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Enhancing Thermal Stability of *Pseudomonas fluorescens* Esterase I by *in Vivo* selection

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Thermal stability of an enzyme increases its half-life and co-solvent compatibility as well as allows its storage, transport and reuse under non-natural conditions, being thus a desirable property for industrial enzymes. A generalized trend in biotechnological industries is the development of directed evolution platforms -complementary to functional meta genomics- to obtain thermo stable variants of the mesophilic enzymes of interest. Indeed, there is a real need to implement more efficient, simple and inexpensive methods that allow both the screening of thermo stable variants and the detection of thermo stable activities from libraries isolated from thermophilic environments.

In the present study, we have targeted *Pseudomonas fluorescens* Esterase I (E.C. 3.1.1.2; PFEI) for thermo stabilization by directed evolution, using an *in Vivo* thermo selection method previously developed in our group [1]. Transformants of *Thermus thermophilus* were selected by growth at 48 h at 68 ºC and 60 and 80 µg/ml kanamycin. After screening a total of 90,000 clones, 34 active mutants were recovered. These PFEI variants were overproduced in *Escherichia coli* and purified to homogeneity. Thermal stability of PFEI variants was evaluated in terms of dynamic stability (melting temperature; Tm) and kinetic stability (half-life). In addition, kinetic parameters were determined with para-nitrophenyl acetate as model substrate. The most thermostable variant showed a Tm of 77.3±0.1 ºC (4.6 ºC higher than the wild-type) and a half-life of over 13 h at 65 ºC (7.9-fold longer than the wild-type), but unchanged kinetic parameters. Stabilizing mutations were incorporated into a previously reported PFEI variant that showed enantio selective activity towards the (-)-enantiomer of the lactam Vince (2-azabicyclo [2.2.1] hept-5-en-3-one). Finally, molecular modeling studies on the improved thermostable mutants were carried out to investigate the effect that introduced mutations had on the structure and thus, on the overall stability.

[1] Chautard H, Blas-Galindo E, Menguy T, Grand'Moursel L, Cava F, Berenguer J, Delcourt M (2007) Nat Methods 4: 919-921.

[2] Torres LL, Schließmann A, Schmidt M, Silva-Martin N, Hermoso JA, Berenguer J, Borscheuer UT, Hidalgo A (2012) Org Biomol Chem 10: 3388-3392.

Biography:

Diana Maté obtained the Bachelor's degree in Chemistry from the Autonomous University of Madrid in 2008. She did her PhD studies in the Dr. Miguel Alcalde group at the Institute of Catalysis (CSIC, Spain) between 2008 and 2013. Her PhD dealt with the engineering of fungal Laccases for biomedical applications. In June 2013 she received the PhD in Molecular Biosciences from the UAM. In 2014 and 2015 she was a postdoctoral researcher at Prof. Ulrich Schwaneberg group at the DWI-Leibniz Institute for Interactive Materials (Germany). There, she was involved in collaborative projects for the application of sortases in microgel synthesis. In 2016 she rejoined Alcalde group where she worked in a project in collaboration with industry. In 2017 she joined the Department of Molecular Biology of UAM, where she currently works as a researcher and collaborates as lecturer. She is co-author of 20 SCI articles, 4 book chapters and 4 patents.