Chaperone-Assisted Expression of Alginate Lyase in *E. coli* and Its Application for Production of Mannuronic Acid

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Recently, alginate polymers and oligosaccharides from alginate with novel physicochemical and physiological functions are sought by biopolymer-based industries in order to expand the application areas of polysaccharide such as functional food additives for cholesterol decrease. Alginate lyase cleaves the glycosidic bonds in alginate by β-elimination, resulting in the formation of D-mannuronic acid and L-guluronic acid. For the mass production of these acids from alginate, the overexpression of alginate lyase is a prerequisite. When the alginate lyase gene (*aly*, 1.19 kb) from *Pseudomonas elyakovii* was expressed in *E. coli*, most of the gene product was found within the cell and no activity was detected both in the medium and intracellular fractions, indicating the formation of insoluble aggregates, inclusion body. To produce the soluble and active alginate lyase in *E. coli*, chaperone teams of GroEL/ES and DnaK/DnaJ/GrpE were coexpressed. The plasmids, pALP4 and pG-KJE6, in which the alginate lyase and DnaK/DnaJ/GrpE genes are under the control of T7 promoter and *araB* promoter, respectively, were co-transformed into *E. coli*. The optimal concentrations of L-arabinose for the expression of DnaK/DnaJ/GrpE were found be 0.5 mg/ml. When arabinose was added at OD_{600} = 0.4 ~ 0.5, the production of active alginate lyase in the soluble fraction of *E. coli* cell was significantly increased about 20 ~ 50%, compared with that without molecular chaperones. When the active alginate lyase enzyme was reacted with alginate, D-mannuronic acid and L-guluronic acid were efficiently produced within 2.0 hr.