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%.