Expressing heterologous cellulases in oleaginous yeast *Yarrowia lipolytica*: Bottlenecks and opportunities

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Outline & Introduction

Recently, *Yarrowia lipolytica* has been shown to have the potential to become one of the model oleaginous yeasts for the development of biofuels, due to the availability of its genome sequence, a reliable genetic transformation system, and the ability to use a range of substrates that include glycerol and industrial fats. However, so far only sugars and agro-industrial wastes have been used to culture these microorganisms, and the use of these carbon-sources inevitably increases the cost of biofuel production.

Initial efforts in expressing Trichoderma reesei CBHII and EGII in *Yarrowia* were successful. However, the expression of *T. reesei* CBH in *Yarrowia* only led to limited enzymatic activity. The aim of this study is to express other types of heterologous CBHs in *Y. lipolytica* and examine their activities and potential for enabling utilization of cellulose, both in vitro and *in vivo*, by the combination of *Yarrowia* CBHII, CBHII, and EGII transformants and their expressed cellulases. More importantly, the bottlenecks and opportunities for co-expressing these cellulase genes in *Yarrowia* to enable utilization of cellulosic substrates were discussed, including the possible roles of promoters and secretion in the control of the cellulase expression.

Materials and Methods

**Y. lipolytica strain**

Fig. 1. Morphology of *Y. lipolytica* Po1g on YPD medium (A) and after being disturbed using deionized and distilled water (B).

**Approaches**

Fig. 2. Diagram for experimental approaches

**Transformation of Yarrowia**

- Selection vector pYLSCL
- *Y. lipolytica* cells were cultured in YPD pH 4.0 broth.
- The transformation of *Y. lipolytica* with the plasmid constructs above was conducted using YLOS One step Transformation system.

CBH, CBHII and EGII expression

**Constructs for expressing cellulases in Yarrowia**

Fig. 3. Domain architecture of endoglucanase (EG) and cellobiohydrolases (CBH) expressed in *Y. lipolytica*.

- Western blot for CBH, CBHII and EGII transformants

Fig. 4. SDS-PAGE and Western blot analysis of *Y. lipolytica* transformants expressing the Trichoderma reesei - Talaromyces emersonii chimeric CBHII, Trichoderma reesei CBHII and EGII.

- Specific Avicelase activity & deglycosylation of purified Tr-Te chimeric CBH

Fig. 5. Specific Avicelase activity of chimeric vs. native *T. reesei* CBH.

- Deglycosylation of chimeric vs. native *T. reesei* CBH

Fig. 6. Deglycosylation of chimeric vs. native *T. reesei* CBH

Consortia culture system of *Yarrowia* transformants

**Avicel cellulose utilization by co-cultures of chimeric CBH, CBHII, and EGII transformants**

Fig. 7. Morphology of co-culturing *Y. lipolytica* transformants expressing heterologous cellulases on mineral medium containing Avicel (2.7% w/v) as sole C source.

**Lipid production by co-culturing of *Y. lipolytica* transformants on Avicel**

Table 1. Co-culturing (120-h) of *Y. lipolytica* transformants expressing heterologous cellulases in 150 mL mineral medium containing 4 g Avicel (equivalent to 2.7% w/v) as sole C source.

<table>
<thead>
<tr>
<th>Cellulose</th>
<th>Glucose</th>
<th>Ethanol</th>
<th>Lipid yield (mg/L)</th>
<th>TPH (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Y. lipolytica</em></td>
<td>1.5</td>
<td>0.5</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td><em>Y. lipolytica</em> CBH</td>
<td>1.5</td>
<td>0.5</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td><em>Y. lipolytica</em> CBHII</td>
<td>1.5</td>
<td>0.5</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td><em>Y. lipolytica</em> EGII</td>
<td>1.5</td>
<td>0.5</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td><em>Y. lipolytica</em> CBH + CBHII</td>
<td>1.5</td>
<td>0.5</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td><em>Y. lipolytica</em> CBH + EGII</td>
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<td><em>Y. lipolytica</em> CBHII + EGII</td>
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</tr>
<tr>
<td><em>Y. lipolytica</em> CBH + CBHII + EGII</td>
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<td>30</td>
<td>24</td>
</tr>
</tbody>
</table>

Challenges and opportunities

- In this study, the secretion yield for chimeric CBH, EGII, and CBHII proteins by *Yarrowia* transformants was between 20 and 40 mg/L, much lower than that in model species *T. reesei*.
- The secretion level for individual cellulases may decrease for co-expressing them.
- To further increase the expression level of CBH and CBHII, we will
  - explore the use of more efficient promoters
  - screen for more efficient signal peptides
  - test different media and growth conditions

Summary

- The results demonstrated the first case of successful expression of a chimeric CBH with essentially full native activity in *Y. lipolytica*.
- It supports the notion that *Y. lipolytica* strains can be genetically engineered, ultimately by heterologous expression of fungal cellulases and other enzymes, to directly convert lignocellulosic substrates to biofuels.