Masters thesis project at Chalmers University of Technology, Division of Industrial Biotechnology, Department of Biology and Biological Engineering

Title: Investigation of the substrate range and catalytic mechanism of bacterial Tannase enzymes.

Duration: 6 or 12 months

Preferred start dates: Late 2021 / early 2022

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Background: Tannin acyl hydrolases (tannases) are a commercially important class of enzymes due to their use in food and beverage processing, such as in the clarification of tea and beer.[1] Their role is to catalyze the hydrolysis of ester bonds, through a conserved catalytic triad of amino acids (His/Asp/Ser), in potentially complex substrates. Tannase enzymes are found across the tree of life, although only a few have been characterized in significant biochemical detail, with even fewer structurally characterized. Therefore, it is not entirely clear what roles certain amino acid residues in these enzymes may play.

Project overview: Our group recently characterized three novel tannases from the bacterium *Clostridium butyricum* (*Cb*Tan1-3), which is the first time three tannases have been found in the same organism. We solved the X-ray crystal structure of one of these. The three *Cb*Tans were found to have only slightly different substrate preferences, and this raised the question of why this organism encodes for 3 distinct tannases.

In this project, the goal is to generate and characterize a library of mutant tannases in order to further elucidate the binding and reaction mechanism of this family of enzymes. Specific mutations will be introduced into the expression vectors harboring the *Cb*Tan genes, followed by protein production in *E. coli*. The resulting enzymes will be purified, and the substrate binding properties and enzyme kinetics for the mutant enzymes determined. The enzymes will also be screened for protein crystallization and structural investigations.

Prerequisites/desired skillset: The student should have undergraduate training in biochemistry / molecular biology / biotechnology / organic chemistry. Direct laboratory experience is not crucial although previous experience in procedures such as cloning, culturing or enzyme assays will be highly valuable.

The project will be a close collaboration with a skilled postdoctoral researcher (Tom Coleman) and give the student relevant training in site-directed mutagenesis, enzyme kinetics, protein structure interpretation, scientific writing and data analysis, while working in a collaborative and international environment.

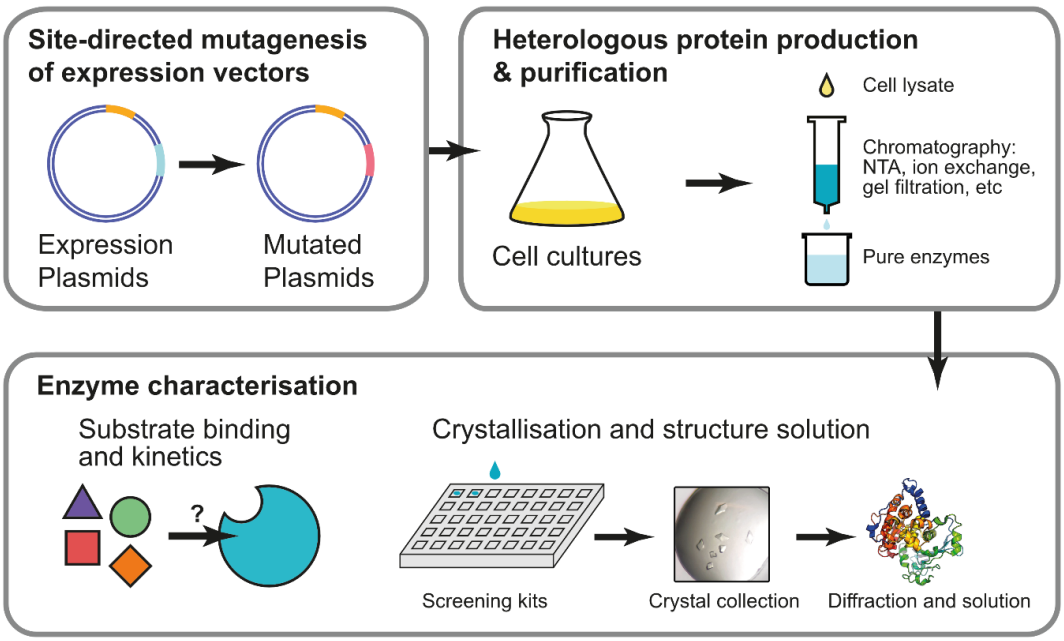

Figure: Project overview. The project will give the student first-hand experience in planning and executing a research workflow in enzyme characterization, from genetics to structural biology.

[1] de las Rivas, B., et al., *Bacterial tannases: classification and biochemical properties.* Applied Microbiology and Biotechnology, 2019. **103**(2): p. 603-623.