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Application of Recombinant Arylsulfatase for Production of Electrophoretic-Grade Agarose from Agar

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To develop a novel enzymatic method for preparation of electrophoretic-grade agarose from agar, the desulfatation activity of arylsulfatase was applied to remove sulfate groups in agaropectin or agar. 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 (D-AAT) 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. To confirm the agarose produced by this enzyme method the quality of agarose should be evaluated by the sulfate content, gel strength, and DNA migration. Low sulfate($\leq$ 0.25%) agarose with appropriate 487,7±0.17 (g/cm^2) gel strength was successfully isolated from agar using the above technique and showed successful DNA migration. The resolution of agarose prepared from agar in this study was compared with a commercially available agarose by running 1 kb or 100 bp DNA ladders. Based on the image analyzer data, these DNA ladders showed similar banding patterns of migration and resolution. This result suggests that arylsulfatase expressed in *E. coli* could be applicable to the production of electrophoretic-grade agarose.