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Cloning, expression and characterization of a hyperthermostable GH family 12 endo-1,4-β-glucanase cloned from *Thermotoga naphthophila* in a mesophilic expression host

Ikram-ul-Haq
GC University, Pakistan

The growing demands of bioenergy have led to the emphasis on novel cellulases to improve the efficiency of biodegradation process of plant biomass for bioethanol production. Therefore, a novel gene TnEgl (936 bp) was cloned from a hyperthermophilic eubacterium Thermotoga naphthophila and overexpressed as soluble endo-1,4-β-glucanase (TnEgl) belonging to glycoside hydrolase family 12 in *Escherichia coli* BL21 codonPlus. Enhancing heterologous expression of the cloned protein using various cultivation and induction strategies. After gene cloning and expression of a thermostable protein enhanced using various modifying cultivation media and induction parameters. After optimal production of TnEgl with a molecular weight of 36 kDa, was purified to homogeneity, and characterized completely. High-cell-density or dry cell weight (DCW) and optimal expression of endo-1,4-β-glucanase were obtained in 3×ZYBM9 medium after 72 h inducement at 22°C, induced the culture either with 0.5 mM IPTG/100 mM lactose after heat shock treatment (42°C for 1h) when OD600nm reached at 0.6. Recombinant extracellular enzyme activity was improved by 7.78 and 6.18 fold in 3×ZYBM9 and ZYBM9, respectively under optimal cultivation conditions. Using M9NG and YNG auto-induction medium, activity was 6.5 and 4.76 fold increased after 72 h incubation at 22°C with agitation (200 rev min-1). Hence, the results showed that the effective process strategy is essential to enhance engineered cell mass (production) and enzyme expression. The purified enzyme was optimally active with 1036 Umg-1 of specific activity against 4% CMC at pH 6.0 and 95°C. The enzyme exhibited great stability over a broad pH (6.0-9.0) and temperature range (80-90°C) and was quite stable for 12 hours at 80°C. The enzyme showed great resistance towards various chemical inhibitors and great affinity towards various substrates. A hyper thermotolerant TnEgl with great catalytic efficiency and independence of various chemical inhibitors, all noteworthy features make TnBglB a suitable candidate for various industrial applications.

director_dric@gcu.edu.pk