

13<sup>th</sup> International Conference on

# Metabolomics and Systems Biology

October 11-12, 2018 | Zurich, Switzerland

## A hyperthermostable multidomain GH family 3 $\beta$ -glucosidase from *Thermotoga naphthophila* RKU-10<sup>T</sup>: Cloning, characterization and thermodynamic analysis

Ikram Ul Haq

Government College University, Pakistan

A novel gene (2,166 bp) was cloned from a hyperthermophilic eubacterium *Thermotoga naphthophila* RKU-10<sup>T</sup> and over-expressed a multidomain  $\beta$ -glucosidase protein (TnBglB) belonging to glycoside hydrolase family 3 (GH3) in *Escherichia coli* BL21 CodonPlus. An extracellular TnBglB enzyme with a molecular weight of 81kDa, was purified to homogeneity. Purified TnBglB showed peak activity at 85°C and pH 5.0 using p-nitrophenyl- $\beta$ -D-glucopyranoside (PNPG) as substrate. Enzyme displayed high thermal stability over a broad range of temperature (60-90°C) and quite stable after 480 min incubation at 85°C. Enzyme activity was enhanced by 175% by the addition of 10mM Ca<sup>2+</sup> cation and in the presence of short chain alcohol (10%v/v) activity was improved by 185%.  $K_i$  value of TnBglB for glucose and xylose inhibition was estimated 150 mM and 200 mM, respectively.  $K_m$ ,  $V_{max}$ ,  $k_{cat}$  and  $k_{cat}/K_m$  values, towards PNPG as substrate, were 0.45mM, 153 mmolmg<sup>-1</sup>min<sup>-1</sup>, 1214285s<sup>-1</sup> and 2698413, respectively. Thermodynamic parameters for PNPG hydrolysis by TnBglB like  $\Delta H^*$ ,  $\Delta G^*$  and  $\Delta S^*$  were calculated at 85°C as 24.09kJ mol<sup>-1</sup>, 46.55kJ mol<sup>-1</sup> and -62.74Jmol<sup>-1</sup>K<sup>-1</sup>, respectively. TnBglB displayed a half-life (t<sub>1/2</sub>) of 4.44 min at 94°C with denaturation parameters of enzyme including  $\Delta H^*D$ ,  $\Delta G^*D$  and  $\Delta S^*D$  were 283.78 kJ mol<sup>-1</sup>, 108.69 kJ mol<sup>-1</sup> and 0.477 kJ mol<sup>-1</sup> K<sup>-1</sup>, respectively. TnBglB showed great affinity towards p-nitrophenyl substrates and cellobiose. Possible catalytic sites involved in hydrolyzing different p-nitrophenyl substrates are proposed based on TnBglB docking studies with its substrates. A hyperthermotolerant TnBglB with great catalytic efficiency and low product inhibition, also exhibited independence of various chemical inhibitors. All noteworthy features make TnBglB suitable candidate for industrial applications.

ikmhaq@yahoo.com