A high-throughput strategy to optimize whole-cell biocatalysis in cyanobacteria

Julia Jodlbauer,[a], Christian Waltl[a], Matthias Schmal[b], Florian Rudroff[a]

[a] Institute of Applied Synthetic Chemistry, TU Wien

[b] Institute of Chemical, Environmental and Bioscience Engineering, TU Wien

Getreidemarkt 9/163, 1060 Vienna / Austria

julia.jodlbauer@tuwien.ac.at

Cyanobacteria emerge as promising hosts for whole-cell biocatalysis, offering a rich environment for relevant processes due to their photoautotrophic metabolism, which provides valuable cofactors, reducing power and oxygen. Their unique ability to utilize light energy and CO_2 as a carbon source presents a promising platform for a sustainable production process [1]. Cyanobacteria have primarily been utilized for single-step biotransformations. Recently, also multiple-step biocatalytic cascades were realized in cyanobacteria [2, 3]. However, despite substantial progress in recent years, performance and productivity still need to catch up with the yields achieved with highly established heterotrophic hosts. In cyanobacteria, the overexpression of recombinant enzymes has been a significant limitation for biocatalytic performances.

Motivated by this shortcoming, we developed a strategy to tackle the challenge of insufficient biocatalyst expression. We investigated three enzymes: a ketoreductase LfSDR1M50 [4], an enoate reductase YqjM [5], and a Baeyer-Villiger monooxygenase $CHMO_{mut}$ [6]. By implementing a high-throughput approach, we generated large-scale expression libraries of our biocatalysts based on distinct promoters, ribosome binding sites (RBSs), and a genetic insulator known as RiboJ that were analyzed by using a combination of fluorescence-activated cell sorting (FACS) and deep sequencing.

By investigating the optimized expression strains, we could improve overall biocatalytic activities for all three enzymes. For recombinant Syn. 6803 LfSDR1M50, we achieved up to 39.2 U g_{CDW}^{-1} (17-fold improvement) towards the substrate 4-methylcyclohexanone. Optimized YqjM strains yielded 58.7 U g_{CDW}^{-1} (16-fold increase) towards 2-cyclohexen-1-one, and improved recombinant $CHMO_{mut}$ strains achieved 7.3 U g_{CDW}^{-1} for the production of ε-caprolactone from cyclohexanone (1.5-fold).

Furthermore, with this study we got detailed insights into the performance of distinct expression tools in different genetic contents.

Lastly, we applied the knowledge gained from this study and applied it to co-express the three enzymes LfSDR1M50, YqjM and $CHMO_{mut}$ to establish a 3-step cascade from 2-cyclohexan-1-ol to ε-caprolactone.

In conclusion, this work outlines an overall strategy to improve cyanobacterial whole-cell biocatalysts by optimizing the recombinant gene expression. By enhancing expression levels and understanding tool performance, we pave the way for rational cascade design, addressing key challenges in whole-cell biocatalysis.

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