SECRETION OF HETEROLOGOUS GLYCOPROTEINS IN ASPERGILLUS: EFFECT OF GLYCOSYLATION PATTERN IN FUNCTIONAL PARAMETERS OF GLYCOSYL HYDROLASES

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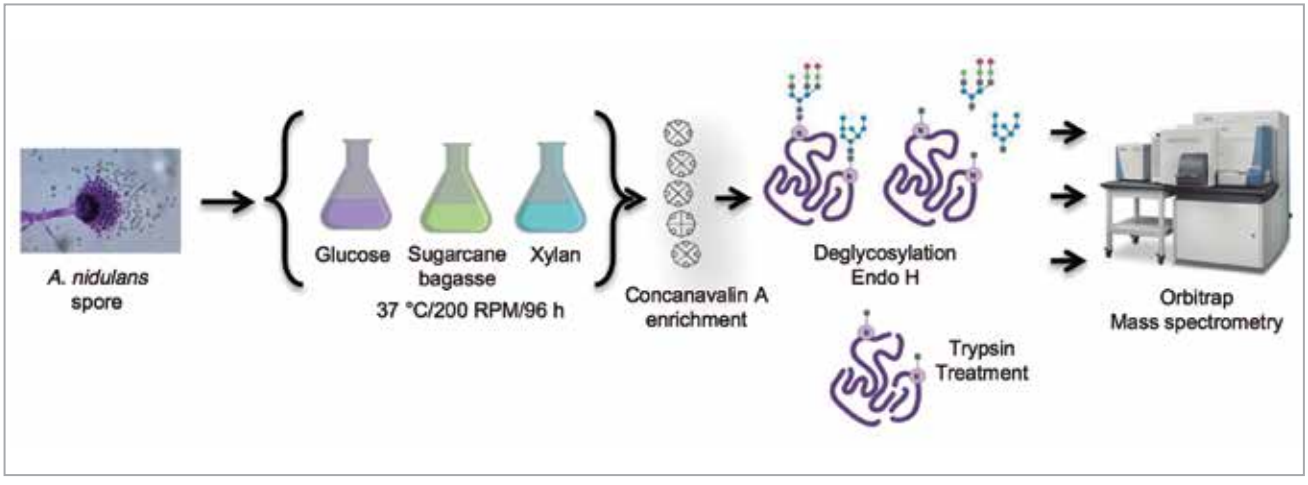

Figure 1. Glycoproteomics pipeline. This approach was applied to the analysis of total secretome of A. nidulans grown in glucose, xylan and sugarcane bagasse. The aim is to identify N-glycosylation sites in Carbohydrate-Active Enzymes (CAZymes) studying possible patterns. Comprehensive analysis of protein glycosylation processes in A. nidulans will assist in a better understanding of glycoprotein structures, profiles, activities and functions.

The use of renewable sources in obtaining fuel becomes an important alternative, by generating fewer pollutants and allows the sustainable development of economy and human society. Alternatively, the use of lignocellulosic biomass, mainly composed of cellulose, hemicellulose and lignin, is a consensus in worldwide, since it is the most abundant renewable energy source in the Earth.

Due to the complexity of plant biomass, which is rich in glycoconjugates, oligo- and polysaccharides, a wide variety of enzymes should act in conjunction to degrade this type of biomasses. The Carbohydrate-Active enZymes (CAZymes) participate in breakdown, biosynthesis or modification of the plant cell wall compounds. In general, CAZymes are structurally constituted by a catalytic domain and some families have an additional carbohydrate-binding module (CBMs). Based on structural and functional features, the CAZy database currently cover five enzyme classes such as glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs).

Heterologous protein expression in filamentous fungi shows interesting advantages compared to other hosts. First, filamentous fungi secrete proteins in large quantities what results in high production levels and facilitates their purification and characterization. Second, most genes from fungi have introns and these organisms are able to recognize and process them correctly. Finally, several fungal proteins are glycosylated, and the protein expression in another filamentous fungus results in a glycosylation pattern that is similar to that of the native fungal enzyme of interest.

We will apply proteomic and transcriptomic approaches to understand how the *A. nidulans* adapts to the high expression and secretion of heterologous proteins by global analysis. We compared four strains of *A. nidulans*, with wild type, an empty plasmid-transformed strain and two heterologous strains producing a GH51 arabinofuranosidase (AbfA) and GH7 celobiohydrolase (CbhI) cloned from *A. fumigatus*. Moreover, there are few studies mapping the global N-glycosylation of CAZymes in filamentous fungi. In this study we will map the profile of N-glycoproteins in *A. nidulans* secretomes trying to understand the patterns of N-glycosylation in CAZymes. The most common and frequent N-glycosylated motifs, an overview of CAZymes glycosylation and the number of mannoses found in N-glycans will be analyzed with this data. Comprehensive analysis of protein glycosylation processes in *A. nidulans* will assist in a better understanding of glycoprotein structures, profiles, activities and functions. This knowledge can allow the optimization of heterologous expression and protein secretion using *A. nidulans* as a model host.

MAIN PUBLICATIONS

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