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Antioxidant and Tumor Growth Inhibitory Activities of DongChongXiaCao Extracts

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DongChongXiaCao (winter worm-summer grass) is a special type of mushroom which is formed on an insect larva infected by the entomogenous fungus. In order to investigate availability of DongChongXiaCao as alternative medicine or natural therapeutics against degenerative diseases, we first carried out antioxidant assay (DPPH, FRAP), α-glucosidase assay and cytotoxicity assay against KATO III (a human gastric carcinoma cell line) and B16 (a mouse melanoma cell line). Among 15 different kinds of DongChongXiaCao extracts, *Cordyceps gracilioides, Cordyceps scara, Cordyceps ochraceostromat, Periostracum tenuipes,* and *Cordyceps cardianlis* showed dose-dependently high antioxidant activity and α-glucosidase inhibitory activity. Moreover, *Cordyceps gracilioides, Periostracum tenuipes, Cordyceps cardianlis* have an inhibitory effect on the proliferation of KATO III and B16 cells. Together, these results indicate that DongChongXiaCao extracts have strong anti-oxidative and anticancer effects *in vitro,* suggesting that the extracts have potential to natural anti-oxidant and/or anti-tumor products, if we find more functions.

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Production of Electrophoretic-Grade Agarose from Agar by *Pseudoalteromonas* Arylsulfatase Overexpressed in *E. coli*

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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) and the transformant 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. The fed-batch cultivation employing Maxybroth-HD medium and additional feeding of glycerol gave about 140 unit/ml of arylsulfatase at 20 h, which is corresponded 5-fold higher level of enzyme activity. 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. 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 overexpressed in *E. coli* could be applicable to the production of electrophoretic-grade agarose.