PB - 7

Purification and characterization of α-agarase produced from a marine bacterium, *Pseudomonas* sp. AP5333

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Introduction

Agar can be degraded by agarose fromseveral bacterial strains in marine environments and other sources. These hydroytic enzymes are classified into two groups according the mode of action on agarose. The α-agarase cleaves the α-L-(1→3) link between 3,6 anhydro-L-galactose at the reducing end. The β-agarase cleaves the β-D-(1→4) link between the D-galactopyranose and the 3,6 anhydro-L-galactopyranose, yielding oligosaccharides with D-galactose residues at the reducing end (Hassairi et al. 2001). Agaro-oligosaccharides obtained from enzyme hydrolysis or acid treatment showed various functionalities. Especially, Agaro-oligosaccharides obtained with enzyme hydrolysis has more functional abilities than those from acid hydrolysis of acid treatment. Agaro-oligosaccharides has been reported its improved functionalities, suppression of the inducible nitric monoxide synthetase (iNOS) expression, suppression of prostaglandin E2 production and TPA-induced edema, suppression of TNF-α production (Kato et al. 2001). In this work, we isolated a marine bacterium producing agarase, identified as *Pseudomonas.* sp. AP5333, and described the purification and characterization of an α- agarase from *Pseudomonas* sp. AP5333.

Materials and methods

The screening medium for agarolytic bacteria was composed of 2.0% agar, 1.0% beef extract, 1.0% peptone and 2.5% NaCl (pH 7.5). The plates were incubated at 25℃ for three days. Colonies that formed pits or clearing zones on agar plate were picked up and isolated further by the same plating method. This strain was culture in the modified marine medium to produce agarase. After a 3 day-culture of *Pseudomonas* sp. AP5333, culture midium was centrifuged at 8000 g for 30 min. The supernatant was diluted with 2 volume of 20 mM Tris-HCl buffer (pH 7.5) and loaded onto a S-Sepharose colume (2.5 × 12 ㎝) equilibrated with 20 mM Tris-HCl buffer (pH 7.5). Unbound fraction of the

S-Sepharose column was load onto a Q-Sepharose column (2.5 × 12 ㎝) equilibrated with 20 mM Tris-HCl buffer (pH 7.5). Enzyme fractions were collected and diluted with 2 volume of equilibration buffer was loaded onto Mono-Q (Phamacia, HR 5/5) column equilibrated with 20 mM Tris-HCl buffer (pH 7.5). The elution was performed with a linear gradient of 0.5 to 1.0 M NaCl in 20 mM Tris-HCl buffer (pH 7.5). The enzyme fraction was further purified by the Mono-Q colum chromatography with previous conditions. The enzyme fractions from Mono-Q column were pooled and loaded onto Superose-6 column attained in FPLC system. With the GPC chromatpgraphy, the agarase was purified and agarase activity was determined by the enzymatic production of reducing sugars from agarose (Somogyi-Nelson 1952).

Result & Abstract

An agarolytic bacterium was isolated from *Capsosi-phon fulvecense* (C. agardh) collected in the Southern coast of Korea and the strain was identified as *Pseudomonas* sp. AP5333 by phylogenetic studies based on analysis of the 16s rDNA gene sequence. Optimal culture conditions for the production of agarase was estimated to be pH 7.5, 2 5℃ and 72 hr incubation. The enzyme was purified 13.5-fold purity against the culture supernatant by a series of ionic exchange chromatographies and gel filteration. The molecular weight of the enzyme was estimated to be 27 kDa by sodium dodesylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The optimum pH and temperature for hydrolysis of agarose were determined to be 7.5 and 50℃, respectively. The enzyme was identified as α-agarase according to the degradation fashion of synthetic substrates. The enzyme showed a specific activity for agar, agarose, however, did not show on sodium alginate, or κ-,ι-, and λ -carrageenan. The enzyme was inhibited by Mn^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, and Zn^{2+}. However, bivalent metal cations such as Mg^{2+}, Li^{2+} did not changed enzyme activity.

References

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