

Development of a thermo-stable β -agarase from marine organism

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Abstract

Neoagaro-oligosaccharides are produced only by enzymatic degradation of agarose by β -agarase.¹⁾

Neoagaro-oligosaccharides inhibit the growth of bacteria, slow the rate of degradation of starch, are used as low-calorie additives to improve food quality, and have macrophage-stimulating activity. Furthermore, neoagarobiose is a rare reagent that has both moisturizing effect on skin and whitening effect on melanoma cells.²⁾ An agar-degrading marine bacterium was isolated from the sea water at the northeast coast in Cheju island, Korea. The strain was gram negative, aerobic, and motile rod. The 16S rRNA of the strain had the closest match of 98% homology, with that from *Agarivorans albus*. On the basis of several phenotypic characters and a phylogenetic analysis, this strain was designated *Agarivorans* sp. JA-1. In solid agar plate, *Agarivorans* sp. JA-1 produced a diffusible agarase that caused agar softening around the colonies. *Agarivorans* sp. JA-1 was cultured for 36 hr in marine broth 2216 (Difco, USA) and the supernatant that containing an extracellular β -agarase was prepared by centrifugation of culture media. The enzyme exhibited relatively strong activity at 40°C and was stable up to 60°C. Using PCR primers derived from the β -agarase gene of *Vibrio* sp., the gene encoding β -agarase from *Agarivorans* sp. JA-1 was cloned and sequenced. The structural gene consists of 2931 bp encoding 976 amino acids with a predicted molecular weight of 107,360 Da. The deduced amino acid sequence showed 98% and 34% homology to agaA²⁾ and agaB³⁾ genes for β -agarases from *Vibrio* sp., respectively. The expression plasmid for β -agarase gene of *Agarivorans* sp. JA-1 is

being constructed and the recombinant enzyme will be biochemically characterized.

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