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 fat wastes. 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 successfully. However, the expression of *T. reesei* CBHI in *Yarrowia* only led to limited enzymatic activity. **The aim of this study** is to express other types of heterologous CBHIs 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* CBHI, 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 cellulolytic substrates were discussed, including the possible roles of promoters and secretion in the control of the cellulase expression.

Materials and Methods

❑ ***Y. lipolytic* strain**

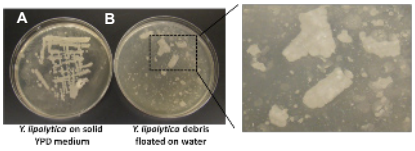

Fig. 1. Morphology of *Y. lipolytic* Po1g on YPD medium (**A**) and after being disturbed using deionized and distilled water (**B**).

❑ ***Approaches***

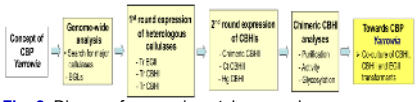

Fig. 2. Diagram for experimental approaches

❑ **Transformation of *Yarrowia***

- Secretion vector pYLSC1
- *Y. lipolytica* cells was 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.

CBHI, CBHII and EGII expression

Constructs for expressing cellulases in Yarrowia

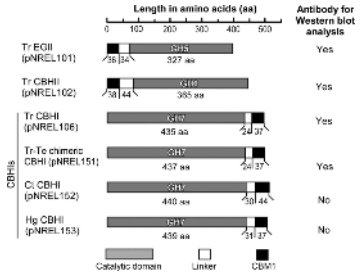

Fig. 3. Domain architecture of endoglucanase (EG) and cellobiohydrolases (CBH) expressed in *Y. lipolytica*.

Western blot for CBHI, CBHII and EGII transformants

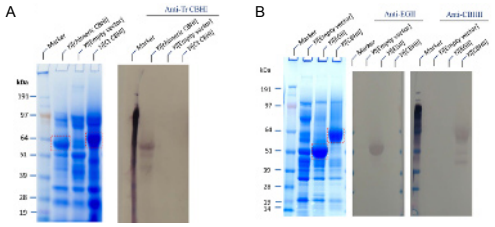

Fig. 4. SDS-PAGE and Western blot analysis of *Y. lipolytica* transformants expressing the *Trichoderma reesei* - *Talaromyces emersonii* chimeric CBHI, *Trichoderma reesei* CBHII and EGII.

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

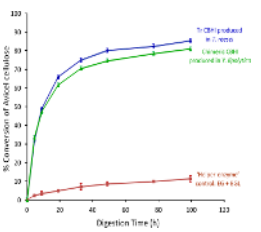

Fig. 5. Specific Avicelase activity of chimeric vs. native *T. reesei* CBHI

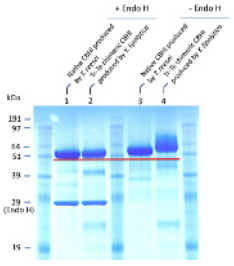

Fig. 6. Deglycosylation of chimeric vs. native *T. reesei* CBHI

Consortia culture system of Yarrowia transformants

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

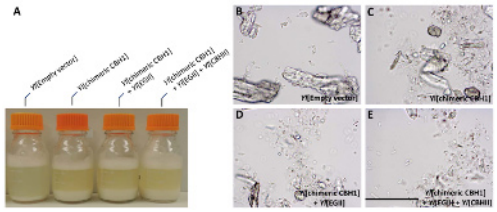

Fig. 7. Morphology of co-culturing *Y. lipolytica* transformants expressing heterologous cellulases on mineral medium containing Avicel (2.7% w/v) as sole carbohydrates.

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.

Culture of transformants *Y. lipolytica* (enzyme)	DW of Avicel-cell pellet g	Avicel consumed%	DW of cells g	Cell mass yield g DW of cells per g Avicel consumed	Total FAME mg in whole pellet
[empty vector] control	4.02	0	0.02	0	N/A
[chimeric CBHI]	3.63	12.0	0.11	0.23	16.3
[chimeric CBHI] + [EGI]	3.41	20.7	0.24	0.29	24.1
[chimeric CBHI] + [EGI] + [CBHII]	3.36	23.5	0.30	0.32	28.2

Challenges and opportunities

- In this study, the secretion yield for chimeric CBHI, 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 CBHI 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 CBHI 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.

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