B-5

Isolation and Characterization of alginate lyase producing marine bacterium, *Vibrio* sp. S21

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Introduction

Alginates distributed in brown marine plants such as *Undaria pinnatifida*, *Laminaria* are acidic polysaccharides composed of (1-4) linked β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G) residues. Alginates arranged in block structures which may be homopolymeric (poly M or poly G) or heteropolymeric, often occurring as random sequences (poly MG) (Haug, A. *et al.*, 1967). The gelling proportion of alginates in the presence of divalent cations are determined by the ratio of the two uronic acids (M:G ratio) within the polysaccharide chains. Highly visous gels are formed with alginates of low M:G ratio, because of the chelate metal ions by the poly G blocks (Gacesa, P., and Wusteman, F. S., 1990). Alginates are widely used for food, pharmaceutical, textile, and oil industries.

In this study, isolation of alginate lyase produging marine bacterium from decayed seaweed and identified as *Vibrio* sp. S21. A purification of alginate lyase from *Vibrio* sp. S21.

Materials and Methods

Material : Strain *Vibrio* sp. S21 was originally isolated from a sample obtained an abalone. The medium used for the isolation of bacteria was minimal medium plate (NH_4Cl 1.0 g, Na_2HPO_4 4.8 g, KH_2PO_4 4.4 g, NaCl 20 g, $MgSO_4 \cdot 7H_2O$ 5.5 g, $CaCl_2$ 0.1g, $FeSO_4 \cdot 7H_2O$ 0.002 g, sodium alginate 1.5 g/L and 1000 ㎖ distilled water pH 7.5). The plates were incubated at 25 ℃ for 2 days. Colonies forming pits or clearing zones on sodium alginate plate were pick up and isolated further by the same plating

method.

Phenotypic tests : Cell morphology was observed by optical microscopy of Gram-stained preparation. Motility of the S21 was examined using wet mounts. Bacteria prepared by gram staining and imaged in the transmission electron microscope. Biochemical tests were analyzed using API 20E (Korean culture center of microorganisms, Korea).

Amplification of 16s rDNA sequencing : Total genomic DNA was prepared using Genomic DNA extract kit (Bioneer, Daejeon, Korea) according to manufactures manual. PCR of 16S rDNA gene was conducted by GeneAmp PCR system 2700(Applied Biosystems, CA, USA) in 16S rDNA of S21.

Purification of Alginate lyase : All step were performed at 4℃. Culture medium was separated with the centrifuge at 8000 x *g* for 30 min, The supernatant was passed through Q-sepharosec column (2.5 x 15 cm) equilibrated with 20 mM Tris-HCl buffer (pH 7.5). The enzyme was eluted with 400 ㎖ of liner gradient ranging from 0.2 to 1.0 M NaCl in the same buffer. The flow rate and fraction size were 100 ㎖/h and 5 ㎖, respectively. Whereas minor proteins were still detected on SDS-PAGE.

Result

Bacteria producing alginate lyase was isolated from brown algae at Southeast coast in Korea. Isolate strain S21 is a Gram-positive, aerobic, polar flagella, rods 1.3 x 0.45 um. This organism grew optimally at 25°C and in the presence of 2% (w/v) NaCl. The 16S rDNA gene sequence from strain S21 was similar to the sequence from *Vibrio* sp. This strain contained large amounts of fatty acids $C_{16:0}$, $C_{18:1}$ ω7c, $C_{12:0}$ and C14:0 . Alginate lyase was purified from *Vibrio* sp. S21 through ionic exchange chromatographies. The enzyme was molecular weight of 38 kDa as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis.

References

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