Molecular Microbiology

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CLONING AND EXPRESSION OF CLOSTRIDIUM THERMOCELLUM CBM3 IN PICHIA PASTORIS

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Cellulose-binding modules (CBMs) have been used to improve the surface/interface properties of cellulose fibers. Glycosylation in fungal CBMs has been suggested essential for the modification of paper pulps properties. In a previous work, the CBM3 from the bacterium *Clostridium thermocellum* scaffolding protein (CipA) was expressed in *Escherichia coli* and conjugated with polyethylene glycol (PEG) to mimetize glycosylation [1]. Recombinant CBM3-PEG conjugate improved the drainability of *Eucalyptus globulus* and *Pinus sylvestris* pulps, but not recombinant CBM3 alone (i.e. non-conjugated CBM3), without affecting the physical properties of the papersheets [1]. Nevertheless, the role of glycosylation on CBMs activity still needs to be elucidated.

The aim of this work is to produce glycosylated CBM3 in the methylotrofic *Pichia pastoris* KM71H yeast strain and to study the effect of the glycans of recombinant CBM3 on the surface properties of cellulose fibers. Two versions of the *cbm*3 gene, both containing codons in preference in *P. pastoris*, were cloned into the pPICZαA plasmid: one with three native potential N-glycosylation sites (N12; N68; N124), and the other with no potential N-glycosylation sites (amino-acid substitutions: N12Q; N68Q; N124Q), to serve as control [2]. pPICZαA is an integrative vector that contains the strong methanol inducible *AOX*1 promoter and the *Saccharomyces* α-factor preprosequence to direct the recombinant protein into the secretory pathway. The integration of the CBM3 coding sequences in the yeast genome was carried out by electroporation and confirmed by colony PCR. Multi-copy recombinants were selected in YPD plates containing 1000 µg/ml Zeocin. Induction was conducted in shake-flasks at 30 °C and 200 rpm, using buffered minimal medium supplemented daily with 0.5% (v/v) methanol, during 3 days. The expression of the recombinant proteins in the culture supernatants was analyzed by SDS-PAGE. The non-glycosylated version of recombinant CBM3 presented a band with its calculated molecular weight (18 kDa), while the putative glycosylated version presented a band with higher molecular weight. N-Glycosylation was confirmed by the reduction of this molecular weight to 18 kDa after digestion with Endoglycosidase H. Both recombinant proteins showed high affinity for cellulose in Avicel-binding assays. The characterization and application of the glycosylated recombinant CBM3 for the modification of pulp and paper properties are now being conducted.

Acknowledgments: This work was financially supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal, through Project GlycoCBMs (ref.: PTDC/AGR-FOR/3090/2012 – FCOMP-01-0124-FEDER-027948).

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