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Purification and Characterization of *Thermotoga maritima* thermostable xylanase B Expressed in *E. coli.*

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Second only to cellulose in natural abundance, xylan is a major component of hemicellulose fraction of the plant cell walls, accounting for up to 35% of total dry weight in higher plants. Xylans are heteropolymeric comprising a backbone of 1,4-linked β-D-xylopyranose resides. Depending on the sources, its backbone may carry various substitutents like arabinose and glucuronic acid. Typically, backbone depolymerization is accomplished by the action of endo-xylanases and β-xylosidases. Xylanase can hydrolyze β-1,4-glycosidic linkages of the xylan backbone to produce short chain xylo-oligosaccharides of varying lengths, hence Endo-β-xylanase is the crucial enzyme components of microbial xylanolytic systems.

Thermotoga maritima MSB8 possesses two different genes of xylanases, *xynA* and *xynB*. The *xynB* gene was isolated from genome DNA of *Thermotoga maritima* MSB8, cloned and expressed in *E. coli.* The xynB was purified to homogeneity by heat treatment, affinity chromatography and anion exchange column chromatography. The purified enzyme, showed a single protein band on SDS-PAGE with a molecular mass of 42 kDa. This enzyme was extremely thermostable, and had a broad pH stability. It was quite stable over the pH range of pH 5.0 -11.4 at 70℃, and stable up to 100℃ at the pH ranging from pH 7.0 to 8.5. The optimum pH was pH 6.14 (at 50℃). XynB was optimally active at 90℃(at optimum pH6.14). Additionally, the enzyme exhibited a broad substrate specificity hydrolysed. K_m and K_{cat} of the purified enzyme for p-nitrophenol-β-D-xylobioside measured at 30 ℃ were 0.0095mM and 16.4 s-1 respectively.

Thermostability is a desirable property for xylanases used in industrial processes. It is apparent that the cloned xynB was extremely thermophilic and thermostable like other xylanases from the *Thermotoga* species. Besides, it was of interest that xynB was apt to be stable in the neutral to alkaline region and relatively less stable in the acid pH region, moreover, this may be attractive feature with regard to industrial applications.