Isolation, Purification, and Enzymatic Activity of iota-Carrageenase from Cultured Marine Bacterium

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1. Introduction

Carrageenans are gel-forming linear sulfated galactans extracted from certain marine red algae. Due to their lower molecular weight compared to native ones, oligosaccharides obtained from cleaving process of carrageenans can show potential anti-tumor activities, anticoagulation, anti-inflammation, anti-oxidation, anti-thrombosis, and viral inactivation. Different chemical and physical methods included enzymatic and acid hydrolysis, and ultra-sonication are applied to degrade carageenans. Because enzymes are highly specific to their substrates and they can generate oligo-derivatives with uniform molecular weights oligosaccharides produced by the action of microbial enzymes are found to be more invaluable than those produced by other methods. The present study was undertaken in an attempt to purify and measure the specificity and activity of ι- carrageenase produced by *Cellulophaga Baltica* Species.

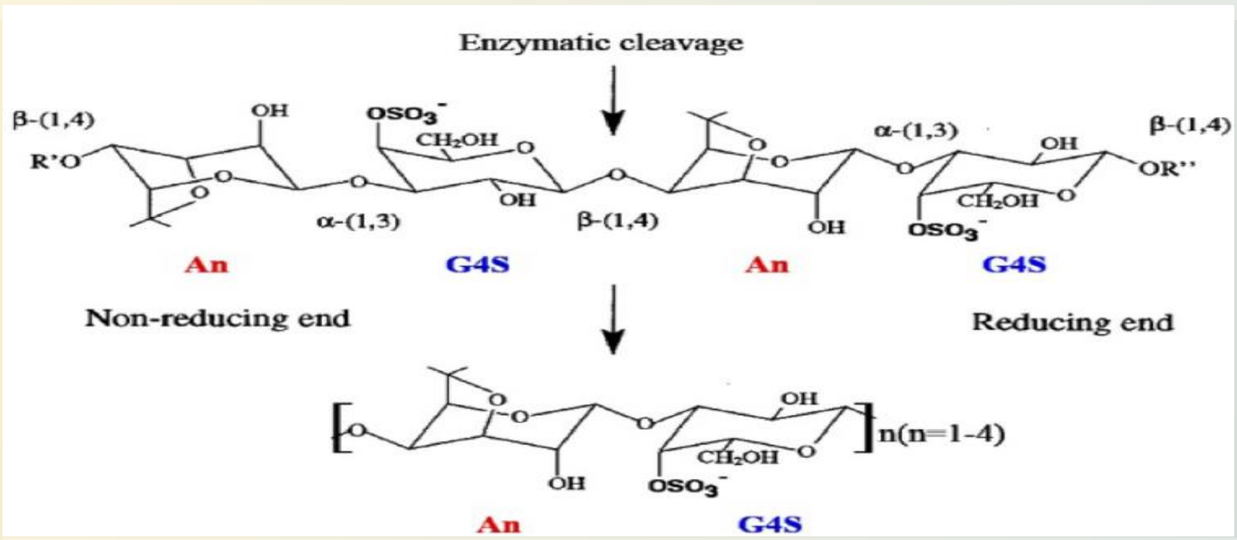

Figure 1. Schematic diagram showing two disaccharide-repeating units of carrageenan, where the site of enzymatic hydrolysis is specific at the internal β-1,4 linkage of the carrageenan.

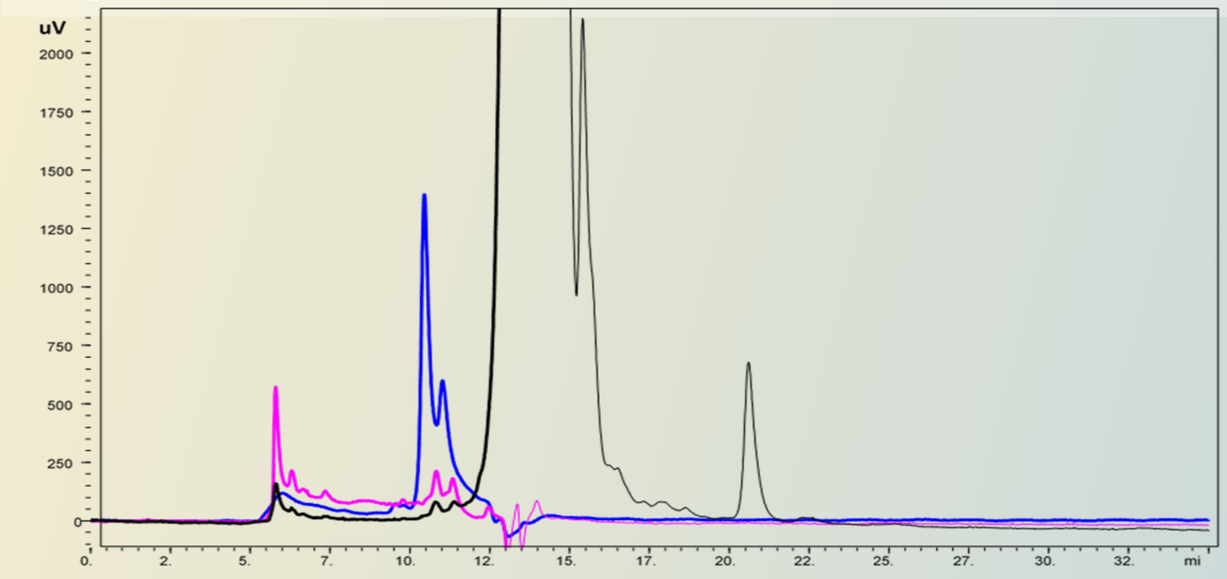

Figure 2. Size Exclusion-High-Performance Liquid Chromatography (SEC-HPLC) for ι-carrageenase. **Black** = Cell-free medium chromatogram, **Pink** = After Ultrafiltration using Sartorius Stedim Vivaflow-200 10,000 MWCO, and **Blue** = after 40%-70% ammonium sulfate precipitation.

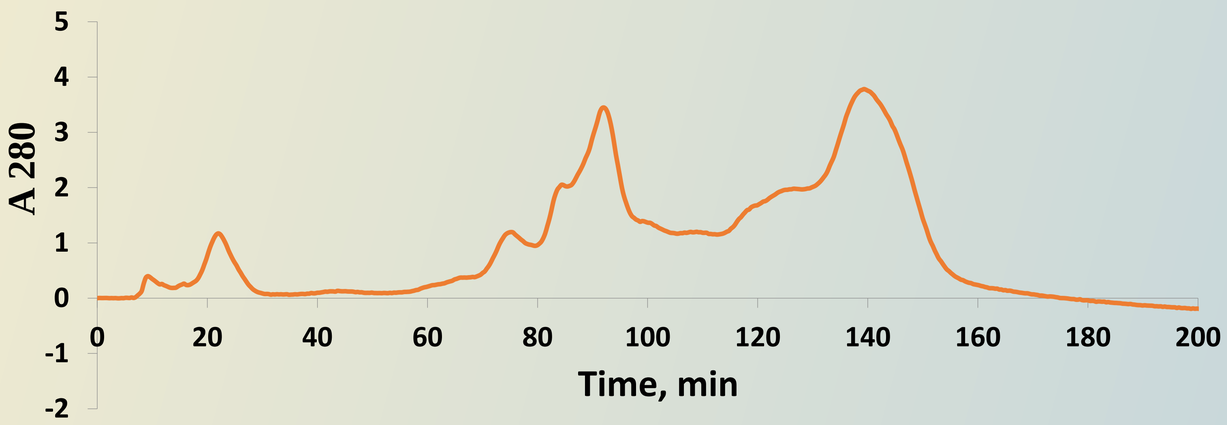

Figure 3. Ion- exchange chromatography of ι-carrageenase. The sample was eluted at a flow rate of 1.0 mL/min with Tris-HCl buffer at pH 7.8. The eluates were monitored for protein (A280).

2. Methods

EXPERIMENTAL METHODS

In this study, the extracellular carrageenase was isolated from the cell-free medium of a culture of marine bacterium *Cellulophaga* species grown on marine broth with 0.16% furcellaran. Active fractions of carrageenase was separated and purified from the mixture by ultrafiltration, ammonium sulfate precipitation **Figure (2)**, and ion-exchange chromatography **Figure (3)**. The enzymatic activity of ι-carrageenase was measured during purification procedure **Table (1)**. As each enzyme is a very specific compound, the enzymatic activity of enzymes against different substrates as carrageenans, agars, and alginates was measured by reducing sugar assay **Figure (5)**.

Table 1. Summary of the purification procedures of ι-carrageenase

Step	Total protein [a] (mg)	Total activity [b] (U)	Yield (%)	Specific activity (U/mg)	Purification Folds
Crude medium (Cell-free medium)	12.03	262.3	100.00	21.81	1.0
Ultrafiltrate	2.82	94.5	36.03	33.54	1.5
$(NH_4)_2SO_4$ precipitation	1.10	89.5	34.14	80.89	3.7
DEAE HP (ion-exchange chromatography)	0.0095	7.5	2.88	793.69	36.4

[a] Protein contents were determined by Bradford method; [b] Carrageenase activity was determined by the reducing power method of Potassium ferricyanide.

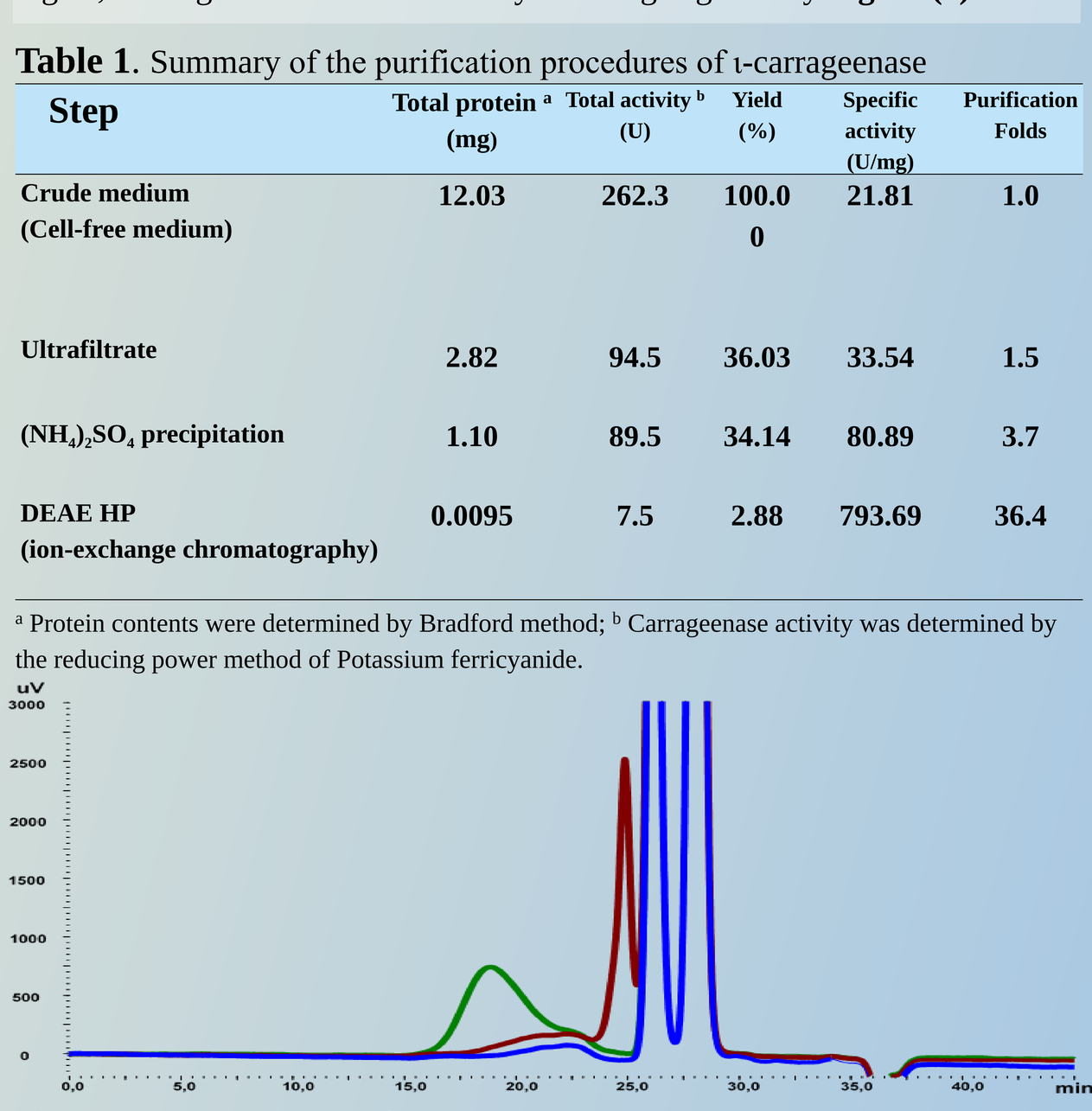

Figure 4. Chromatograms of the enzymatic hydrolysis ι-carageenan polysaccharide monitored by refractive index detector. **Blue** = Enzyme + Tris-HCl buffer pH = 7.8 as substrate, **Green** = Inactivated Enzyme (by heating for 20 min at 99.5°C) + ι-carageenan polysaccharide substrate dissolved in Tris-HCl buffer pH = 7.8, **Red** = Enzyme + ι-carageenan polysaccharide substrate dissolved in Tris-HCl buffer pH = 7.8. Enzyme and substrate in each of the three samples where incubated in thermo-shaker for 30min at 40 °C and 1000 rpm.

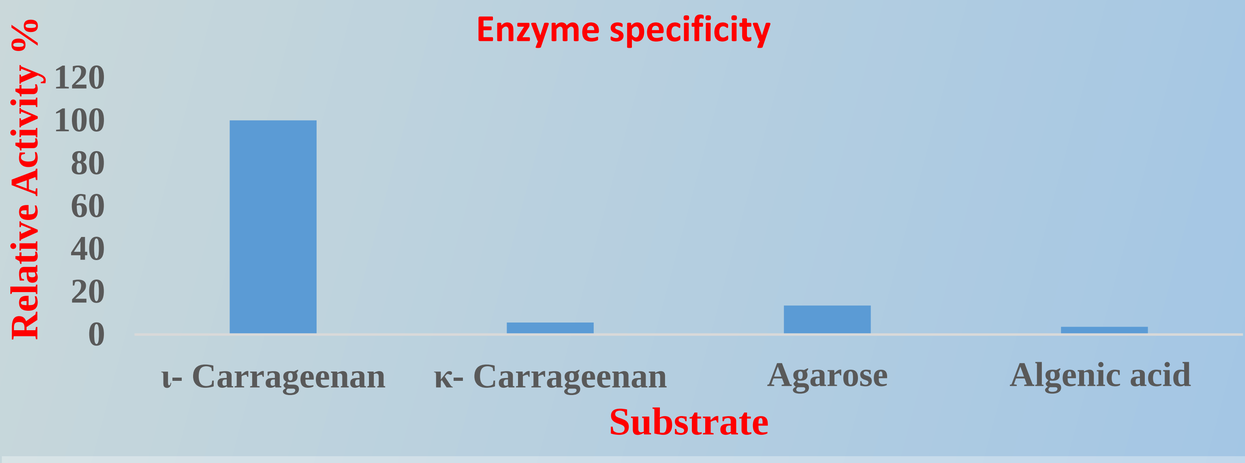

Figure 5. Substrate Specificity Studies of ι-Carrageenase. Enzyme and each substrate where incubated in thermo-shaker for 30min at 40 °C and 1000 rpm.

Results

ι- carrageenase was successfully purified by a series of purification procedures and finally yielded a significantly high activity and specificity against ι -carrageenan polysaccharides. Depending on results obtained by enzymatic activity assay, The extracellular ι-carrageenase in the last purification step showed 36 fold increase in specific activity comparing to that of the cell -free medium.

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