Production of 3−hydroxypropionic acid in engineered *Methylobacterium extorquens* AM1and application of CRISPR interference to rapidly mine a new phytoene desaturase for carotenoid biosynthesis

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Methylobacterium extorquens AM1, a facultative methylotrophic α-proteobacterium, is capable of utilizing methanol as the sole carbon and energy source. Here we constructed a malonyl-CoA pathway by heterologously overexpressing the *mcr* gene to convert methanol into an important platform chemical 3-hdroxypropionic acid (3-HP) in *M. extorquens* AM1. The engineered strains demonstrated 3-HP production with initial titer of 6.8 mg/l in shake flask cultivation, which was further improved to 69.8 mg/l by increasing the strength of promoter and *mcr* gene copy number. *In vivo* metabolic analysis showed a significant decrease of the acetyl-CoA pool size in the strain with the highest 3-HP titer, suggesting the supply of acetyl-CoA is a potential bottleneck for further improvement. Notably, 3-HP was rapidly degraded after the transition from exponential phase to stationary phase. Metabolomics analysis showed the accumulation of intracellular 3-hydroxypropionyl-CoA at stationary phase with the addition of 3-HP into the cultured medium, indicating 3-HP was first converted to its CoA derivatives. *In vitro* enzymatic assay and β-alanine pathway dependent ^{13}C-labeling further demonstrated that a reductive route sequentially converted 3-HP-CoA to acrylyl-CoA and propionyl-CoA, with the latter being reassimilated into the ethylmalonyl-CoA pathway. The deletion of the gene META1_4251 encoding a putative acrylyl-CoA reductase led to reduced degradation rate of 3-HP in late stationary phase.

Moreover, we optimized a CRISPRi system that the expression of sgRNA was under control of strong promoter P_{mxaf} and expression of *Streptococcus pyogenes* Cas9-derived dcas9 was under control of promoter $P_{R/tetO}$. This CRISPRi system has been shown to effectively knockdown the expression of exogenous fluorescent protein gene *mcherry* as well as endogenous genes *glyA* and *crtI* in *M. extorquens* AM1. We then used CRISPRi technology in a sgRNAs pool format to mine essential genes involved in biosynthesis of carotenoid. We rapidly identified a novel phytoene desaturase (encoded by META1-3670) involved in carotenoid biosynthesis. The function of this gene was further confirmed by gene deletion and complementation experiments. We then used CRISPRi to interfere the *shc* (encoding for a squalene-hopene cyclase) to channel more flux to enhance the titer of carotenoid without disturbing the cell growth. The sgRNA-1547 can significantly repress the transcriptional level of *shc* by 65% to the control sgRNA, achieving 2.2-fold increase of carotenoid production.

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Research Interests:

Methylotrophs and Methanotrophs

Metabolic Engineering

OMICS analysis

Selected publications

1. Yang et al. Unusual and Highly Bioactive Sesterterpenes Synthesized by Pleurotus ostreatus during Coculture with Trametes robiniophila Murr. 2019, Jul 1;85(14).
2. Yang et al., Metabolic engineering of Methylobacterium extorquens AM1 for the production of butadiene precursor. 2018, Microb Cell Fact. 2018 Dec 20;17(1):194.
3. Yang et al., Production of 3-hydroxypropionic acid in engineered Methylobacterium extorquens AM1 and its reassimilation through a reductive route. Microb Cell Fact. 2017 Oct 30;16(1):179.
4. Yang et al. Comprehensive molecular characterization of Methylobacterium extorquens AM1 adapted for 1-butanol tolerance. Biotechnol Biofuels. 2016 Apr 11;9:84.