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**β -Agarase Gene from a Marine Bacterium, *Pseudoalteromonas* sp.
JT-6: Cloning, Expression and Enzymatic Properties**

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A gene (*agaA6*) encoding an extracellular agarase from a marine bacterium, *Pseudoalteromonas* sp. JT-6, was cloned, sequenced and expressed in *Escherichia coli*. It comprised an open reading frame of 1338 base pairs and encodes a protein of 445 amino acids with a predicted molecular weight of 50,150 daltons. The entire amino acid sequence of this agarase gene showed 99% identity with the *agaA* gene from *Janthinobacterium* sp. SY12. It consists of a signal peptide, a glycoside hydrolase family 16 β -agarase domain and a carbohydrate-binding domain. The recombinant agarase was overexpressed and purified to homogeneity. Enzyme activity analysis revealed that the optimum temperature and pH for the purified recombinant enzyme were around 40°C and 9.0. The enzyme was an endo-type β -agarase and hydrolyzed agarose to several oligosaccharides.

Key words: Agarase; *Pseudoalteromonas* sp. JT-6; cloning; expression

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**Optimization for Mass Production of Carboxymethylcellulase from
Psychrobacter aquimaris LBH-10 isolated from seawater
by Orthogonal Array Method**

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Rice bran and peptone were found to be the best carbon source and nitrogen sources for the production of carboxymethylcellulase (CMCase) by *Psychrobacter aquimaris* LBH-10 isolated from seawater. Optimal conditions for production of CMCase such as concentrations of carbon and nitrogen sources, initial pH of medium and temperature were investigated using $L_{25}(5^3)$ -orthogonal array method with five distinct levels. The highest production of CMCase by *P. aquimaris* LBH-10 was 371.7 u/ml from 5.0% (w/v) rice bran and 3.0% (w/v) peptone under initial pH of 8.0 and temperature of 30°C. The optimal concentration of mineral salts for the highest production of CMCase from *P. aquimaris* LBH-10 using Orthogonal array were found to be 0.00% K_2HPO_4 , 0.05% NaCl, 0.01% $MgSO_4 \cdot 7H_2O$ and 0.03% $(NH_4)_2SO_4$. Optimal agitation speed and aeration rate for the production of CMCase by *P. aquimaris* LBH-10 in a 7L bioreactor was 300 rpm and 1.0 vvm, respectively. Under these conditions, production of CMCase by *P. aquimaris* LBH-10 in a 7L bioreactor was 322.4 u/ml for 72hr culture and that in a 100L bioreactor with the inner pressure of 0.4 kgf/cm² was 288.7 u/ml.

Key words: Carboxymethylcellulase; Orthogonal Array; Rice bran