Production of βglB – ACBM and βglB – BCBM, chimeric proteins presenting "beta-glucosidase" and "cellulose binding" domains

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Introduction

- Cellobiose is an inhibitor product of celluloses' catalytic degradation process carried by cellulases. These inhibitors act in the surface of cellulose crystal.
- Wild - type beta-glucosidases can decrease the presence of cellobioses in the reactional system.
- Chimeric beta-glucosidases presenting a cellulose binding domain (CBD) can act directly in the surface of cellulose fibers, accelerating this process even more.
- Therefore, industries which processes depend on cellulose degradation (ethanol industries, for example) can be interested in those types of reactions.

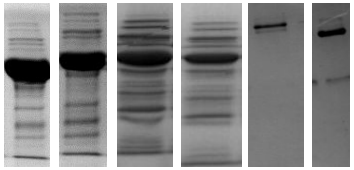
Main goals

- Express and purify chimeric enzymes βglACBM and βglBCBM (chimeric forms).
- Verify catalytic efficiency compared to their respective wild - type ones

Methodology

- Expression was carried with pLate51 vector in BL21DE3 bacterias (*E.coli)*
- Purification was carried in Ni-NTA agarose resin, with additional ionic exchange chromatography (IEC) step for the chimeric ones.
- Activity assays were carried using Avicel as substrate, cellobiohydrolase (CBHI) and the purified enzymes. Negative controls of CBHI and enzymes were also carried..
- DNS was used for reductive power measurement, whereas TGO was used for glucose measurement using absorbance as reference.

Experimental results



βglA/βgl ACBM	15,06
βglB/βgl BCBM	11,07

Image 1: SDS- Page gels: βglA βglB ; βglACBM before IEC; βglBCBD before IECa; βglACBM after IECa; βglBCBM after IEC Table 1: Enzymatic activity relation between wild - types and their respective chimeric ones

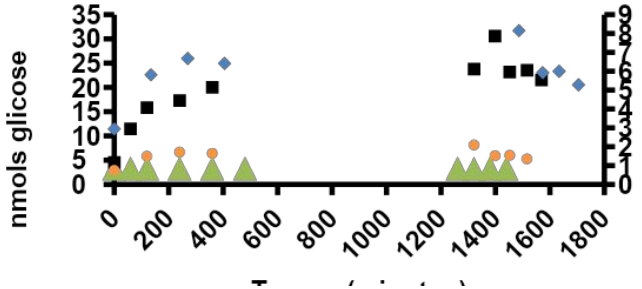


Image 2: Production of glucose using cellulose as substract for βglA + CBHI (blue), βglACBD + CBHI (orange), βglB + CBHI (black) e βglBCBD + CBHI (green). O left axis refers to βglA ; the right axis, to βglB.

Conclusions

- Chimeric enzymes expression was succeeded, something that cannot be affirmed for their purification results.
- More studies are necessary for a real comparison between wild type enzymes and their respective chimerics..
- βglA presented higher values of activity than βglB .