Metabolic Engineering of Saccharomyces Cerevisiae: amino acid metabolism for production of products of industrial interest

Saccharomyces cerevisiae is widely used in microbial production of chemicals, metabolites and proteins, mainly because genetic manipulation of S. cerevisiae is relatively easy and experiences from its wide application in the existing industrial fermentations directly benefit new S. cerevisiae-based processes. This study has focused on metabolic engineering of the amino acid metabolism in S. cerevisiae for production of two types of chemicals of industrial interest. The first chemical is δ-(L-α-aminoadipyl)–L-cysteinyl–D-valine (LLD-ACV). ACV belongs to non-ribosomal peptides (NRPs), which are synthesized by specific peptide synthetases and have a broad range of biological and pharmacological properties. Due to the scarcity of the production of NRPs in nature and the difficulties in their chemical synthesis, it was initiated here to develop S. cerevisiae as a platform for microbial production of one type of NRPs – ACV. Production of ACV was achieved by introducing the Penicillium chrysogenum gene pcbAB, which encodes ACV synthetase (ACVS), and the Aspergillus nidulans gene npgA, which encodes phosphopantetheinyl transferase (PPTase) required for activation of ACVS, into S. cerevisiae on a high-copy plasmid. Several possible factors that could improve ACV production were investigated. Lowering the cultivation temperature from 30 to 20 °C led to a 30-fold enhancement. The ACVS and PPTase encoding genes were also integrated into the yeast genome. The second chemical is isobutanol, which is regarded as an important next generation biofuel. As a substitute for liquid fossil fuels, isobutanol is better than ethanol due to its higher energy density, lower hygroscopicity and lower vapor pressure. Isobutanol is also better than its isomer n-butanol due to a higher octane number. In this study, by simultaneous overexpression of biosynthetic genes ILV2, ILV3, and ILV5 in valine metabolism in S. cerevisiae, the isobutanol yield was improved from 0.16 to 0.97 mg per g glucose in anaerobic fermentation in mineral medium. Isobutanol yield was further improved by two times by the additional overexpression of BAT2. Additional overexpression of ILV6 in the ILV2 ILV3 ILV5 overexpression strain decreased isobutanol production yield by three times. The stoichiometric genome-scale model of S. cerevisiae was applied to find genetic manipulation targets. The BioOpt software was used for in silico cell metabolism simulation. A reasonable agreement was obtained between the experimental data and the in silico simulation results. Through the application of single gene overexpression and deletion options of BioOpt in the overexpression strain ILV2356_XCY605, several target genes are suggested for further overexpression or deletion.

General information
Publication status: Published
Organisations: Department of Systems Biology
Contributors: Chen, X.
Publication date: Jun 2011

Publication information
Place of publication: Kgs. Lyngby, Denmark
Publisher: Technical University of Denmark (DTU)
ISBN (Print): 978-87-9149499-4
Original language: English
Electronic versions:
_XC_phd_thesis.pdf
Source: orbit
Source-ID: 313776