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HIDDEN METHANOL ASSIMILATION PATHWAYS IN THE METHYLOTROPHIC YEAST PICHIA PASTORIS

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Pichia pastoris (Komagataella phaffii) is able to grow on methanol as the sole energy and carbon source. Methanol can be electrochemically produced from CO_2 and some alternative methanol assimilation pathways may capture CO_2 as well, which makes a production process more sustainable. Besides the known methanol pathway for yeast, there are other methanol assimilation pathways in nature known for prokaryotes, but not for eukaryotes. These are being investigated within this study for their activity in P. pastoris. Metabolic pathway candidates with CO_2-coassimilation are the bacterial serine cycle or the reductive glycine pathway.

In both pathways, methanol is dissimilated to formate, which enters the tetrahydrofolate pathway, ending up in methylenetetrahydrofolate. Glycine is either de-novo synthesized by the glycine-cleavage system through the reaction of methylenetetrahydrofolate with CO_2 or provided by the serine cycle. Serine is formed by a second methyl group of methylenetetrahydrofolate, which is transferred to glycine by glycine hydroxymethyl transferase. Serine is then used as a precursor for all biomass formation. All enzymes in this pathway leading to serine are present in P. pastoris.

In our study, the known, natural xylulose5-phosphate assimilation pathway was deleted by knocking out DAS1 and DAS2. The knockout strain did not increase in optical density over 20 days of cultivation on methanol. With ^{13}C-methanol labeling and an advanced GC-HRMS metabolomics methodology, we could still prove the uptake of methanol through a hidden methanol assimilation pathway. Based on the GC-HRMS results, we showed that the reductive glycine pathway is the active, hidden methanol assimilation pathway. With additional knockouts within the reductive glycine pathway, we proved that the detected alternative methanol assimilation pathway is indeed the reductive glycine pathway. However, the native flux of the reductive glycine pathway seems to be too low to support cell growth.

To additionally analyze the very early methanol fixation within the tetrahydrofolate cycle, further research is being conducted to develop suitable LC-MS methods and folate extraction procedures.