ENHANCEMENT OF LIPASE SELECTIVITY BY SITE DIRECTED MUTAGENESIS

Katja Zorn, Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, Greifswald University, Greifswald, Germany
katja.zorn@uni-greifswald.de
Isabel Oroz-Guinea, Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, Greifswald University, Greifswald, Germany
Henrike Brundiek, Enzymicals AG, 17489 Greifswald, Germany.
Uwe T. Bornscheuer, Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, Greifswald University, Greifswald, Germany

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Lipases belong to the α/β-hydrolase fold family and naturally catalyze the hydrolysis of fats and oils into glycerol and fatty acids. This class of enzymes displays numerous features that make them useful biocatalysts, including (i) broad substrate spectrum, (ii) excellent chemo-, regio- and stereoselectivity, (iii) high stability towards harsh reaction conditions, (iv) independence of cofactors and, furthermore, (v) a wide variety of lipases is commercially available. Due to all these advantages lipases have been widely applied in industrial processes such as dairy, baking, and detergent industry. Furthermore, they can be used for the production of trans-fatty acid free margarines and biodiesel [1-3]. However, despite their great applicability, each industrial application needs particular reaction conditions (e.g. substrate selectivity or stability towards temperature, pH and/or organic solvents) that should be borne by the biocatalyst. Therefore, protein engineering can be applied in order to obtain enzymes that meet the required parameters [4].

The present study focuses on the enhancement of lipase selectivity by protein engineering and its application for the enrichment of long chain fatty acids from natural oils, which are interesting building blocks for the chemical industry. Hence, a broad spectrum of commercial lipases was screened to identify those that already displayed the desired selectivity. Furthermore, lipases with interesting structural features were selected from literature as candidates for rational design [5, 6]. The most promising candidates were overexpressed in Pichia pastoris and Escherichia coli and subsequently purified to test their hydrolytic activity towards different p-nitrophenyl fatty acid esters. The best candidate found was subjected to molecular modelling to examine the potential hotspots to perform saturation mutagenesis. Three different amino acids present in the binding pocket were identified, allowing the design and creation of three combinatorial mutant libraries. Once the libraries were transformed into E. coli, the hydrolytic activity of more than 4500 clones was screened by using the fully automatized robotic platform LARA [7]. The most selective variants were chosen and used for confirmation of their activity and selectivity towards both, different chain length p-nitrophenyl fatty acid esters and several oil fractions.

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