

ENHANCEMENT OF ACTIVITY AND THERMOSTABILITY OF A *GEOBACILLUS* ENDOGLUCANASE VIA A UNIQUE SELF-TRUNCATION PROCESS

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The complete utilization of lignocellulosic biomass requires the hydrolysis of cellulose fibers via the synergistic action of three enzymes: exoglucanase, endoglucanase and beta-glucosidase. GsCelA is a 368-amino-acid endoglucanase secreted from a thermophilic *Geobacillus* sp. 70PC53 that was isolated from a rice straw compost in south Taiwan. GsCelA belongs to the glycosyl hydrolase family 5 and has a typical TIM barrel structure. This enzyme has excellent lignocellulolytic activity and high thermostability, with optimal temperature at 60°C and pH at 5.0. The purified GsCelA is capable of carrying out a unique self-truncation process at temperature higher than 10 °C with optimal pH at 6-7. This self-truncation process is not due to the action of contaminating proteases and it can be suppressed by EDTA and EGTA, and enhanced by divalent metal ions. This self-truncation process also takes place *in vivo* in *Geobacillus* sp. 70PC53. The spontaneous or engineered C-terminal truncation up to 60 amino acids from the C-terminus improves GsCelA specific activity and renders the enzyme more thermostable. To investigate the importance of specific amino acids on the enzymatic activity of GsCelA, site-directed mutagenesis and protein engineering approach were employed to alter amino acid residues unique to this enzyme. It was demonstrated that point mutations Y195T , D55S, G288T and D289L replacements increase the activity of this enzyme by 30%.