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N-glycan profiling of proteins secreted by *Ashbya gossypii* in different culture conditions**Tatiana Quinta Aguiar¹, Merja Penttilä², Lucília Domingues¹**¹IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Portugal; ²VTT Technical Research Centre of Finland, Espoo, Finland

The filamentous hemiascomycete *Ashbya gossypii*, used for the industrial production of riboflavin (vitamin B₂), has recently been explored as a new fungal host system for the expression and secretion of proteins. Therefore, the interest in the carbohydrate structures (glycans) attached to the glycoproteins produced by this fungus has been raised. Currently almost no information is available on the size distribution and structural characteristics of the *N*-glycans of *A. gossypii* derived glycoproteins. From a phylogenetic point a view, *A. gossypii* is more closely related to yeast than to other filamentous fungi, nevertheless, expression of heterologous glycoproteins in *A. gossypii* has indicated that the recombinant glycoproteins obtained appear to be less extensively glycosylated than those from *Saccharomyces cerevisiae*. A better understanding of the *N*-glycosylation profile of *A. gossypii*'s native proteins may possibly be of practical impact in the production of heterologous proteins. Therefore, in this study, using MALDI-TOF mass spectrometric profiling, we have examined the *N*-glycans present in *A. gossypii*'s native glycoproteins secreted under different culture conditions. *N*-glycan profiling revealed that the major glycan species derived from *A. gossypii*'s secreted proteins are high-mannose type glycans containing core-type structures with eight to eleven mannoses (Man₈₋₁₁GlucNac₂). Smaller structures (Man₅₋₇GlucNac₂) were also present in lower abundance and only minor relative amount of glycans were shown to have structures larger than Man₁₂GlucNac₂. Growth in defined minimal medium also resulted in charged glycan structures that were slightly bigger (Man₁₃₋₁₅GlucNac₂) and phosphorylated. In contrast, no charged glycans could be detected when complex rich medium was used, implying that small glycan species (Man₅₋₁₂GlucNac₂) may be the general feature of *N*-glycans of the secretory pathway in *A. gossypii* when growing in these conditions.

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