Modification of Fatty Acid Selectivity of *Pseudozyma antarctica* Lipase A by Error-prone PCR

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The aim of the experiment was to modify *Pseudozyma antarctica* lipase A (CAL-A) fatty acid selectivity by generating mutant libraries using epPCR.

Mutations were induced by epPCR using the GeneMorphII kit (Stratagene). Modified inserts were cloned into a plasmid vector pETBlue-2 (Novagen) (Tuner™(DE3)p*lac*I expression cells).The screening of lipolytic activity and selectivity towards CLA was performed by using a *p*-nitrophenol assay and analysis of the released fatty acids using gas chromatography, respectively.

Two libraries containing 400 and 600 clones were obtained, with an average and a low mutation rate, respectively. The clone MA39 from the average mutation rate library was selected and compared to the native protein of CAL-A. Lipase MA39 and CAL-A indicated the highest intracellular activity against *p*-NP butyrate and *p*-NP laurate, 5 U/mg; 21 U/mg, and 1 U/mg; 15 U/mg, respectively.

The degree of CLA-TAG hydrolysis increased from 1.1 % for CAL-A to 8.8 % for MA39 lipase. Constant selectivity to *cis*-9, *trans*-11 and *trans*-10, *cis*-12 did not differ among the native protein and the protein after mutation (α=0.01 and α=0.13, respectively).

The commercial enzyme preparation Novozym 735 had a selectivity towards *cis*-9, *trans*-11, *trans*-10, *cis*-12, *cis*-10, *cis*-12 and *trans*-9, *trans*-11 and *trans*-10, *trans*-12, α=0.24, α=0.13, α=0.21 and α=0.01, respectively (degree of CAL-TAG hydrolysis 13.2%).

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