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Characterizations of A Bifunctional Cellulase and Its Structural Gene (CEL Gene): An Exo- and Endo-Glucanase Activity from *Bacillus* sp. D04

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A cellulase from *Bacillus* sp. D04 was purified and characterized. The molecular weight of the purified cellulase was about 35 kDa. This cellulase degraded CM-cellulose, cellotetraose, cellopentaose, pNPC, and avicel PH101. Based on the HPLC analysis of degradation products, cellulase of *Bacillus* sp. D04 cleaved randomly β-1,4-glycosidic bond in cellotetraose and cellopentaose as an endo-glucanase, and also hydrolyzed only agluconic bond in pNPC and cleaved avicel to cellobiose as an exo-glucanase. Therefore the purified cellulase had both endo- and exo-glucanase activities. Cellobiose inhibited the pNPC degrading activity as a competitive inhibitor but not CM-cellulose degrading activity. The 10 mM pCl-HgBzOH completely inhibited pNPC degrading activity but not CM-cellulose degrading activity. The CM-cellulose did not inhibit but increased pNPC degrading activity, vice versa. But MUC strongly inhibited pNPC degrading activity. These results suggested that this cellulase had active site of endo- and exo-glucanase, respectively. The cellulase gene (cel gene), 1461 bp in the length, of *Bacillus* sp. D04 was cloned. The cel gene had potential promoter regions (-35 (TGACA), -10 (TACAAT)), and a Shine-Dalgarno sequence (AAGGAGG). The 30 amino acids in the N-terminus of the cel gene had a common signal peptide structure of *Bacillus* species. The cel gene has a high nucleotide sequence homology with those of *Bacillus subtilis* DLG and *Bacillus subtilis* BSE616.