Design and Synthesis of a Library of Chromogenic Chemical Entities for the Discovery of New Thermostable Enzymes Relevant for the Chemical Industry

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Introduction

The impact of thermophylic enzymes in the industry

Biotechnology is everywhere, its impact on industries is much bigger than what previously envisioned on several diverse industries, such as feed and food production, biofuel and energy generation as well as sustainable production of high-value chemical compounds. Conditions in an industrial process are often far from standard biocatalyst's properties. Hence, there is considerable demand for a new generation of stable enzymes that are able to reach this goal by replacing or supplementing traditional chemical processes[1].

Extremozymes, enzymes derived from extremophilic microorganisms, are an attractive alternative to perform complex chemical processes because they are adapted to harsh living conditions, such as reactions in non-aqueous environments, water/solvent mixtures, extremely high pressures, acidic and alkaline pH, temperatures up to 140 °C, or near the freezing point of water[2].

One of the main objective of the Marie Curie HOTDROPS project was to look for new thermostable esterases and lipases. Once they were isolated, a specific library of compounds was designed and synthesized in order to screen and characterize their activity.

Objectives

Development of a chromogenic synthetic library

The literature describes several high-throughput enzyme assays that are applied as tools to visualize a particular enzyme class. Several of them were described for the particular case of lipases and esterases and the evaluation of their activities by using fluorescence or chromogenic substrates[3].

Chromogenic substrates are generally more convenient than fluorogenic substrates. Firstly, their reactions can be detected visually, implying that they are suitable for routine activity control without instruments. Secondly, UV-spectrometers are more broadly available than fluorometers[4].

Therefore in this work a library of p-nitrophenol esters was synthesized in order to have a method that allows us a quick characterization of the enzymes.

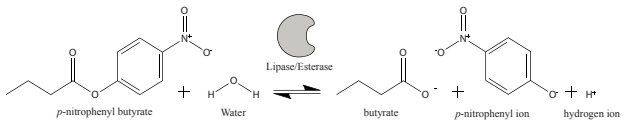


Illustration 1: *p-nitrophenol containing substrates library*

Results and Discussion

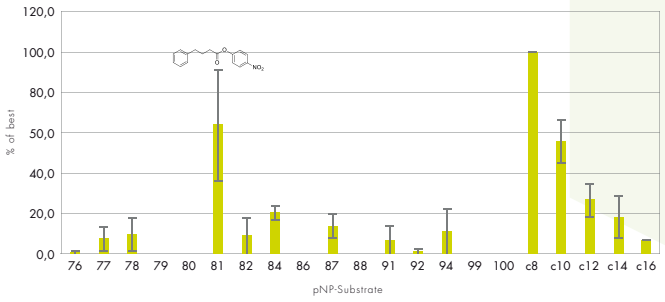


Illustration 2:
Substrate specificity of Klest3S

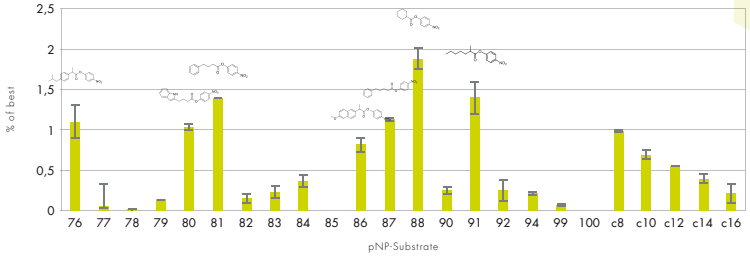
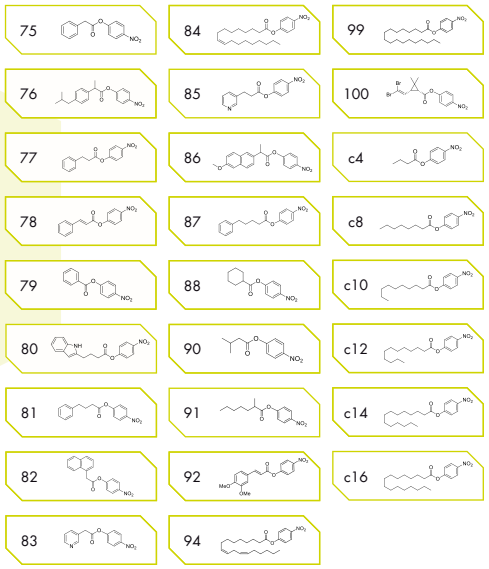


Illustration 3:
Substrate specificity of LipD11



Conclusions

A library of 26 chromogenic esters has been synthesized and applied to the characterization of the enzymes LipD11 and Klest3S. The library includes structural motifs that lie beyond the usual 'classic' fatty acid esters, including aromatic and heteroaromatic rings with a direct industrial application. As summary, the use of this library:

- Allows for a more complete characterization of the enzymes catalytic activity.
- Helps to gather additional data about the substrate specificity of the catalytic site.
- Allows the discover of new substrates for the enzymes with potential industrial applications.
- Shows that novel, different substrates are a useful 'bait' to find new enzymes with affinity for non-classical substrates.

References

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[2] *Current Opinion in Biotechnology* 2013, *17*, 215-220.
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[4] *Tetrahedron: Asymetry* 2004, *15*, 2981-89.

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