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Profiling and structural analysis of *Ashbya gossypii* N-glycans

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Ashbya gossypii is a filamentous hemiascomycete used for the industrial production of riboflavin (vitamin B2) that has recently been explored as a new fungal host system for the expression and secretion of proteins. However, the secretory pathway of *A. gossypii* remains poorly characterized, although evidences indicate that the level of secretion in *A. gossypii* is more similar to yeast than to other filamentous fungi; and that recombinant glycoproteins produced by *A. gossypii* are less extensively glycosylated than those produced by *Saccharomyces cerevisiae*. A better understanding of the *A. gossypii* glycobiology offers a basis to characterize its protein secretory processes and may possibly be of practical impact in the production of heterologous proteins.

As a starting point on the elucidation of *A. gossypii* N-glycosylation, in this study we performed a global analysis of whole mixtures of N-glycans attached to *A. gossypii* native secreted glycoproteins. Through combined analysis of N-glycan spectra obtained by MALDI-TOF mass spectrometry and ¹H NMR spectroscopy we report here that the major N-glycan species derived from *A. gossypii* secreted proteins are high-mannose type glycans containing core-type structures (Man8-9GluNac2). Smaller core structures (Man5-7GluNac2) are also present in lower abundance and N-glycans with long outer chains are only present in minor relative amounts. All identified acidic N-glycans contain a phosphate residue. Among the most abundant

acidic structures are short phosphorylated outer chain glycans (PhMan13-15GluNac2).

Our data present the first report on the composition, size distribution and structural characteristics of *A. gossypii* N-glycans.

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