Expanding the potential of phenolic acid decarboxylase from *Bacillus subtilis* by use of non-conventional solvents

Anna K. Schweiger[a], Nicolás Ríos-Lombardía[b], Christoph K. Winkler[c], Sandy Schmidt[a], Francisco Morís[b], Wolfgang Kroutil[c], Javier González-Sabín and Robert Kourist[a]

[a]Institute of Molecular Biotechnology, Graz University of Technology, 8010 Graz, Austria
[b]Entrechem, S.L., Vivero Ciencias de la Salud, 33011 Oviedo, Spain
[c]Department of Chemistry, Organic & Bioorganic Chemistry, University of Graz, 8010 Graz, Austria

Phenolic acid decarboxylase (PAD) from *Bacillus subtilis* is a highly interesting cofactor-free enzyme. Thus, the enzyme only relies on the intrinsic substrate prerequisites to convert coumaric acid derivatives into 4-hydroxy styrenes [1]. Recently, several examples of incorporation of PAD into chemo-enzymatic cascades have highlighted its potential, as alkenes represent ideal targets for further chemical transformations [2]. However, the expansive application of PAD is still hampered by the exceptional low solubility of coumaric acids and their derivatives in aqueous media, which requires elaborate strategies to overcome this bottleneck.

Herein, we report on the activity of wildtype PAD and different enzyme variants in classical aqueous medium and in deep eutectic solvents (*DES*s). Common limitations of aqueous systems, like substrate solubility and product stability, were tackled by use of *DES*s [3]. The exceptional performance of PAD in pure *DES* and *DES*-water mixtures allowed the conversion of substrate loads far beyond solubility limits in buffer (up to 300 mM), and moreover disclosed fascinating differing reactivity patterns depending on the used solvent system.

[1] R. Kourist, J.-K. Guterl, K. Miyamoto, V. Sieber, ChemCatChem 2014, 6, 689–701.

[2] Á. Gómez Baraibar, D. Reichert, C. Mügge, S. Seger, H. Gröger, R. Kourist, Angew. Chemie Int. Ed. 2016, 55, 14823–14827.

[3] V. Gotor-Fernández, C. E. Paul, J. Biotechnol. 2019, 293, 24–35.