Engineering Conferences International
ECI Digital Archives

Microbial Engineering II | Proceedings

4-3-2022

Pathway design for mixotrophic production of biochemicals from CO2 and methanol in yeasts

Golnaz Memari

Özge Ata

Diethard Mattanovich

Follow this and additional works at: https://dc.engconfintl.org/microbial_ii

PATHWAY DESIGN FOR MIXOTROPHIC PRODUCTION OF BIOCHEMICALS FROM CO_2 AND METHANOL IN YEASTS

Golnaz Memari, Austrian Center for Industrial Biotechnology (acib), University of Natural Resources and Life Sciences (Boku), Austria
golnaz.memari@boku.ac.at
Özge Ata, Austrian Center for Industrial Biotechnology (acib), University of Natural Resources and Life Sciences (Boku), Austria
Diethard Mattanovich, Austrian Center for Industrial Biotechnology (acib), University of Natural Resources and Life Sciences (Boku), Austria

Key Words: *Pichia pastoris, Komagataella phaffii,* Serine Cycle, CO_2 fixation, CRISPR-Cas9

Autotrophy and mixotrophy in industrial strains have the potential to contribute to the mitigation of climate change, and they are therefore of a great interest towards a more sustainable production of biochemicals. In previous studies, functional autotrophy was engineered in yeasts by integrating the Calvin-Benson-Bessham (CBB) cycle in *Pichia pastoris* (*Komagataella phaffii)* (Gassler et al. 2020), and similarly in *Escherichia coli* (Gleizer et al. 2019), allowing growth on CO_2 as the sole carbon source. The autotrophic *P. pastoris* strain uses methanol for energy supply. In this project, we aim to design alternative pathways for assimilation of both CO_2 and methanol for the conversion of CO_2 into value-added compounds. Using methanol in both assimilatory and dissimilatory pathways lowers the overall methanol demand. A blueprint for such a mixotrophic pathway is the bacterial serine cycle and modifications thereof (Yu and Liao 2018).

By using methods for synthetic biology such as Golden Gate Assembly and CRISPR-Cas9, we are aiming to design thermodynamically feasible and energetically favorable pathways for the production of organic acids from a mixed feed of methanol and CO_2. For this purpose, additional enzymatic loops are added to the serine cycle by either introducing bacterial genes or activating native yeast pathways. Functionality of the novel cyclic pathways is assessed by the capacity to produce the target molecules and intermediates from methanol and CO_2.

Gassler T. et al. 2020. The industrial yeast *Pichia pastoris* is converted from a heterotroph into an autotroph capable of growth on CO_2. Nature Biotechnology 38: 210-216.

Gleizer S, Ben-Nissan R, Bar-On YM, Antonovsky N, Noor E, Zohar Y, Jona G, Krieger E, Shamshoum M, Bar-Even A, Milo R. 2019. Conversion of *Escherichia coli* to Generate All Biomass Carbon from CO_2. Cell. 179:1255-1263.e12.

Yu H, Liao JC. 2018. A modified serine cycle in *Escherichia coli* coverts methanol and CO_2 to two-carbon compounds. Nat Commun. 9(1):3992.