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Poster Presentation

N-glycosylation differences between wild-type and recombinant strains affect catalytic properties of two model enzymes: β-glucosidase and phosphatase

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Background

Glycosylation is involved in many proteins' properties and functions such as conformation, thermostability, solubility, protection against proteolysis, intra-cellular migration, secretion. Surexpression and production of proteins by yeasts already constitute a fabulous technological tool but some *in vivo* dysfunctions are frequently reported (half-life time, allergy...) [1].

Study of recombinant glycoproteins glycosylation is then of crucial importance: not only for better understanding of the relationship between the glycoprotein properties and its oligosaccharides but also for being able to orientate the glycosylation process of a given microorganism.

This study compares N-glycosylation of two model enzymes, β-glucosidase and phosphatase, between wild-type yeast strains and heterologous hosts, *Pichia pastoris* and *Schizosaccharomyces pombe*. It also tries to correlate glycosylation differences to enzyme catalytic properties and to distinguish which part of glycosylation could be attributed to the nature of microorganism or to the glycoprotein itself (sequence, number of glycosylation sites...).

Results

Gene sequences analysis assumes 11 potential N-glycosylation sites for the β-glucosidase and 9 for the phosphatase.

Glycans part is around 30% of total molecular weight for the native and recombinant phosphatase while β-glucosidase shows quantitative glycosylation differences since it represents 45% of total molecular weight for the native form and 30% for the recombinant β-glucosidase.

Monosaccahrides composition and oligosaccharides type and branching were studied by capillary electrophoresis for both proteins [2].

Mannose appears as the major component of N-oligosaccharides from wild-type and *Pichia pastoris* strains while *Schizosaccharomyces pombe* N-oligosaccharides composition dispays a larger range of monosaccharides such as ribose and galactose [3]. Trace of N-acetylglucosamine are also encountered in all proteins.

N-oligosaccharides profiles (polymerisation degree) are different between strains and proteins. Nevertheless, for a given protein, oligosaccharide structure (type of linkages between monosaccharides units) and composition are markedly the same between wild-type strain and *Pichia pastoris* whereas *Schizosaccharomyces pombe* exhibits higher chains and different linkages.

Those observed N-glycosylation differences affect enzyme catalytic properties, mainly thermostability.

Conclusion

Schizosaccharomyces pombe appears as a singular heterologous host since for both proteins expressed its glycosylation is widely different from the one of wild-type and *Pichia pastoris* strains. The nature of the microorganism itself seems to be of major importance for determining monosaccharides composition while the gene sequence and culture conditions affect mainly oligosaccharides profiles.

References

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