**INTRODUCTION**

The recombinant production of glycosylated proteins is one of the main limitations of yeast expression systems. Glycosylation, especially N-glycosylation, is very important in multiple aspects like correct folding, half-life, immunogenicity, and therapeutic protein functionality. Because of this, the next study is focused on N-glycosylation type reproduced by *Pichia pastoris*. *Phycocyanin* incorporates less mannos-type glycans on the protein structure and has some advantages compared with animal cell culture. One of them is its larger potential production scale.

**OBJECTIVE**

Reproduce the mammalian glycosylation pattern in a glycoengineered yeast. For this purpose, it is necessary to use a reporter protein with a terminal sialylated site.

**RESULTS**

**Engineered glycosylation pathway**

[Diagram showing the pathway of engineered glycosylation in yeast, including the manipulation of the reporter gene's glycosylation sites and the introduction of sialylation at the terminus.]

**METHODOLOGY**

**Main technique: combinatorial genetic libraries**

- Genetic library searching for yeast’s transmembrane domain
- Genetic library searching for mammalian’s catalytic domain
- Primer design for bp deletion and restriction sites
- PCR and digestion by A and B restriction enzymes
- PCR and digestion by B and C restriction enzymes
- Termination marker
- Auxotrophy marker
- Expression vector
- Screening by optimized localization and activity
- Multiple combinations of genetic elements
- LIKE LEGO!

**Promoters and expression vectors**

- **Promoter**
  - GADPH: Constitutive promoter
  - AOX1: Methanol inducible promoter
  - PMA1: Constitutive promoter

- **Expression vectors**
  - pJN329
  - pKD53, pTC53, pRCD54

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Features</th>
<th>Expression vectors</th>
<th>Origin</th>
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<tbody>
<tr>
<td>GADPH</td>
<td>Constitutive promoter</td>
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<td>pJN329</td>
<td>PCR-2.1-TOPO</td>
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<td>Mannose</td>
<td>D. melanogaster: Drosofila melanogaster</td>
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<tr>
<td>N-acetylglucosamine</td>
<td>D. melanogaster: Rattus norvegicus</td>
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<td>Galactose</td>
<td>M. musculus: Homo sapiens</td>
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<tr>
<td>Sialic acid</td>
<td>M. musculus: Mus musculus</td>
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**DISCUSSION AND CONCLUSIONS**

A glycoengineered strain of *Pichia pastoris* is obtained in this study by serial integrations of expression vectors in the genome. These vectors express chimeric proteins which make possible to obtain erythropoietin with the desired glycosylation pattern. Therefore, synthetic and metabolic engineering provide an efficient and easy method for this purpose. The robust platform developed could give rise to a great large scale production system. Even so, industry level new has nearly come to a standstill since 2006. More studies to resolve the bottlenecks of the system are needed.

**References**