Ameliorating the metabolic burden of the co-expression of secreted fungal cellulases in a high lipid-accumulating *Yarrowia lipolytica* strain by medium C/N ratio and a chemical chaperone

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Supplementary Materials and Methods

Nucleotide sequences of the cellulase expression cassesttes of construct 162.

Note that the nucleotide sequence ((length: 7780 bp) was codon-optimized based on the codon bias of *Y. lipolytica*, with the SalI site (GTCGAC) and PmlI site (CACGTG) at the 5' end, and the KpnI site (GGTACC) at the 3' end for cloning into the vector pUC57 and pYLEX (see the Methods section for details).

>pNREL162_construct_CBH I-CBH II-EG II

GTCGACCACGTGAGAGACCGGGTTGGCGGCGCATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAAT
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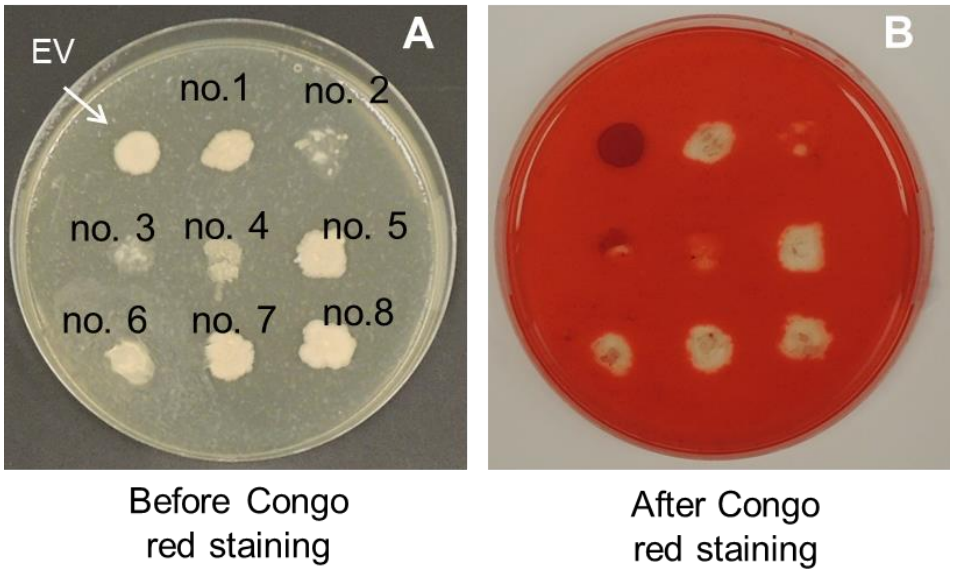
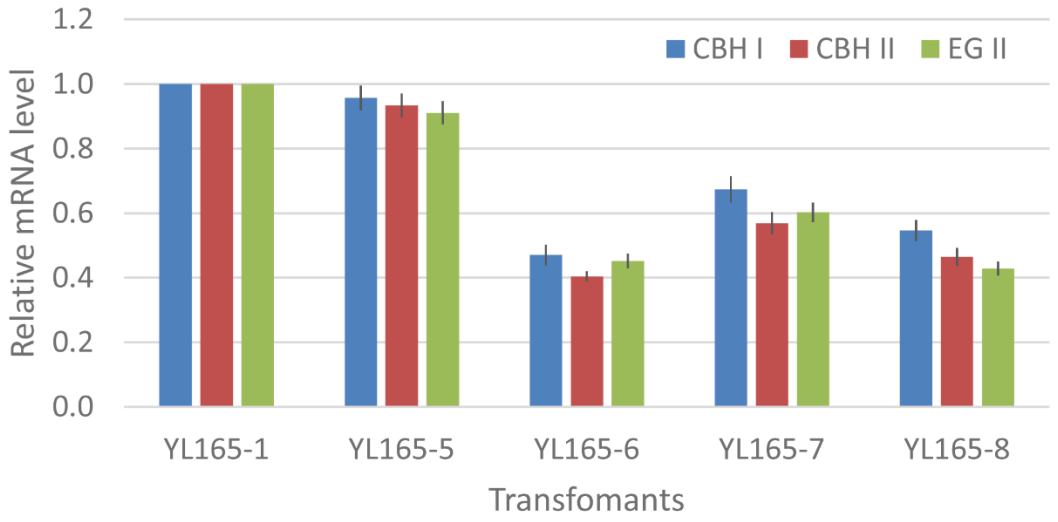
ATCCCTTGGTTTGTTCGTTATACGGTGTACAAGTATGTACGATCGGTACC,

Supplementary Table S1. **Primer sequences for real-time RT PCR.**

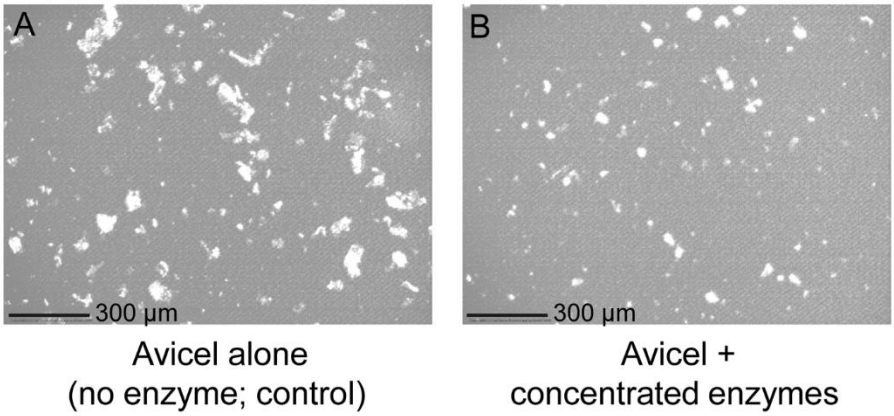
Target genes	Primer sequences	Amplicon size (bp)
Actin	Yl-Actin-1-F: TCCAGGCCGTCCTCTCCC Yl-Actin-1-R: GGCCAGCCATATCGAGTCGCA	141
chimeric CBH I	CBH I-2-F: TTCACCGCATCTAACCCACC CBH I-2-R: GGTAGTTCCAGACGCACACA	159
CBH II	CBH II-1-F: GACCCCACTCATGGAGCAAA CBH II-1-R: TTTGTACTTGGCGACCCCTC	166
EG II	EG II-3-F: TGACCATCTTCCGACTCCCT EG II-3-R: ACGATGCAGTAAGCACCCAA	136



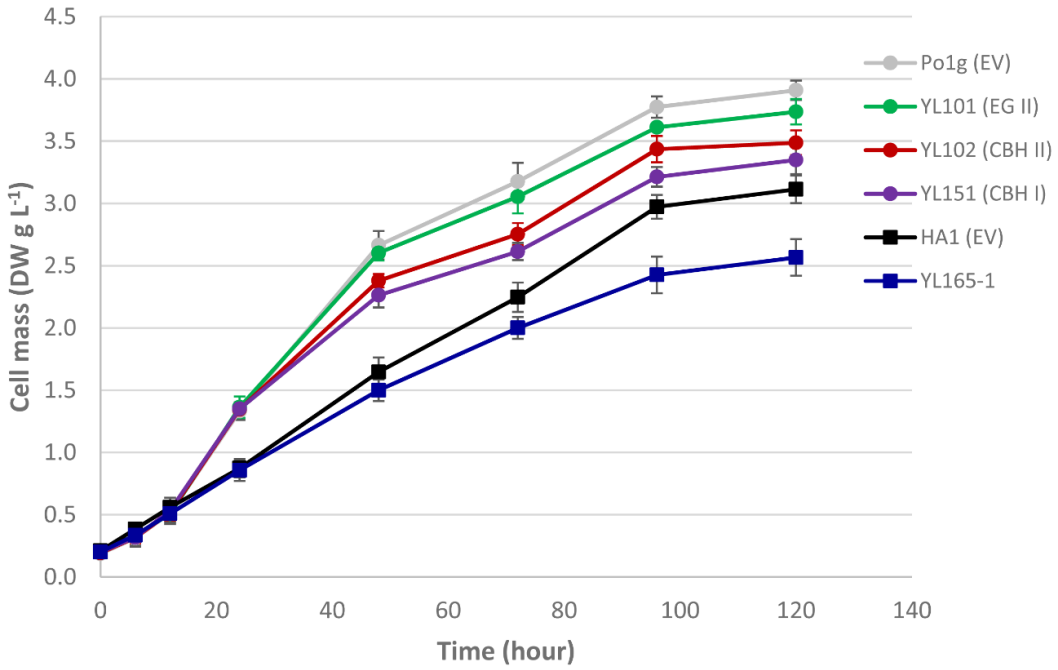
Supplementary Figure S1. Growth of *Y. lipolytica* transformants co-expressing CBH I, CBH II, and EG II on PASC-YPD agar plate. Eight transformants were grown on PASC-YPD agar plate (6 d) before (**A**) and after the Congo Red staining (**B**). EV, parent strain transformed with empty vector.



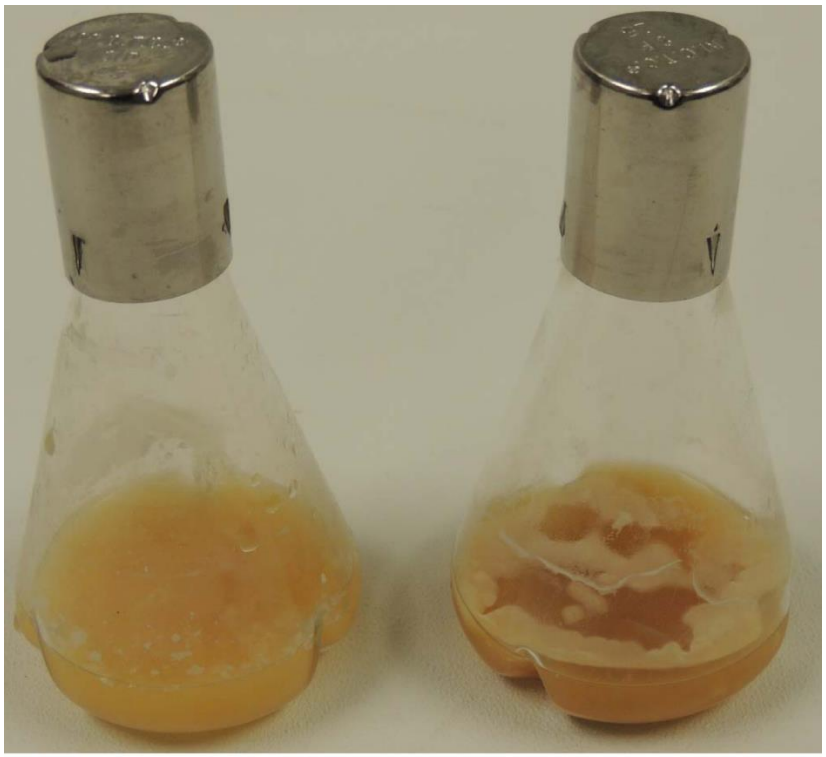
Supplementary Figure S2. Transcript levels relative to YL165-1 for CBH I-CBH II-EG II co-expressed in *Y. lipolytica* transformants YL165-1, -5, -6, -7, and -8. Data from three biological replicates, with error bars indicating the standard error of the mean (SEM).



Supplementary Figure S3. Effects of concentrated crude enzymes from transformant YL165-1 on the morphology of wet ball-dispersed Avicel particles. Morphological comparison of wet ball-dispersed Avicel granules after 5 d incubation with no enzymes **(A)** versus with 35x concentrated crude enzymes of *Y. lipolytica* strain YL165-1 **(B)**. The scale bar represents 300 µm.



Supplementary Figure S4: The timeline profiling of cell mass weight of *Y. lipolytica* transformants expressing multiple cellulases and the control strain HA1 (EV) in shake flasks.



Supplementary Figure S5. Floating cell mats of *Y. lipolytica* HA1 (EV) and transformant YL165-1. The cells were cultured in YPD-3% glucose medium in 250-mL baffled flasks at 200 rpm, 28°C for 6 d.