High-Throughput characterization of bacterial feruloyl acid esterases

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Background

- Ferulic Acid Esterases (FAEs) represent an appealing group of biocatalysts susceptible to considerable roles for various industrial and medicinal applications. Feruloyl esterases (FAEs, E.C. 3.1.1.73), represent a subclass of the carboxylic acid esterases (E.C. 3.1.1) that catalyze the hydrolysis of the ester linkage of hydroxycinnamic acids (ferulic acid and p-coumaric acid) and diferulates (diFAs) present in plant cell walls. These enzymes have the ability not only to deconstruct plant biomass, but also to synthesize a broad range of novel bioactive components for use in food, cosmetic and pharmaceutical industries.
- Compared with fungal FAEs, bacterial FAEs are a diverse group of enzymes that remain relatively unexplored. Within CAZy (www.cazy.org), FAEs are predominantly found in Carbohydrate Esterase family 1 (CE1). A total of > 4000 sequences of bacterial CE1 FAEs was analyzed to select a subset of 480 enzymes representing the diversity observed within the family for characterization.

Aims

- An High-throughput Gene synthesis, Cloning, Expression and Protein purification platform was used to produce 480 bacterial enzymes representative of CE1 family.
- Enzymes were characterized for substrate activity using different substrates and biochemical conditions.

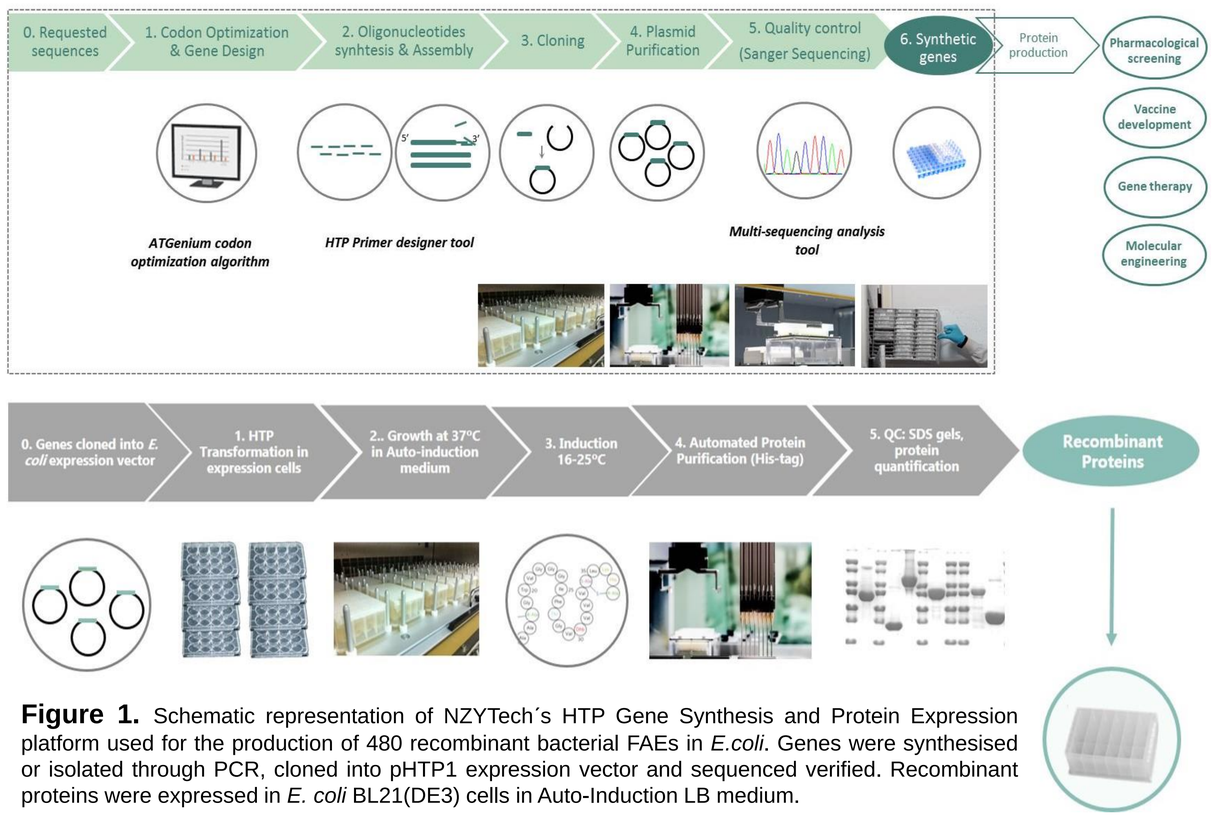
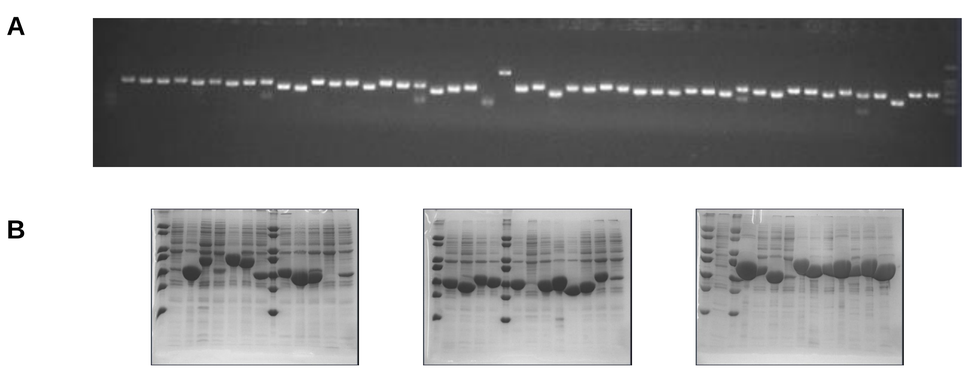


Figure 1. Schematic representation of NZYTech´s HTP Gene Synthesis and Protein Expression platform used for the production of 480 recombinant bacterial FAEs in *E.coli*. Genes were synthesised or isolated through PCR, cloned into pHTP1 expression vector and sequenced verified. Recombinant proteins were expressed in *E. coli* BL21(DE3) cells in Auto-Induction LB medium.

Cloning, Expression and Purification of 480 FAEs



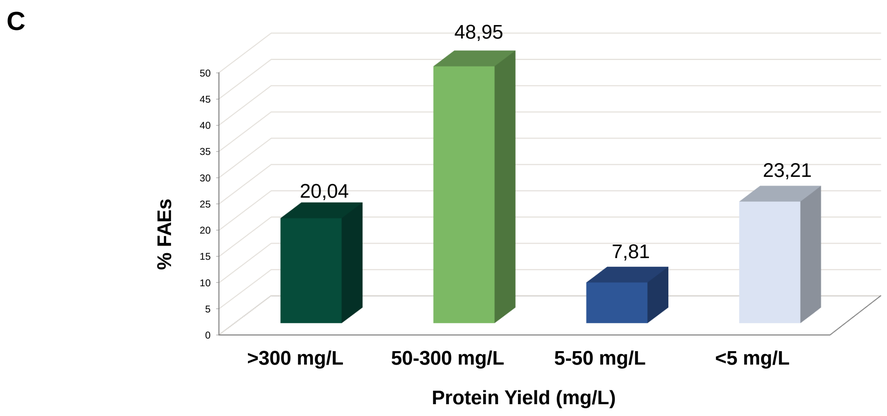


Figure 2. Efficacy of the platform used for the cloning and expression of 480 bacterial FAEs in *E. coli.* **A**. Agarose gel electrophoresis of a sample of the 480 genes isolated through PCR or synthesized *in vitro*. **B.** Genes were cloned into pHTP1 and recombinant plasmids used to transform *E. coli*; the majority of the generated recombinant BL21(DE3) derivative strains expressed high levels of recombinant protein when grown in Auto-Induction LB medium. **C.** The majority of bacterial FAEs (235) present a protein yield of 50 to 300 mg/L medium.

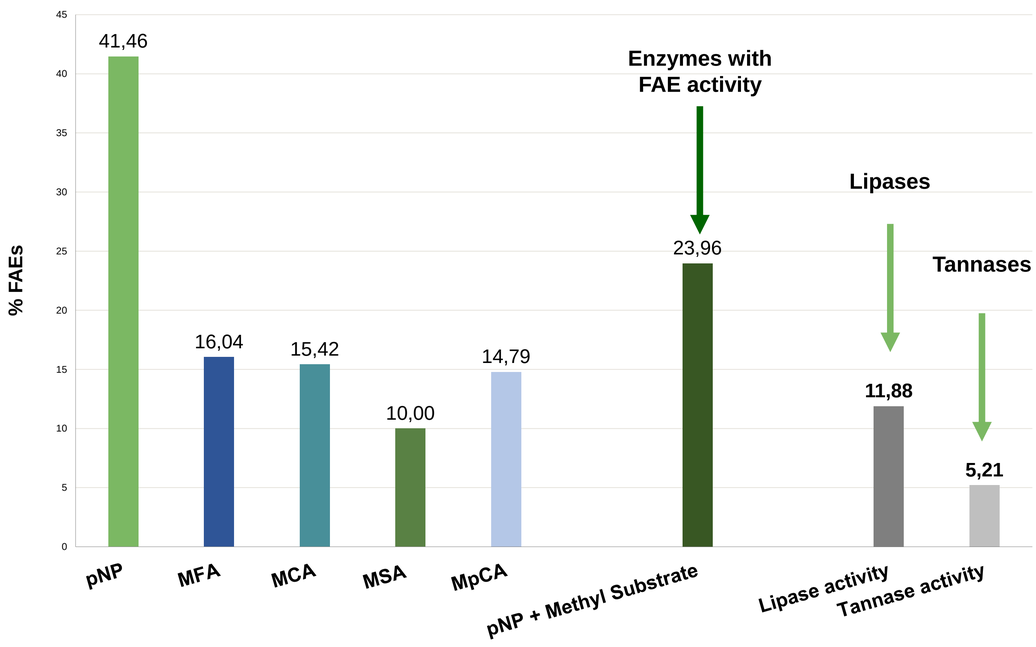


Figure 3. Percentage of enzymes displaying activity against pNP-ferulate and methylated cinnamic acids. Enzymes displaying cumulative pNP-ferulate activity is highlighted. Percentage of enzymes also displaying lipase & tannase activity is also displayed. A total of 199 out of 480 FAEs present activity for pNP-ferulate. In addition, 115 out of 480 FAEs have activity for both pNP-ferulate & methylated cinnamic acid. The screening allow identifying 57 lipases and 25 tannases.
Abbreviatures: pNP - pNP-ferulate, MFA – Methyl ferulate, MCA – Methyl caffeate, MSA – Methyl sinapinate, MpCA – Methyl p-coumarate

Screen of 480 bacterial FAEs for pNP-ferulate activity

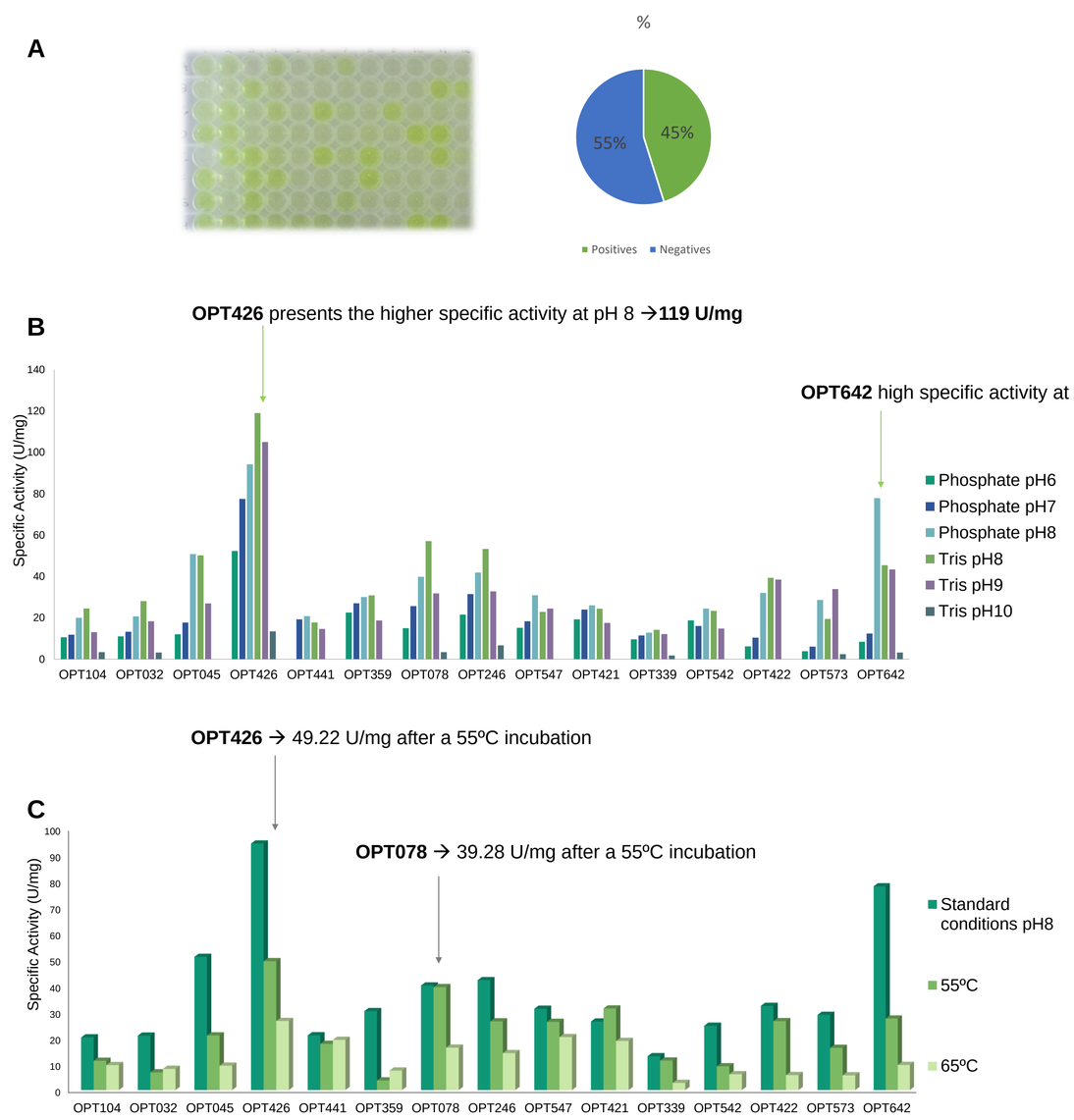


Figure 4. Screen of 480 bacterial FAEs for pNP-ferulate activity. **A.** The assay was performed under standard conditions (95µL substrate plus 5µL enzyme, with 0,6mM pNP-ferulate in 50 mM NaPi pH 7, at 25 °C) in plates. **B.** The best 15 bacterial FAEs were tested for optimum pH (pH 6-10). **C.** Thermostability at both 55ºC and 65ºC was evaluated for the best 15 bacterial FAEs.

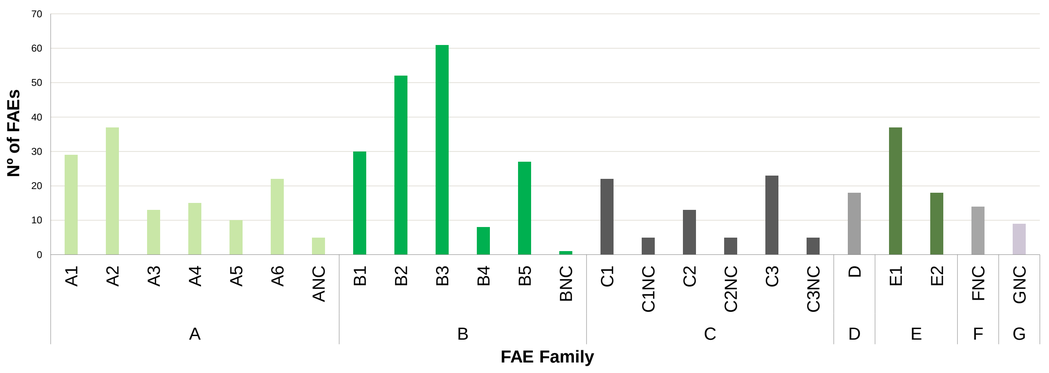


Figure 5. FAEs were organized into 7 Families, based on their primary sequence similarity. Currently relationship between the family and enzyme activity is being explored.

Concluding Remarks

- A significant proportion of the bacterial FAEs were shown to be expressed at high levels in *E. coli* and to be biologically active.
- Several novel recombinant FAEs of bacterial origin might be optimal biocatalysts when comparing with reference FAEs and potential comprise a wealthy repertoire of enzymes to exploit industrially.
- Determination of activity against different carbohydrate-ester substrates is ongoing as well as different structure function studies

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