Influence of N-glycosylation in The Secretion and Functional Properties of *Aspergillus nidulans* β-xylosidase AN8401

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Introduction Lignocellulosic biomass is one of the greatest biotechnological potential nowadays; it is mainly composed by cellulose, hemicellulose and lignin. A wide range of enzymes is necessary for the bioconversion of lignocellulosic biomass to fermentable sugars and high valuable compounds. Filamentous fungi are the main targets for enzymes prospection due to the great enzymatic repertoire and the high protein secretion. *Aspergillus nidulans* stands out to be a model of filamentous fungi for genetics and cellular biology studies. It is estimated that more than two-thirds of eukaryotic enzymes are N-glycosylated, so undergoes co- or post-translational changes, which attaches an oligosaccharide to nascent protein. N-glycosylation is related to folding, intracellular trafficking, secretion and biophysics properties of glycoproteins. The attachment of oligosaccharide occurs into asparagine present in a consensus sequence, Asn-X-Ser/Thr. Furthermore, the position and number of glycans attached to protein influences the secretion and functional properties. Therefore, the validation of N-glycosylation sites becomes an important approach to the study of proteins secretion in heterologous systems. **Objectives** In order to investigate the influence of N-glycosylation in the secretion of proteins in *A. nidulans* a β-xylosidase (AN8401) was selected. **Results and Discussion** β-xylosidases (EC 3.2.1.37) are glycoside hydrolases that assist the plant biomass degradation releasing xylose from hemicellulose. AN8401 was selected from *A. nidulans* glycoproteomics data after induction by xylan and sugarcane bagasse. In a first step five N-glycosylation sites were validated in AN8401 by LC-MS/MS. We designed a glycomutant by the deletion of these five N-glycosylation sites by site-directed mutagenesis and intriguingly the absence of these sites did not interfere in the secretion process. This mutant will be further evaluated for functional properties and enzymatic kinetics parameters. **Conclusion** The knowledge and modulation of *A. nidulans* N-glycosylation process is a strategy that can allow the improvement of heterologous expression and secretion of target proteins from different organisms.

Key words: β-xylosidase; N-glycosylation; *Aspergillus nidulans*.

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