ENZYMATIC DECARBOXYLATION IN DEEP EUTECTIC SOLVENTS: FROM STABILITY ENGINEERING TO APPLICATION IN CONTINUOUS FLOW

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Enzymatic decarboxylation of bio-based hydroxycinnamic acids gives access to phenolic styrenes for adhesive production. Phenolic acid decarboxylases are proficient enzymes that have been applied in aqueous systems, organic solvents, biphasic systems, and deep eutectic solvents, which makes stability a key feature. Stabilization of the enzyme would increase the total turnover number and thus reduce energy consumption and waste accumulation associated with biocatalyst production. In this study, we used ancestral sequence reconstruction to generate thermostable decarboxylases. Investigation of a set of 16 ancestors resulted in the identification of a variant with an unfolding temperature of 78.1 °C and a half-life time of 45 hours at 60 °C. Crystal structures were determined for three selected ancestors. Structural attributes were calculated to fit different regression models for predicting the thermal stability of variants that have not yet been experimentally explored. As bacterial PADs do not convert sinapic acid, simultaneous saturation mutagenesis was used for an expansion of the substrate scope of the ancestor of the highest stability.

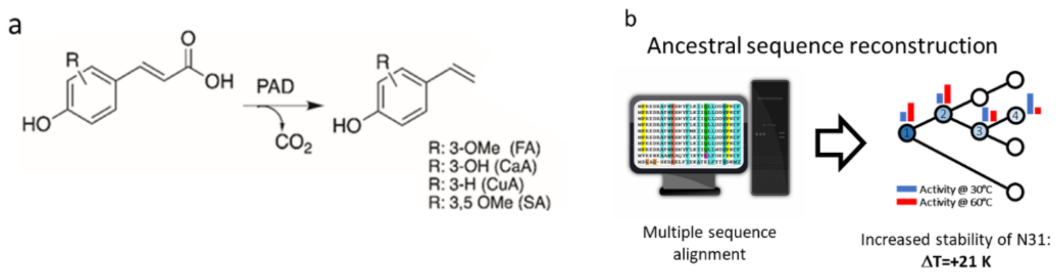

Figure 1: Ancestral sequence reconstruction for the improvement of the stability of phenolic acid decarboxylase.

A further stabilization of the enzyme was achieved by the application of mixtures of natural deep eutectic solvents and buffer. This approach substantially improves productivity, rendering our approach a straightforward option for enhancing the industrial application of the process..

[1] K. Myrtollari, E. Calderini, D. Kracher, S. Galušić, A. Slavica, A. Taden, D. Mokos, A. Schrüfer, G. Wirnsberger, K. Gruber, B. Daniel, R. Kourist (2024), Stability increase of phenolic acid decarboxylase by a combination of protein and solvent engineering unlocks applications at elevated temperatures, *ACS Sust. Chem. Eng.*, *accepted manuscript*