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Purification and Characterization of Carboxymethylcellulase from *Bacillus subtilis* subsp. *subtilis* A-53

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The extracellular enzyme produced by *B. subtilis* subsp. *subtilis* A-53 was purified by ammonium sulfate saturation of supernatant of culture broth after removal of cells and column chromatography through HiTrap™ QXL ion exchange column and Mono Q ion exchange column. The molecular weight of purified enzyme was estimated to be about 56 KDa by SDS-PAGE. Carboxymethylcellulose (CMC), xylan, cellobiose, filter paper, cellulose, avicel and *p*-nitrophenyl-β-D-glucopyranoside (pNPG) as a substrate for the purified enzyme were tested. Among them, CMC was found to be the best substrate. Optimal temperature and pH for the CMCase were determined to be 50℃ and 6.5. The activity of CMCase was stable between pH 6.0 and pH 9.0 and it retained over 40% of its original activity within the pH 6.0 to 9.0 for 8 hr. The K^+, Ni^+ and EDTA increased the activity of the CMCase, while Co^{2+}, Hg^{2+} decreased the activity.

Key words: *Bacillus subtilis,* CMCase, purification, characterization

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Expression of Cellulase Gene of *Bacillus subtilis* subsp. *subtilis* A-53 in E. coli and its Production

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Bacillus subtilis subsp. *subtilis* A-53 was isolated from the seashore of the Kyungsang province in Korea. The highest production of cellulase was obtained when concentrations of rice hull and yeast extract were 5.0% (w/v) and 0.0% (w/v). Optimal initial pH of the medium and temperature were 6.8 and 37℃. Under these conditions, optimal agitation speed and aeration rate in a 7L bioreactor were found to be 400 rpm and 1.0 vvm. The gene coding cellulase in *B. subtilis* subsp. *subtilis* A-53 was isolated and cloned. The deduced amino acid sequence of the cellulase showed high identity to cellulase from other *Bacillus* species. *E. co*li JM109 was used as a host strain for cloning and maintenance of plasmid. The production of cellulase by *B. subtilis* subsp. *subtilis* A-53 and its transformant from rice bran as a carbon source was 68.7(U/ml) and 868.8(U/ml),respectively.

Key words: Cloning, sequencing, cellulase