4-3-2022

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Diethard Mattanovich

REFACTORING YEAST CENTRAL METABOLISM TO REVERSE GROWTH PHENOTYPES AND PRODUCT FORMATION

Diethard Mattanovich, University of Natural Resources and Life Sciences Vienna (BOKU), Austria
diethard.mattanovich@boku.ac.at

Keywords: synthetic biology, yeast, *Pichia pastoris*, autotrophic growth, Crabtree effect

Re-engineering of growth phenotypes, substrate utilization and product formation in non-model production organisms has been hugely accelerated by deep understanding of their biology, and by the recent development of synthetic biology tools. Working with the methylotrophic yeast *Pichia pastoris* (*Komagataella phaffii*), a host commonly used in the manufacture of industrial enzymes and pharmaceuticals, we have recently engaged in creating synthetic strains that overcome barriers in substrate use and metabolite production.

Like most biotechnological production hosts, *P. pastoris* is heterotrophic, growing on organic feedstocks. By addition of eight heterologous genes and deletion of three native genes, we engineered the peroxisomal methanol-assimilation pathway of *P. pastoris* into a CO_2 fixation pathway resembling the Calvin-Benson-Bassham (CBB) cycle, the predominant natural CO_2 fixation pathway. The resulting strain can grow continuously with CO_2 as a sole carbon source at a μ_{max} of 0.008 h^{-1}. The specific growth rate was further improved to 0.018 h^{-1} by adaptive laboratory evolution. This engineered *P. pastoris* strain may promote sustainability by sequestering the greenhouse gas CO_2 and by avoiding consumption of an organic feedstock with alternative uses in food production. First results towards the production of bulk chemicals will be discussed.

In another approach we have overcome the glycolytic flux limitation due to the Crabtree negative phenotype of *P. pastoris* which is limiting rates and thus productivities for primary metabolites. Natural evolution of the Crabtree positive phenotype is considered having been a complex process involving numerous interconnected events. However, we could show that overexpression of a single Gal4-like transcription factor is sufficient to convert Crabtree-negative *P. pastoris* into a Crabtree positive yeast. Upregulation of the glycolytic genes and a significant increase in glucose uptake rate due to the overexpression of the Gal4-like transcription factor caused an overflow metabolism, triggering both short-term and long-term Crabtree phenotypes. Implications on the production of primary metabolites will be discussed, as well as potential applications of this strain for low alcohol fermented beverages.