CLONING, EXPRESSION AND PURIFICATION OF CARBOHYDRATE BINDING MODULE IN *PICHIA PASTORIS*

Functionalization of biomedical materials using a carbohydrate binding domain

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The enzymes responsible for carbohydrate degradation are, usually, compose of two distinct modules: catalytic and a substrate binding module. Since these two modules are functionally independent, the CBMs (carbohydrate binding modules) can be fused with bioactive molecules to drive them to starch based biomaterials. In this work, the CBM cloned belongs to human phosphatase laforin, which is involved in metabolism of the glycogen. Aiming at the optimization of large scale expression, CBM peptide production was done by cloning CBM coding sequence in two different systems of *Pichia pastoris*: pGAPZα C which has a constitutive promoter and pPICZα C which has an inductive promoter. Both expression systems have the secretion signal α- factor. The integration of the CBM coding sequence, in yeast genome and the gene transcription were confirmed by slot-blot and northern-blot, respectively. The fermentation conditions for different *P. pastoris* clones were optimized and recombinant protein was purified from fermentation medium by affinity chromatography. Purified protein was analysed by western-blot. The CBM was obtained at low expression level and its starch affinity was not confirmed. Glycosilation of the expressed protein may explain the reduced functionality. Studies are underway to confirm this hypothesis.

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