

Purification and characterization of algae eucheuma polysaccharide degrading enzymes from Micro bacterium

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Abstract. A micro bacterium was used for degradation of eucheuma polysaccharide in this study. The purification and identification of the polysaccharide-degrading enzyme from fermented algae eucheuma extract was developed. Under the ammonium sulfate precipitation, DEAE ion exchange chromatography procedure, enzyme was purified. SDS-PAGE gel electrophoresis result showed the crud enzyme was complex with four components. With the TLC and molecular exclusion chromatography method, eucheuma polysaccharide degrading activity of the crud enzyme was tested.

Keywords: Eucheuma polysaccharide; lyase; oligosaccharide.

1. Introduction

Eucheuma also known as agar, carrageenan, carrageenan, in natural classification, eucheuma belongs to rhodophyta, gigartinales, Hongling cabombaceae, Eucheuma^[1]. With high gel content and main component of carrageenan, eucheuma is also rich in cellulose, hemicellulose, vitamins and minerals^[2]. Due to the high biological activity of antitumor, antiviral, antibacterial, reducing radiation damage and inhibition of urinary stones and other biological activity, eucheuma oligosaccharide became a popular health product. Because of the stereochemical variability of carbohydrates, polysaccharide-degrading enzymes are essential tools for resolving the structure of these complex macromolecules.

As a major source of carrageenan extraction, the chemical structure of eucheuma consists of calcium, potassium, sodium, ammonium sulfate of polysaccharide sulfate with galactose and galactose in the dehydration. This type of sulfate mainly exists in the cell wall, and the content can reach to 50% of the cell dry weight. Carrageenan is based on sulfuric acid or sulfuric acid radical of galactose and 3, 6-dehydration of galactose by the alpha-1,3 and beta-1,4 glycosidic bond alternately connected, with one sulfuric acid radicals on the unit of C₄ in the 1,3 connection D-galactose. The molecular weight can be over 200000. According to the number and position of the group of sulfate and the different binding forms of sulfate, carrageenan can be divided into K type (kappa), I type (Iota) and L type (Lambda). Modern pharmacological studies shown that eucheuma oligosaccharides owned main biological activity compared with polysaccharide^[6]. Tradational alkali and acid degrading procedure for eucheuma oligosaccharide extraction is easy to cause inactivation of oligosaccharides and environmental pollution.

In this study, a strain of *microbacterium* was used for degradation of algal polysaccharides. The ectoenzyme induced by eucheuma polysaccharide were primary studied.

2. MATERIALS AND METHODS

2.1. Materials

Strains: A5 micro bacterium, preserved in our lab.

LB medium, Peptone 10g/L, Yeast 5g/L, NaCl 10g/L;

Fermentation medium, Eucheuma 6g/L, Na₂HPO₄ ·12H₂O 2g/L, NaH₂PO₄ 2H₂O 2g/L, MgSO₄ ·7H₂O 2g/L, FeSO₄ ·7H₂O 2g/L, the pH adjusted to pH=5.0.

2.2 Methods

Separation and purification of crude enzyme: After removal of cells and solid impurities by centrifugation ($10,000\times g$ for 20 min at 4°C), the supernate was collected. Then ammonium sulfate precipitation was used for protein precipitation; the mixture was freeze-dried and redissolved in 20mM phosphate buffer solution. Then the mixture was chromatographed on a column (2.0×25 cm) of Toyopearl DEAE-650S and monitored for total protein absorbance at 280 nm^[12]. Washed the column with gradient NaCl solution, kept the elution rate at 1ml/min and collected protein elution peak.

SDS-PAGE test for enzyme: Preparation of 12% separation gel and 5% spacer gel and keep constant current 25mA in the whole process^[13].

Substrate degradation activity of the crude enzyme: The crude lyase was incubated in a mixture containing different substrates (glucose, lactose and euclidean polysaccharide) and phosphate buffer, pH 5.0, for 5h at 30°C . The reaction was analyzed for products on TLC^[14] respectively.

Expansion agent (mobile phase): $V_{\text{butanol}}: V_{\text{isopropyl alcohol}}: V_{\text{acetic acid}}: V_{\text{water}}=7:5:2:4$;

Chromogenic agent: aniline (4ml)-two aniline (4g)-phosphoric acid (20ml) dissolved in 200ml acetone^[15].

MS analysis of products: The crude lyase was incubated in a mixture containing euclidean polysaccharide and phosphate buffer, pH 5.0, for 5h at 30°C . In order to analyse the Mr of products, electrospray ionization mass spectrometry was used. Removed the protein by the method of sevag^[16] before the experiment and made the detection range at 0-1500 Da.

3. Result and discussion

3.1 Growth curve of microbacterium

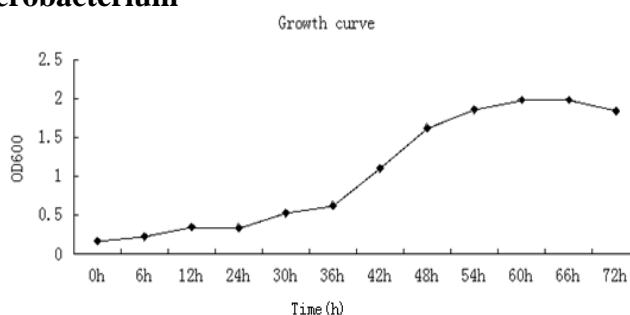


Fig. 1 growth curve of microbacterium

The culture of 60hours used for enzyme extraction.

3.2 Separation and purification of euclidean degrading enzyme with Toyopearl DEAE-650S ion exchange

With the NaCl gradient elution, two protein elution peaks were resulted. 0.5MNaCl, get elution peak ①, 1.0MNaCl, get elution peak ② (Fig 2).

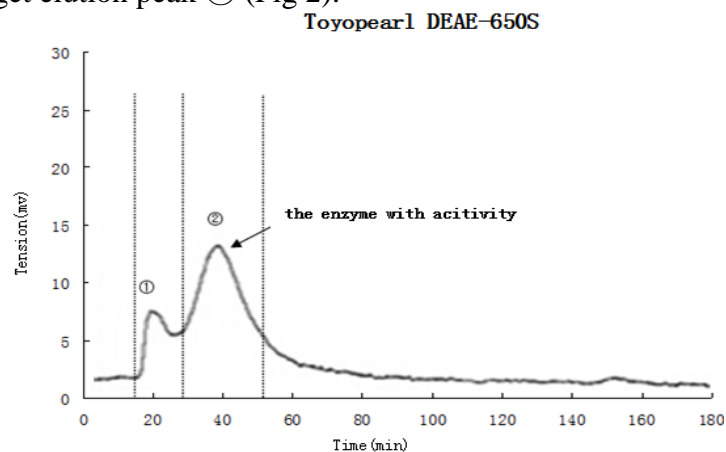


Fig. 2 Plot of toyopearl DEAE-650S ion exchange chromatography

3.3 SDS-PAGE of two elution peaks from DEAE-650S

The elution peak ② was a complex enzyme with four electrophoretic bands, the molecular mass of bands were 43 kDa, 55kDa, 72kDa and 80 kDa.

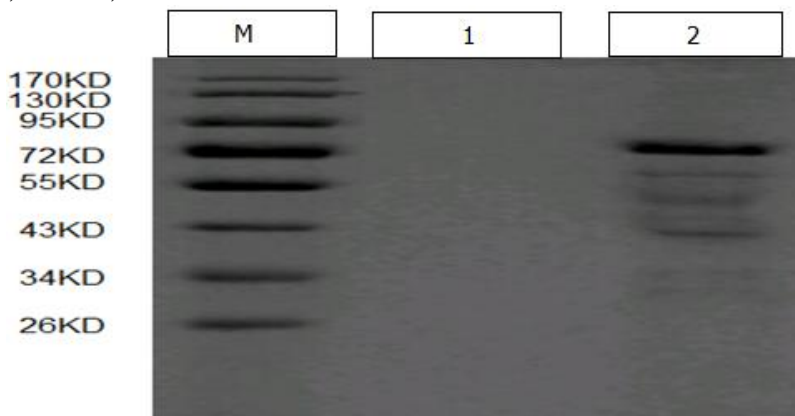


Fig. 3 the determination of relative molecular mass

M, Marker, Lane 1, elution peak ①, Lane 2, elution peak ②.

3.4 Substrate degrading activity of the crude enzyme

The TLC result (Fig 4) showed that euclidean polysaccharide could be degraded into four kinds of components, which were different from glucose and lactose. They might be euclidean oligosaccharides.

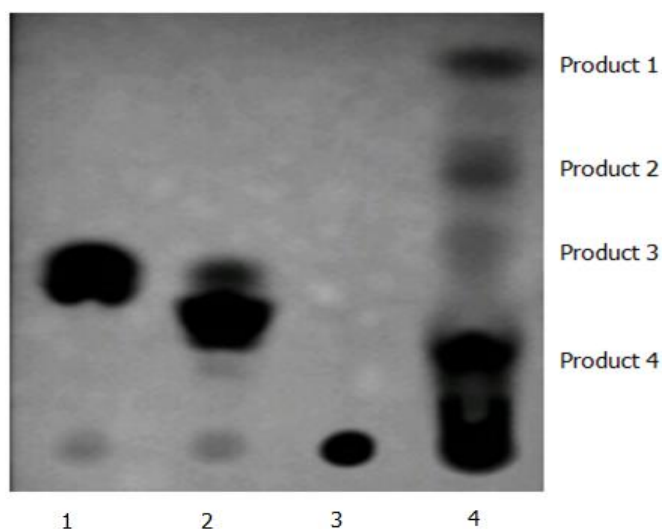


Fig. 4 detection of enzyme activity

Note: lane 1 was glucose, lane 2 was lactose, lane 3 was euclidean polysaccharide without degradation with enzyme, and lane 4 was euclidean polysaccharide after degradation with enzyme.

4. Conclusion

In this study, the euclidean polysaccharide degrading crude enzyme was purified from microbacterium fermentation culture. The SDS-page test result showed the one protein elution peak from DEAE-650S ion exchange chromatography was a complex enzyme with four bands. TLC results confirmed the euclidean polysaccharide degradation activity of the crude enzyme.

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