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Purification and Characterization of Recombinant Arylsulfatase Cloned from *Pseudoalteromonas carrageenovora*

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Arylsulfatase (aryl-sulfate sulphohydrolase, E.C.3.1.6.1) activity has been identified to be involved with desulfatation of sulfated polysaccharides in a marine aerobic Gram-negative bacterium, *Pseudoalteromonas carrageenovora*. The gene encoding arylsulfatase (*astA*) from *Pseudoalteromonas carrageenovora* was subcloned into the expression vector pET21a. The constructed plasmid pAST-A1 was introduced into *E. coli* BL21 (DE3), and the recombinant arylsulfatase was overproduced after IPTG induction. The expressed recombinant arylsulfatase was purified from the crude cellular periplasmic extract through series of column chromatography methods; i) DEAE-cellulose anion exchange chromatography, ii) Heparin-sepharose affinity chromatography, and iii) Gel filtration chromatography. Arylsulfatase activity was assayed by monitoring the enzymatic hydrolysis of phenolic ester substrate (*p*-nitrophenyl-sulphate), which produces chromogenic product, *p*-nitrophenol in the reaction. The purified arylsulfatase exhibited an estimated molecular mass of 31 kD in SDS-PAGE analysis, indicating that the precursor polypeptide (36 kD) was processed by releasing a potential signal peptide in the cell. The signal peptide located at the N-terminal of the protein is believed to direct the arylsulfatase to the periplasmic space of the cell. The purified arylsulfatase will be useful to produce desulfated agarose with a superior grade due to its activity of desulfatation of agaropectin. Here, we will present currently established protocols of the protein purification, and will discuss the results.