

**Consolidated bioprocessing of corn cob-derived hemicellulose: engineered industrial  
*Saccharomyces cerevisiae* as efficient whole cell biocatalysts**

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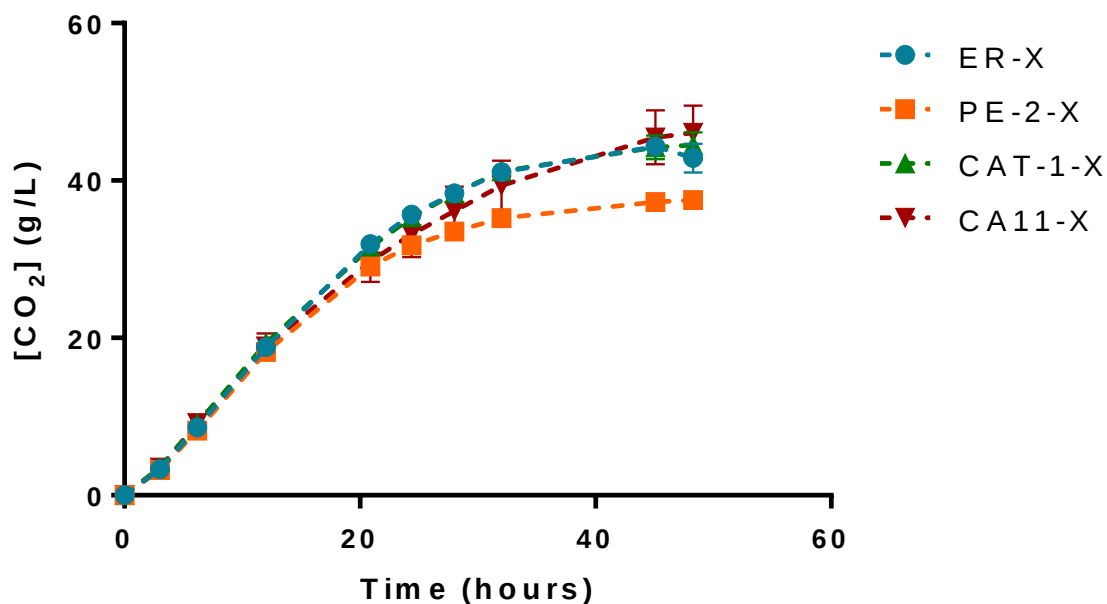
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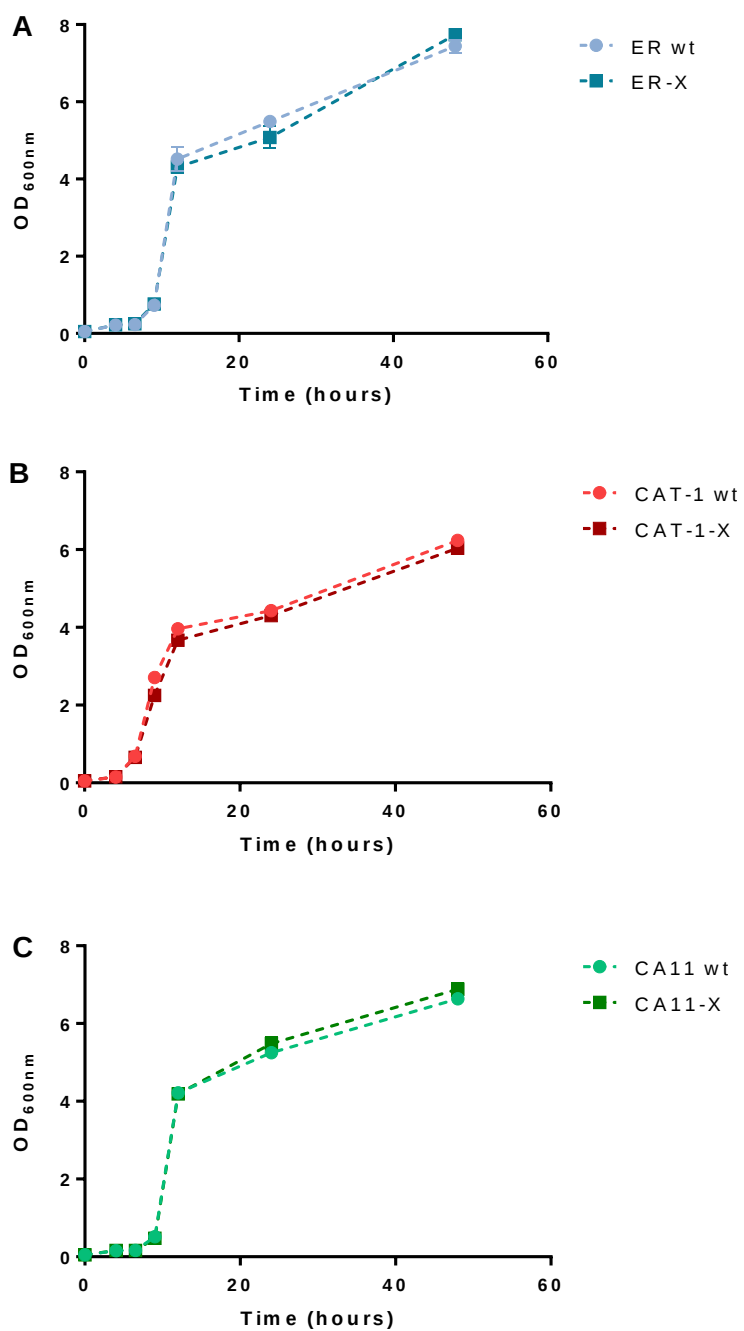
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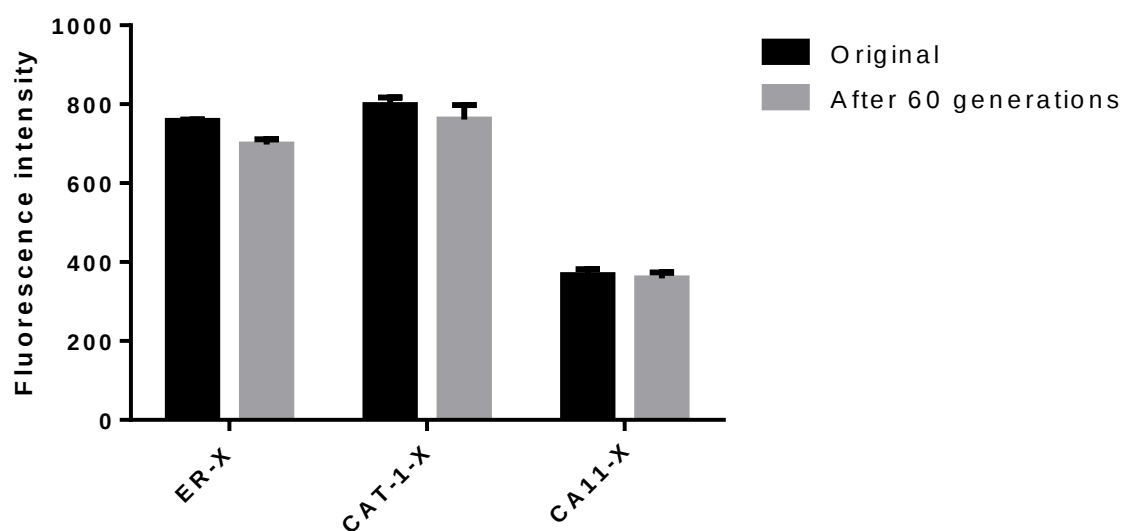
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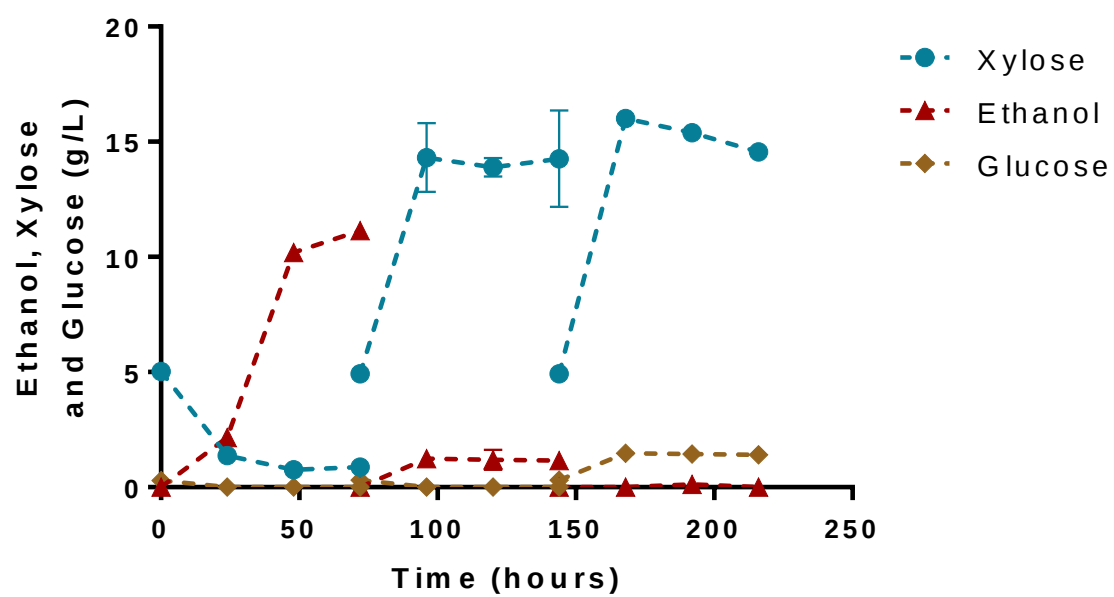
**Fig. S1.** Fermentation in corn cob liquor 29X<sub>Pot</sub> (75 %) supplemented with 100 g/L glucose at 40 °C to evaluate the metabolic capacity of the different strains at higher temperature. Cells were inoculated at 10 g wet cells/L in flasks fitted with glycerol lock. The fermentation was performed at 40 °C and 150 rpm for 48 hours and was monitored by measuring the reduction of mass loss resulting from CO<sub>2</sub> production. Data represents the average  $\pm$  SD from two biological replicates.



**Fig. S2.** Growth profiles of the wild type *S. cerevisiae* strains, ER, CAT-1 and CA11, and of the strains with cell surface display of hemicellulolytic enzymes, ER-X, CAT-1-X and CA11-X, in YPD. Cells were inoculated at an OD<sub>600nm</sub> of 0.05 and grown for 48 hours at 30 °C and 200 rpm. Data represents the average  $\pm$  SD from two biological replicates.



**Fig. S3.** Stability evaluation of the integration at the  $\delta$ -sequences after growth for 60 generations. Cells were grown in YPD liquid medium for 60 generations. The fluorescence intensity of the cells before and after 60 generations (normalized to an  $OD_{600nm}$  of 0.2) was measured in a microplate fluorometer set at 480 nm (excitation) and 510 nm (emission). Data represents the average  $\pm$  SD from two biological replicates.



**Fig. S4.** Fermentation in corn cob liquor 32X<sub>pot</sub> with ER-X-2P strain and 2 cycles of cell recycling. Data represents the average  $\pm$  SD from two biological replicates.

**Table S1.**  $\beta$ -glucosidase 1 (BGL1),  $\beta$ -xylosidase A (XYLA) and endoxylanase II (XYN) enzymatic activities.

| Strain | BGL1 (U/g DCW) | XYLA (U/g DCW) | XYN (U/g DCW)  |                |
|--------|----------------|----------------|----------------|----------------|
|        |                |                | 30 °C          | 40 °C          |
| ER     | 39.1           | 87.8           | 139 $\pm$ 24   | 263 $\pm$ 2    |
| PE-2   | 71.1           | 155            | 93.1 $\pm$ 2.2 | 162 $\pm$ 1    |
| CAT-1  | 50.6           | 50.2           | 76.4 $\pm$ 3.7 | 140 $\pm$ 2    |
| CA11   | 71.3           | 47.8           | 41.0 $\pm$ 8.0 | 57.9 $\pm$ 1.1 |

**Table S2.** Plasmids and primers used for subcloning steps in this study. Lower case sequences indicate addition of homologous regions for plasmid assembling.

| Primers          | Sequence (5'→3')                                                                      | Aim                                                                                                                                 |
|------------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| pAll_fw          | CGCATCAGGAAATTGTAAACG                                                                 | Amplification of pIBG-SSA for construction of pI23-BGL1-kanMX; Amplification of pIU5-TeCBH1c-SSS for construction of pI5-CBH1-NatMX |
| pBGL1_CBH2_rv    | CGTCAGGTGGCACTTTTC                                                                    | Amplification of pIBG-SSA for construction of pI23-BGL1-kanMX                                                                       |
| I23-BGL1_fw      | <u>aagtgcac</u> ctgacgACAGAGAAGGACAAGGCTGAAG                                          | Amplification of intergenic region 23 from ER for construction of pI23-BGL1-kanMX                                                   |
| I23-BGL1_rv      | <u>cgacctgcagc</u> gtacGACACAGGTGACAATAAAGTTTCC                                       | Amplification of intergenic region 23 from ER for construction of pI23-BGL1-kanMX                                                   |
| kanMX-BGL1_fw    | GTACGCTGCAGGTCGACAAC                                                                  | Amplification of <i>kanMX</i> resistance marker for construction of pI23-BGL1-kanMX                                                 |
| kanMX-BGL1_rv    | <u>caatttcctgatgcg</u> ATAGGCCACTAGTGGATCTGATATC                                      | Amplification of <i>kanMX</i> resistance marker for construction of pI23-BGL1-kanMX                                                 |
| I23_FW           | ATAATGAGTTCCGAGTCTGTTGGTG                                                             | Confirm integration in intergenic region 23                                                                                         |
| I23_RV           | CGAGATAAGGCATGGGGTTCTG                                                                | Confirm integration in intergenic region 23                                                                                         |
| pI5-CBH1_rv      | CAGGTTGTGCTCACTGTATATAGTC                                                             | Amplification of pIU5-TeCBH1c-SSS for construction of pI5-CBH1-NatMX                                                                |
| Nat-pI5-CBH1_fw  | <u>agtgcacaca</u> acctgGACATGGAGGCCCAGAATAC                                           | Amplification of <i>natMX</i> resistance marker for construction of pI5-CBH1-NatMX                                                  |
| Nat-pI5-CBH1_rv  | <u>caatttcctgatgcg</u> CAGTATAGCGACCAGCATTC                                           | Amplification of <i>natMX</i> resistance marker for construction of pI5-CBH1-NatMX                                                  |
| pI5-NatMX_fw     | TGCTGGCGTTTTTCCATAG                                                                   | Amplification of pI5-CBH1-NatMX for construction of pI5-XylA-NatMX                                                                  |
| pI5-NatMX_rv     | CAGCCTGAATGGCGAATG                                                                    | Amplification of pI5-CBH1-NatMX for construction of pI5-XylA-NatMX                                                                  |
| XylA_fw          | <u>tcgccattcaggctg</u> CTTCGCTATTACGCCAGATTG                                          | Amplification of pIK-BX-SSS for construction of pI5-XylA-NatMX                                                                      |
| XylA_rv          | <u>ggaaaaaacgccagca</u> CGAATTGGGTACCTTTGATTATG                                       | Amplification of pIK-BX-SSS for construction of pI5-XylA-NatMX                                                                      |
| H-73             | TCTCTCTTGCACCAGCCATT                                                                  | Confirm integration in intergenic region I5                                                                                         |
| H-75             | CGGAATCGCATCAGGTCTT                                                                   | Confirm integration in intergenic region I5                                                                                         |
| pδW_fw           | CTGAGAAATGGGTGAATGTTGAG                                                               | Amplification of pδW-EX-SSS for construction of pδW-XYN-kanMX                                                                       |
| pδW_rv           | CGGGGGATCCACTAGTTCTAG                                                                 | Amplification of pδW-EX-SSS for construction of pδW-XYN-kanMX                                                                       |
| kanMX-UkG1_fw    | ctagtggatcccccgGACACACAAAATATCCCTCCTACTC                                              | Amplification of kanMX-UkG1 cassette for construction of pδW-XYN-kanMX                                                              |
| kanMX-UkG1_rv    | tcaccatttctcagGGTGTGACAACCCTTAATATAACTTC                                              | Amplification of kanMX-UkG1 cassette for construction of pδW-XYN-kanMX                                                              |
| pCRE_fw          | CCATCTTGCACTTCAATAGCATATC                                                             | Amplification of pBF3060 for construction of pCRE-hyg6                                                                              |
| pCRE_rv          | GTATGAGTATTCAACATTTCGGTGTC                                                            | Amplification of pBF3060 for construction of pCRE-hyg6                                                                              |
| hyg6_fw          | <u>gttgaatactc</u> atacGACATGGAGGCCCAGAATAC                                           | Amplification of <i>hyg6</i> resistance marker for construction of pCRE-hyg6                                                        |
| hyg6_rv          | <u>tgaagtgc</u> aagatggCAGTATAGCGACCAGCATTAC                                          | Amplification of <i>hyg6</i> resistance marker for construction of pCRE-hyg6                                                        |
| Plasmids         | Relevant features                                                                     | Source                                                                                                                              |
| pIBG-SSA         | SED1p–SED1ss– <i>A. aculeatus</i> BGL1–SAG1a–SAG1t, <i>HIS3</i>                       | [1]                                                                                                                                 |
| pIU5-TeCBH1c-SSS | SED1p–SED1ss– <i>T. emersonii</i> CBH1–SAG1a–SAG1t, <i>URA3</i>                       | [2]                                                                                                                                 |
| pI5-CBH1-NatMX   | SED1p–SED1ss– <i>T. emersonii</i> CBH1–SAG1a–SAG1t, <i>NatMX</i>                      | This work                                                                                                                           |
| pIK-BX-SSS       | SED1p–SED1ss– <i>A. oryzae</i> XYLA–SED1a–SAG1t, <i>LYS2</i>                          | [3]                                                                                                                                 |
| pδW-EX-SSS       | SED1p–SED1ss– <i>T. reesei</i> XYNII–SED1a–SAG1t, <i>TRP1</i> , $\delta$ -integration | [3]                                                                                                                                 |
| pBF3060          | CRE recombinase, <i>URA3</i>                                                          | [4]                                                                                                                                 |

## References

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