced by different species have certain differences. Most enzymes have milder working conditions, the optimum temperature is between 30-50℃, the optimum pH is 7.0-8.0, neutral and alkaline. But there are exceptions. For example, in 2016, InoueA et al., the alginate lyase NitAly produced by the strain *Nitratiruptor* sp.SB155-2 isolated interestingly, its optimal temperature could reach 70°C (Inoue, 2016). Metal ions and their concentrations also have an important impact on the enzymatic activity of alginate lyases. Wang et al. reported in a 2019 study that for *Vibrio* sp. SY01, its alginate lyase Aly08 produced by it will enhance its activity in an environment containing NaCl, and will reach a peak activity at 300 mM, which is about more than 8 times in the non-NaCl environment. In the presence of other metal ions such as NH_4^+, Li^+, Zn^{2+}, Ba^{2+} and Co^{2+}, Aly08 has no obvious activation effect. Because of Ca^{2+} and Mn^{2+}, its enzyme activity increases greatly, while the surfactants SDS and EDTA seriously weakened the activity of alginate lyase, and the relative activity decreased by more than 50%.

Table 1: List of differences of alginate lyase.

No.	Source	Gene	PL	Molar mass (kDa)	Alginate lyase specificity	Cleavage mode Exo/Endo	Temperature (℃)	pH	Activity (U/mg)	Reference
1	*Nitratiruptor* sp. SB155-2	NitAly	7	29	pM	Endo	70	6	1620	(Inoue, 2016)
2	*Streptomyces coelicolor* A3(2)	OUC-ScCD6	6	52	pM	Endo	50	9	31.6	(Cheng, 2020)
3	*Vibrio* sp. W13	Algb	7	55.05	Alg, pMG	Endo	30	8	457	(Zhu, 2015b)
4	*Haliotis discus hannai*	HdAly	14	28.9	pM	Endo	45	8	28.9	(Shimizu, 2003)
5	*Flavobacterium* sp. S02	AlyS02	7	36.5	pM, pG	Endo	30	7.6	442.84	(Zhou, 2020)
6	*Flavobacterium* sp. H63	rSAGL	7	32	pM	Endo	45	7.5	4044	(Li, 2018)
7	*Pseudomonas* sp. E03	AlgA	5	40.4	pM	Endo	30	8	222	(Zhu, 2015a)
8	*Vibrio* sp. SY01	Aly08	7	35	pG	Endo	45	8.35	841	(Wang, 2019)
9	*Zobellia galactanivorans*	AlyA5	7	42	pG	Exo	-	7	449.3	(Thomas, 2013)
10	*P. carrageenovora* ASY5	Aly1281	7	40.65	pM, pG	Endo	50	8	1.15	(Zhang, 2020)
11	*Shewanella* sp. YH1	rAlgSV1-PL7	7	33.22	Alg, pM, pG	Endo+ Exo	45	8	96	(Yagi, 2017)
12	*Haliotis discus hannai*	HdAlex	14	32	pM	Exo	42	7.1	684	(Suzuki, 2006)

3 APPLICATION

Degradation of alginate into oligosaccharides is the current major trend and key step in the use of seaweed polysaccharides. Enzymatic hydrolysis prepared by different alginate lyases, the products are also different. As early as the 1990s, studies have shown that alginate oligosaccharides could promote growth of plant roots. Yoshihiba et al. prepared alginate oligosaccharides by enzymatic hydrolysis. According to the experimental results, it was confirmed that alginate oligosaccharides had a significant growth-promoting effect on plant rhizomes. In 2020, a study conducted by Aitouguinane et al. pointed out that alginate polysaccharides and its oligosaccharides derivatives trigger plant defense responses and show a significant ability to induce PAL activity in tomato seedling leaves(Aitouguinane, et al., 2020). These results help marine life as a potential biological resource to protect plants from plant pathogens in the context of ecologically sustainable green technology. Studies have found that extracellular polysaccharides are important pathogenic factors for some pathogenic bacteria to cause lung infections. It is well known that alginate exists in biofilms as a key role in the failure of immunotherapy and antibiotic therapy. In 2020, a study by wan et al. reported a new silver nanocomposite, which is used to deliver drug compounds, alginate lyase and ceftazidime. Due to the principle of alginate degradation of alginate lyase, it has a strong inhibition and degradation effect on the *Pseudomonas aeruginosa* PAO1, and it can also eradicate *Pseudomonas aeruginosa* in the lung to achieve the purpose of treatment(Wan, 2020).

With the consumption of petroleum and other non-renewable energy, the development of new clean energy has been put on the agenda. There have been many articles reporting from alginate. The main principle of the research progress in the preparation of bioethanol from alginate is to prepare bioethanol through the synergistic action of the alginate lyase endonuclease and exonuclease and continuous reaction saccharification of alginate.

4 CONCLUSION

Alginate lyases have become new tool enzymes because of its unique biological activity and high efficiency, clean and controllable properties. Recently, the research on alginate lyases have gradually shifted from the early description of its characteristics to the biological study of its mechanism. Meanwhile, with the rapid development of molecular biology, a variety of alginate lyase genes can be cloned, sequenced and selected for heterologous expression to construct efficient engineering strains. It can be predicted that the application of alginate lyases will be more and more extensive in the future.

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