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Production of 3-hydroxypropionic acid from glucose and xylose by metabolically engineered *Saccharomyces cerevisiae*

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A B S T R A C T

Biomass, the most abundant carbon source on the planet, may in the future become the primary feedstock for production of fuels and chemicals, replacing fossil feedstocks. This will, however, require development of cell factories that can convert both C6 and C5 sugars present in lignocellulosic biomass into the products of interest. We engineered *Saccharomyces cerevisiae* for production of 3-hydroxypropionic acid (3HP), a potential building block for acrylates, from glucose and xylose. We introduced the 3HP biosynthetic pathways via malonyl-CoA or β-alanine intermediates into a xylose-consuming yeast. Using controlled fed-batch cultivation, we obtained 7.37 ± 0.17 g 3HP L⁻¹ in 120 hours with an overall yield of 29 ± 1% Cmol 3HP Cmol⁻¹ xylose. This study is the first demonstration of the potential of using *S. cerevisiae* for production of 3HP from the biomass sugar xylose.

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2. Materials and methods

2.1. Strains and chemicals

The xylose utilizing S. cerevisiae strain was described before (Scalcinati et al., 2012). Recombinant yeast strains were selected and maintained on synthetic drop-out agar without tryptophan and with 2% xylose as the sole carbon source. Chemicals were purchased from Sigma-Aldrich. 3HP was purchased from Tokyo Chemical Industry Co. (TCI). Pfu Turbo DNA polymerase was from Agilent Technologies Inc.

2.2. Strain construction

The yeast strains and plasmids are listed in Table 1. The primers and biobricks are listed in Supplementary Tables 1 and 2, respectively. The xylose-consuming CMB.GS010 strain was made auxotrophic for uracil, histidine and leucine as following. First, the ORF of URA3 gene was replaced with the KanMX cassette using homologous recombination. As the KanMX cassette is flanked by LoxP sites, the KanMX cassette was removed by Cre-LoxP-mediated selection marker loop-out as described previously (Jensen et al., 2014a). Next, the ORF of LEU2 and HIS3 genes were replaced by the URA3 from Kluuyveromyces lactis (KIURA3) and KanMX cassettes, respectively. Finally, the KIURA3 and KanMX cassettes were looped-out using Cre-LoxP system to generate the final strain ST2488. The yeast transformations were performed using the lithium acetate protocol (Gietz and Schiestl, 2007). The elimination of the selection markers was verified by PCR in addition to phenotypic test. To construct 3HP-producing strains, expression vectors carrying the genes involved in 3HP biosynthesis were linearized with NotI and then transformed into ST2488. The transformants were selected on synthetic drop-out xylose medium without uracil, histidine, leucine and tryptophan.

2.3. Cultivation of yeast

For testing 3HP production, the strains were cultivated in mineral medium with 20 g L⁻¹ glucose or xylose as carbon-source in 96-deep well plate as described earlier (Borodina et al., 2015).

2.4. Analysis of biomass and metabolites

The growth was measured by optical density at 600 nm and cell dry weight. Extracellular metabolites, such as 3HP, glucose, xylose, ethanol, acetate, glycerol, and succinate, were analyzed by HPLC as reported in (Borodina et al., 2015).

3. Results and discussion

3.1. Engineering 3HP pathways into the xylose-consuming yeast

The xylose consuming yeast CMB.GS010 was constructed previously by transforming CEN.PK 113–3C with the centromeric vector pRS314-X123 carrying xylose reductase (XR), xylitol dehydrogenase (XDH) and xylulokinase (XK) from Pichia stipitis under control of strong constitutive promoter TDH3 and evolving the resulting strain on mineral medium with xylose as the sole carbon source (Scalcinati et al., 2012). The evolved strain had an increased containing 50 g L⁻¹ of xylose, 5 g L⁻¹ of (NH₄)₂SO₄, 3 g L⁻¹ of KH₂PO₄, 0.5 g L⁻¹ of MgSO₄ · 7H₂O, 0.05 mL of antifoam, 1 mL of a vitamin solution and 1 mL of a trace metal solution. The medium used to prepare the pre-cultures in shake-flasks was the same as above with the following modifications: no antifoam, 7.5 g L⁻¹ of (NH₄)₂SO₄, 14.4 g L⁻¹ of KH₂PO₄ and the pH was adjusted to 6.5 with NaOH before autoclaving. Each fermenter was inoculated with an initial OD₆₀₀ of 0.5 using a pre-culture obtained by cultivating a single colony of the desired strain in 30 mL of mineral medium in a 100 mL shake-flask at 200 rpm in an orbital shaker kept at 30 °C. The batch and fed-batch fermentations were performed in 2.7-L DASGIP Bioreactors (DASGIP, Jülich, Germany). The working volume for batch fermentations was 1 L, the temperature set-point was controlled at 30 °C, the airflow was set at 1vvm (gas volume flow per unit of liquid volume per minute), the pH was maintained at 5 by feedback controlled addition of 10% NH₄OH, the dissolved oxygen was kept above 30% of saturation by feedback control of the stirring speed from 600 rpm until a maximum of 1200 rpm. The concentration of O₂ and CO₂ in exhaust gas was monitored by a DASGIP GA4 exhaust analyzer. The fed-batch cultures were initiated as batch cultures using 20 g L⁻¹ xylose. An exponential feeding rate of xylose was designed to keep the growth rate at 0.03 h⁻¹.

Table 1

| Strains | Genotype | Reference |
|---------|----------|-----------|
| CEN.PK 113-7D | MATα URA3 HIS3 LEU2 TRPI MAL2-8′ SUC2 | Euroscarf |
| CMB.GS010 | MATα URA3 HIS3 LEU2 TRPI1 MAL2-8′ SUC2 | Euroscarf |
| ST2488 | CMB.GS010 Trp1 ura3A his3A leu2A | This study |
| ST2546 | ST2488/pCB380/ pCB382/ pCB474 | This study |
| ST2547 | ST2488/pCB743/ pCB800/ pCB799 | This study |
| ST2808 | ST2488/pCB743/ pCB801/ pCB799 | This study |

The three pre-cultures were grown under different conditions: (ST1) evolved for grown on xylose, (ST2) with centromeric URA3 and (ST3) under control of strong constitutive promoter TDH3.
maximal specific growth rate compared with the non-evolved parent strain, i.e. 0.18 h\(^{-1}\) compared with 0.02 h\(^{-1}\), and showed a 15-fold increase in the xylose consumption rate. As the CMB.GS010 strain has no selectable markers available for further genetic modifications, three auxotrophic mutations (\(\text{ura3}^{\Delta}\), \(\text{leu2}^{\Delta}\) and \(\text{his3}^{\Delta}\)) were made in strain CMB.GS010 to obtain strain ST2488.

In ST2488, we introduced two different pathways for 3HP production, one via malonyl-CoA (P-I) and another via \(\beta\)-alanine (P-II) (Jensen et al., 2014a; Borodina et al., 2015) (Fig. 1). The strain with P-I (ST2546) carried overexpression cassettes for 5 genes: malonyl-CoA reductase from \(\text{Chloroflexus aurantiacus}\) MCR, phosphorlation insensitive variant of acetyl-CoA carboxylase \(\text{ACC1}^{S659A, S1157A}\) (\(\text{ACC1}^{**}\)), aldehyde dehydrogenase (ALD6), acetyl-CoA synthase from \(\text{Salmonella enterica}\) (\(\text{SEACS}\)), and pyruvate decarboxylase (\(\text{PDC1}\)). MCR and \(\text{ACC1}^{**}\) were integrated into TY4 retrotransposon regions using degradation-tagged URA3 selection marker as described in (Borodina et al., 2015) to ensure multiple integration events. The pathway P-II either used a NADH-dependent route (P-IIa) or a NADPH-dependent route (P-IIb). The strain with P-IIa (ST2808) carried over-expression cassettes for 5 genes: pyruvate carboxylase (\(\text{PYC1, PYC2}\)), \(\beta\)-alanine-pyruvate aminotransferase from \(\text{Bacillus cereus}\) (\(\text{BcBAPAT}\)), aspartate 1-decarboxylase from \(\text{Triboleum castanium}\) (\(\text{TcPAND}\)), and NADH-dependent 3-hydroxyisobutyrate dehydrogenase (HIBADH) from \(\text{Pseudomonas putida}\) (\(\text{PpHIBADH}\)). In the strain with P-IIb (ST2547) the last gene was replaced by NADPH-dependent 3-hydroxypropionate dehydrogenase (HPDH) from \(\text{E. coli}\) (\(\text{EcYdfG}\)). In the strains with P-IIa and P-IIb pathways, all the genes were integrated into the genome in a single copy, with exception of \(\text{TcPAND}\) gene, which was inserted into TY4 regions. Because the resulting transformants may have different copy numbers of the vectors integrating into different TY4 regions, we chose to screen 15 transformants of each strain for 3HP production on glucose and xylose. The average titer on glucose was highest for the strain with P-I (1.44 ± 0.14 g L\(^{-1}\)), however this strain had a very poor 3HP production on xylose (0.17 ± 0.11 g L\(^{-1}\)) (Fig. 2). The 3HP titers for the P-IIa strain were comparable on glucose and xylose (0.60 ± 0.03 and 0.49 ± 0.16 g L\(^{-1}\)) and the specific yield on xylose was nearly twice as high as that on glucose. The best titer was obtained for strain with P-IIb pathway, 1.00 ± 0.06 g L\(^{-1}\) on glucose and 1.84 ± 0.23 g L\(^{-1}\) on xylose and the specific yield on xylose was nearly 2.7-fold higher than that on glucose. We speculate that the reason for low 3HP production from xylose via P-I is the lack of overflow metabolism (Crabtree effect), where a large part of the carbon is channeled via pyruvate decarboxylase into ethanol. As was shown in a previous study (Borodina et al., 2015), 3HP accumulation largely occurs during the ethanol consumption phase during cultivation on glucose, however only very limited amounts
of ethanol are produced during growth on xylose. The absence of the Crabtree effect had however a positive effect on the 3HP production via the β-alanine pathway, which begins with the anaplerotic reaction from pyruvate to oxaloacetate, and hence competes for pyruvate with pyruvate decarboxylase.

3.2. Production of 3HP in controlled batch and fed-batch reactors

The best 3HP-producing strains ST2547 and ST2808 were fermented in batch mode on mineral medium with xylose as the sole carbon source (Fig. 3). The pH was maintained at 5 during the fermentation. In batch mode about 50 g L$^{-1}$ of xylose was consumed in 140 hours, resulting in 6.09 ± 0.33 g L$^{-1}$ 3HP by ST2547, compared with 2.3 ± 0.09 g L$^{-1}$ 3HP by ST2808. Ethanol concentration did not exceed 1 g L$^{-1}$ at any point of fermentation. ST2547 strain was further characterized in fed-batch mode. In fed-batch mode after 120 hours of fermentation, the 3HP concentration reached 7.37 g L$^{-1}$. In 140 hours, resulting in 6.09 g L$^{-1}$ 3HP by ST2547.

4. Conclusions

3HP production via two different pathways has been established in the evolved xylose-utilizing yeast, where the 3HP pathway via β-alanine resulted in higher product titer on xylose than the pathway via malonyl-CoA. The study lays the basis for development of the yeast strain for producing 3HP from lignocellulosic feedstocks at low pH.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.meteno.2015.10.001.

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Appendix A. Supplementary material

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