Optimization of 3-HP biosynthesis in *Saccharomyces cerevisiae* through cofactor balancing and enzyme engineering

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3-Hydroxypropionic acid (3-HP) is a platform chemical of industrial relevance, employed in the production of polymers, solvents, and other high-value products. The yeast Saccharomyces cerevisiae has emerged as an interesting chassis for the bioproduction of 3-HP through the heterologous expression of malonyl-CoA reductase (MCR), an enzyme catalyzing the conversion of malonyl-CoA into 3-HP. Nevertheless, the efficiency of this pathway is often limited by the availability of metabolic precursors and cofactors, such as NADPH and malonyl-CoA, as well as by the catalytic performance of MCR. The most widely adopted MCR variant reported in the literature is an engineered form of the *Chloroflexus aurantiacus* wild-type enzyme, referred to as CaMCR***, that consists in the bifunctional protein split into two independent domains (N- and C-terminal) with three amino acid mutations introduced to enhance its catalytic efficiency. To address these metabolic bottlenecks, *S. cerevisiae* strains were genetically engineered using CRISPR/Cas9 technology to improve the intracellular availability of key cofactors and precursors. The modifications included the genomic integration of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPN), a mutated variant of acetyl-CoA carboxylase (ACC1**), aldehyde dehydrogenase (ALD6), and acetyl-CoA synthetase (ACS), along with the separate integration of the N- and C-terminal domains of CaMCR***, and deletion of the OCA5 gene. Shake flask cultivation of this strain resulted in 2.6 ± 0.53 g/L of 3-HP after 48 hours. The additional copy number of ACC1** could be a key contributor to the improved production, due to its catalytic role in the conversion of acetyl-CoA to malonyl-CoA, an important precursor in the 3-HP pathway. In a complementary study conducted by the group, five novel MCR variants were tested. Among them, the MCR3 variant demonstrated 3-HP levels comparable to CaMCR***. Subsequent mutagenesis of MCR3 resulted in an approximate tenfold increase in 3-HP production. As future perspectives, *S. cerevisiae* strains co-expressing the optimized MCR3 and the previously engineered modifications will be constructed and evaluated for 3-HP production using various carbon sources — glucose, ethanol, and xylose — coupled with specific metabolic engineering strategies for each substrate.

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