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Thermostable β-Agarase from a Deep-sea *Microbulbifer* Isolate

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Microbulbifer sp. strain JAMB-A3, isolated from the sediment in Sagami Bay, Japan, at a depth of 1174 m, was found to produce a novel β-agarase. The agarase gene was cloned and sequenced. It encodes a protein of 602 amino acids with a calculated molecular mass of 65,017 Da. The deduced amino acid sequence showed similarity to those of known β-agarases in glycoside hydrolase family 16, with 34-55% identity. Tandem sequences similar to a carbohydrate binding-like module were found in the C-terminal region of the enzyme. The recombinant agarase was hyper-produced extracellularly using *Bacillus subtilis* as the host, and the homogeneously purified enzyme had a high specific activity of 528 U/mg at pH 7.0 and 50°C. The optimal temperature and pH for activity were 54°C and around 7, respectively. The recombinant enzyme was thermostable with a half-life of 8.7 h at 50°C. It was very stable during incubation with EDTA, Na^+, K^+, Mg^{2+}, Ca^{2+} ions, and surfactants at high concentrations. *N*-Bromosuccinimide abolished the enzymatic activity, and agarose oligosaccharides protected the enzyme from inactivation by this chemical, suggesting that a tryptophan(s) residue is involved in the catalysis of the enzyme. The pattern of agarose hydrolysis showed that the enzyme is an endo-type β-agarase, and the final main product is neoagarotetraose.

Key words: *Microbulbifer*, β-agarase, glycoside hydrolase family 16, neoagaro-oligosaccharide

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