**BIOCHEMICAL CHARACTERIZATION OF A MESOPHILIC AND A THERMOPHILIC BETA-GLUCOSIDASE FROM GH1 FAMILY**

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Beta-glucosidases can cleave and synthesize glycosidic bonds, which makes them an essential enzyme class in biological pathways in all living organisms. Due to this versatility, they have many biotechnological applications, making the characterization of their structure-function relationship interesting for future use. Here we present the biochemical characterization of a mesophilic beta-glucosidase B from *Paenibacillus polymyxa* (bgliB) and a thermophilic beta-glucosidade A from *Thermotoga maritime* (bgliTm). Gene sequences that codify those proteins were subcloned on vector pLate51 and transformed on E. coli BL21 (DE3), and protein expression was induced with addition of IPTG 1mM. Protein purification with Ni-NTA agarose was confirmed in SDS-PAGE and western-blotting. Enzymatic activity upon substrates as p-nitrophenil-β-D-glucopiranoside (pn-gluco) and p-nitrophenil-β-D-fucopiranoside (pn-fuco) was followed through production of p-nitrophenolate. Melting temperature ($T_m$) was determined by circular dichroism (208nm, 215nm and 222nm) and Differential Scanning Fluorimetry, using temperatures from 20°C to 90°C. Thermal inactivation kinetics was evaluated ($K_{obs}$) by measuring the relative remaining enzymatic activity after different time incubation at 47°C. Stern-Volmer constant ($K_{sv}$) was determined for acrylamide quenching of tryptophan intrinsic fluorescence. For bgliB the following parameters were obtained: $T_m = 42±0.7°C; K_{obs} = 0.08\text{min}^{-1}; K_{sv} = 5.15 \text{M}^{-1}; K_m$ and $V_{max}$ for pn-Gluco were $6.1±0.5 \text{mM}$ and $1.45±0.05 \text{nmol/min}$, respectively; $K_m$ and $V_{max}$ for pn-Fuco were $6.4±0.7\text{mM}$ and $0.46±0.02 \text{nmol/min}$, respectively. For bgliTm the following values were obtained: $K_{obs} = 0.004\text{min}^{-1}; K_{sv} = 5.2 \text{M}^{-1}; K_m$ and $V_{max}$ for pn-Gluco were $0.63±0.07 \text{mM}$ and $1.46±0.06 \text{nmol/min}$, respectively; $K_m$ and $V_{max}$ for pn-Fuco were $2.2±0.2\text{mM}$ and $2.6±0.06 \text{nmol/min}$, respectively. As expected, due to its thermal stability, we were not able to calculate the bgliTm $T_m$. In conclusion the $K_{sv}$ parameters indicate that tryptophan residues of bgliB and bgliTm are similarly exposed. Moreover bgliTm has higher specificity for both substrate and it exhibits higher thermal stability.

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