Constitutive Overexpression of Arylsulfatase by Fed-Batch Cultivation of *E. coli*

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The arylsulfatase gene (*astA*, 984 bp ORF) from *Pseudoalteromonas carrageenovora* genome was subcloned into the pHCE-IA vector, in which the hyper-constitutive expression (HCE) promoter from the D-amino acid aminotransferase gene of *Geobacillus toebii* was employed. When the constructed plasmid pHCE-AST (4.8 kb) was introduced into *E. coli* BL21(DE3), the transformant on LB plate showed the hydrolyzing activity for 4-methylumbelliferyl-sulfate and *p*-nitrophenyl-sulfate. When *E. coli* BL21(DE3)/ pHCE-AST was grown on Maxybroth-FC or Maxybroth-HD medium, arylsulfatase activity reached about 5.9 unit/ml or 12.8 unit/ml after 16 h culture, respectively. In the fermentor batch culture of *E. coli* cell on LB medium containing 2% glycerol, the activity of the enzyme reached about 16.0 unit/ml. With Maxybroth-HD medium containing 2% glycerol, *E. coli* cell showed 2-fold higher expression level of arylsulfatase, ca. 30.0 unit/ml. The enzyme activity was increased with a growth-associated manner and found predominantly within the cell, expecially in the periplasmic space, during whole culture period. The fed-batch cultivation employing the additional feeding of glycerol gave 2-5 fold higher levels of cell growth and enzyme activity. The resolution of agarose prepared from agar by this enzyme was compared with a commercially available agarose by running DNA ladders. The result suggests that arylsulfatase overexpressed in *E. coli* could be applicable to the economic production of electrophoretic-grade agarose.