MetaProcess: How enzyme engineering supports the sustainable synthesis of chiral amino alcohols

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In the past years, our working group has been focusing on the enzymatic production of chiral amino alcohols as precursors for drugs. As showcase, the enzymatic two-step reaction towards metaraminol, an active pharmaceutical ingredient in hypotension treatment, was successfully established. This enzymatic route comprises first the decarboxylation of pyruvate and subsequent carboligation with 3-hydroxy benzaldehyde by the pyruvate decarboxylase of *Acetobacter pasteurianus* (*Ap*PDC). Secondly, the amine transaminase of *Chromobacterium violaceum* (*Cv*ATA) transfers the amino group of L-alanine to the product of the carboligation, (*R*)-3-hydroxy phenylacetylcarbinol ((*R*)-3-OH-PAC), leading to the target product metaraminol [1]. To shift the unfavoured reaction equilibrium towards the product metaraminol, the reductive amination of (*R*)-3-OH-PAC is coupled with an *in situ* liquid-liquid extraction reaching up to 69 % yield after three extraction steps [2].

Although we were able to establish a synthesis route from second generation feedstocks with very good conversions, the applied biocatalysts exhibit low stability against their substrates as well as their products and therefore cannot be reused in the process nor very high product concentration can be achieved. Within our project “MetaProcess”, biotechnologists, bioinformaticians and process engineers work together striving to enhance the operational stability of the applied carboligase as well as amine transaminase. To reach this goal, random mutagenesis and computer-aided rational design are combined to generate more stable enzyme variants. Currently, two promising *Ap*PDC variants derived from the first random mutagenesis round are characterized regarding their stability against 3-hydroxy benzaldehyde. In parallel, the inactivation mechanism is to be elucidated to further evolve the *Ap*PDC by rational design approaches.

In the future, the best performing enzyme variants regarding stability and reusability are implemented into the process building the foundation of a green alternative for the conventional chemical metaraminol production route.

[1] Mack, K., Doeker, M., Grabowski, L., Jupke, A., Rother, D. *Green Chem*., 2021, 23, 4892–4901.

[2] Doeker, M., Grabowski, L., Rother, D., Jupke, A. *Green Chem*., 2022, 24, 295–304.