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Isolated of ι-carrageenase producing bacterium and it's culture conditions for the producing of ι-carrageenase

Hang-Sun Shim, Jong-Oh Kim*, Moon-Chul Bae, Deuk-Moon Cho**, Dae-Seok Byun, Hyeung-Rak Kim

Department of Food and Life Sciences, College of Fisheries Sciences, Pukyong National University, Busan, Korea*Department of Food Service Management, Dong-Pusan College, Busan, Korea

Introduction

Carrageenan is a structural polysaccharide in red algae and consists of a linear backbone of galactopyranose residues linked by alternating and linkges (Tristan B. et al, 2000). The carrageenans have three main branches named kappar, iota, lambda which are well differentiated in linkage of sulfate groups on their galactan backbone. They exhibit unique rheological properties and are widely used as texturing and moisturizing agents in food and pharmaceutical industries.

Enzymes that degrade carrageenanas, namely κ-, λ- and ι-carrageenases, have been isolated from various marine bacteria (Gurvan et al., 2001). ι-Carrageenase were purified from a marine Gram-negative bacterium, *Alterodomonas. fortis* (Gurvan et al., 2001) and *Zovellia galactanovora.* Enzymes identified as endohydrolases that cleave the internal linkages of carrageenans yielding products of the neocarrabioses. These two proteins are homologous and bear no significant sequence similarities to any other polysaccharidase, including κ-carrageenase. They cleave the internal linkage of ι-carrageenan with overall inversion of the anomeric configuration, whereas κ-carrageenase proceed with retention of configuration. It was proposed that ι-carrageenases constitute a new structural family of glycoside hydrolases, referred to as family 82.

In this study, a novel marine bacteria identified as Pseudomonas sp. HS5322 was isolated and culture conditions for the production of ι-carrageenase was determined.

Materials and Methods

A seaweed sample (*Geildium amansii*) was collected at coast of Busan in Korea and spread onto modified ZoBell 2216E plate (10 g peptone, 10 g yeast

extract, 20 g ι-carrageenan, 250 ml distilled water, 750 ml filtered seawater, pH 7.0). Bacterial colonies showing a hole on palate after 4 days incubation at 25°C were pick up and isolated by the same plating method. For liquid culture, the strain was culture in the modified ZoBell medium containing 0.5% ι-carrageenan (ME). Biochemical tests were analyzedusing VITEK GNI Card (Biomerieux, Marcy-l'Eto, France). Gram staining was done by Gram Stain Kit (DIFCO, Detroit, USA).

Total genomic DNA was prepared using Genomic DNA extract kit (iNtRon, Kyungki-Do, Korea) according to manufacture's manual. PCR 16S DNA gene was conducted by GeneAmp PCR Systems 2700 (Applied Biosystems. CA, USA)

Optimum culture conditions for the growth of isolate and ι-carrageenase activity were determined with ME. Cell growth was determined by measuring turbidity at 600 nm (Ultrospec 3000, Pharmacia). The ι-carrageenase activity was determined using ρ-nitrophenyl -D-galactopyranoside. The ι-carrageeanse activity (unit) was expressed as 1 mol ρ-nitrophenol liberated per min per ml of enzyme solution at 37 °C.

Results

A bacterial strain producing ι-carrageenase was isolated from the Southeast coast in Korea. The isolated strain, *Pesudomonas* sp. HS5322 is aerobic, gram-negative and motile. The organism was grown optimally at NaCl concentration of 1~2%. The sequence similarity of the 16s rDNA with those of known *Pseudomonas* sp. was found to be at most 94%, implying that the isolate was a new *Pseudomonas* species. Optimum culture conditions for the producing of ι-carrageenase were determined to be 20°C and pH 8.5 for 72 hr incubation.

References

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