Directed evolution of a type A feruloyl esterase for increased thermostability and organic solvent tolerance

Julien Durand[1], Victor Chaumeton[1], Olga Gherbovet[1], Mélanie Ragon[1], Peter Jütten[2], Joana L.A. Bras[3], Catarina I.P.D. Guerreiro[3], Carlos M.G.A. Fontes[3], Alexander Piechot[2], Régis Fauré[1], Sophie Bozonnet[1], Michael J. O'Donohue[1]

1- LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France ; 2- Taros Chemicals GmbH & Co. KG, Dortmund, Germany; 3- NZYTech genes & enzymes, Lisboa, Portugal

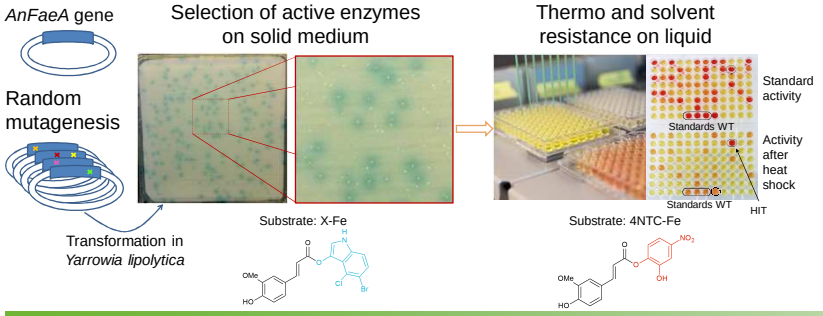
Background

Feruloyl esterases (FAE) are carboxylic esterases (EC 3.1.1) that cleave the bond between ferulic acid and L-arabinofuranosyl residues of arabinoxylans. These enzymes, in addition to helping the deconstruction of lignocellulosic biomass, have a natural capacity to synthesize a wide range of bioactive molecules with interesting properties (notably anti-oxidant) in the fields of food, pharmaceuticals or cosmetics.

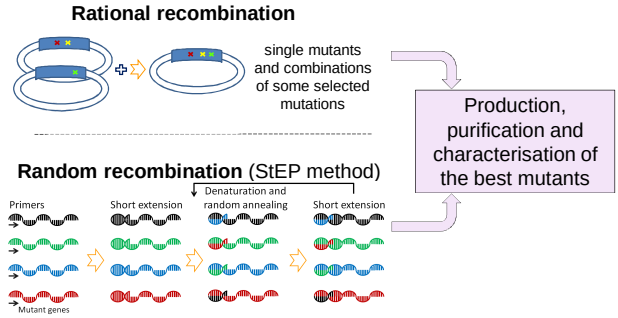
Within the **OPTIBIOCAT European project**, a methodology to express, generate and screen diversity for *Aspergillus niger* feruloyl esterase A was developed, tested and implemented in a high-throughput fashion, using *ad hoc* chromogenic probes [1]. A total of ~10,000 mutant clones were generated and screened to isolate 13 mutants with improved thermal and/or solvent stability. The most interesting mutations were individually studied while their random recombination was performed in a second round of evolution through Staggered Extension Process (StEP) [2].

Experimental workflow

1st round of evolution



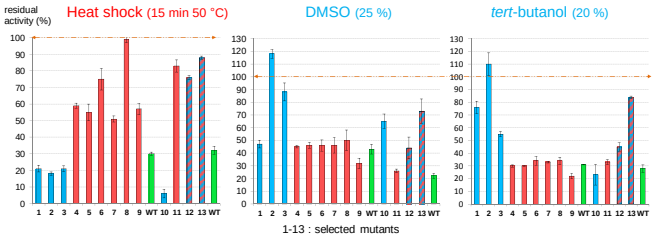
2nd round of evolution



Results

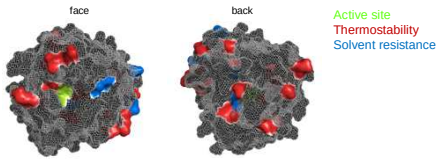
1st round of evolution

ePCR on *AnFaeA* gene



Sequencing of 13 improved mutants

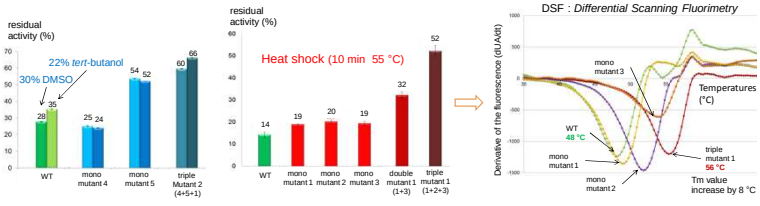
Localisations of the **23 mutations** on *An*FaeA



2nd round of evolution

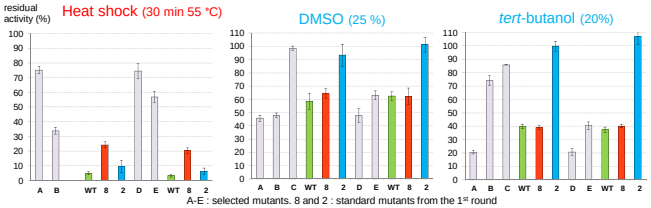
Rational recombination of 7 selected mutations

Residual activity and increase of the Tm values (on purified enzymes)



Random recombination

StEP with the 13 selected mutant genes from 1st round



Conclusions and outlook

This directed evolution work applied to *Aspergillus niger* feruloyl esterase A allowed the identification of mutations conferring:

- better thermostability with a gain of 8 °C of the Tm for the triple mutant 1,
- improved solvent resistance, the triple mutant 2 is twice less affected by the presence of 30% DMSO or 22% of *tert*-butanol than the wild-type enzyme. This mutant is also twice less affected than the wild-type enzyme after a heat shock of 10 minutes at 55 °C which gives to this mutant the dual property of improved thermostability and resistance to solvents.

Beyond the direct applicative interest of these mutant enzymes, the study of their structural modifications will lead to a better fundamental understanding of the mechanisms involved in temperature and solvent resistance for this family of enzymes.

[1] O. Gherbovet *et al.* (2016) *J. Mol. Catal. B: Enzym.* 126:24–31
[2] H. Zhao et W. Zha (2006) *Nat. Protocols*. 1:1865-1871.

This work was supported by the European Union, Grant agreement no: 613868 (OPTIBIOCAT).

LISBP • Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés