IndZyme: Novel inhibitor-resistant lignocellulolytic enzymes from Indian fungal resources

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Background

New energy solutions are needed to meet the increasing global energy demand and solve the environmental and societal concerns related to the use of fossil energy. Exploitation of lignocellulosic residues, such as wheat straw, for fuel production via pretreatment, enzymatic hydrolysis and microbial fermentation, is an attractive solution. The enzyme performance and price still remain a bottleneck to the process, partially due to the inhibition of the enzymes by compounds released from lignocellulose in the pretreatment. However, some of these compounds may also have an activating effect on certain enzymes. This project focuses on discovery and characterization of novel inhibitor tolerant or activated enzymes, cellobiohydrolases (CBHs) and lytic polysaccharide monooxygenases (LPMOs) from fungal species, which according their growth habitat are expected to produce robust cellulolytic enzymes.

Pretreatment liquor and inhibition of cellulases

Finnish wheat straw was hydrothermally pretreated at VTT and phenolic fraction (PF) of the pretreatment liquor was isolated using Amberlite XAD7 resin. Ca 130-150 different types monomers and dimers could be detected in PF (Figure 1). The PF was found to inhibit/ activate commercial cellulases in concentration dependent manner (Figure 2). The CBHs of *Trichoderma reesei* , which are essential in hydrolysis of crystalline celluloses, were found to be sensitive towards the inhibition (VTT and University of Tartu).

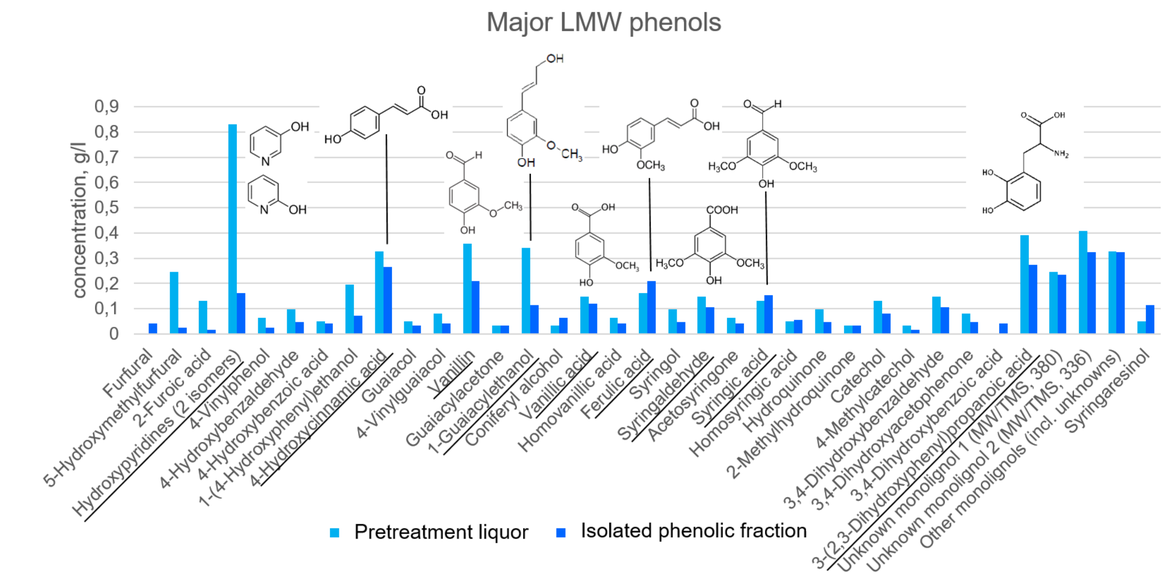

Figure 1. Composition of soluble LMW phenols in wheat straw pretreatment liquor and isolated phenolic fraction identified by GC/MS. Only monomeric and dimeric compounds are amenable to GC/MS, higher oligomers were not detected in this analysis.

Figure 2. Inhibition of cellulase cocktail CellicCTec2 (Novozymes) by phenolic fraction isolated from wheat straw pretreatment liquor. Hydrolysis reactions were performed at 45 ˚C in 50 mM NaAc buffer pH 5.0, cellulose (Avicel) concentration in reaction was 10 g/l, enzyme (CellicCtec2) dosage was 10 mg/g Avicel, phenolic fraction concentration varied 0-5 g/l.

Screening of inhibitor tolerant enzymes

173 fungal strains were screened in Vivekananda Institute of Tropical Mycology for inhibitor tolerant cellulase activity using a dotblot technique (Figure 3). Based on the results, 15 fungal strains were transferred from India to VTT. Strains were cultivated on media inducing expression of cellulose-acting enzymes. mRNA was isolated and sequenced (Figure 4). 30 novel enzyme genes have been selected for production in *T. reesei* to enable characterization of the enzymes including their tolerance to inhibitors (VTT, University of Tartu)

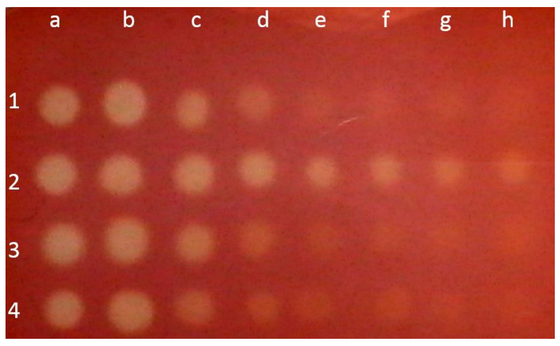

Figure 3. Screening for inhibitor tolerant cellulolytic activity using dotblot technique. Size of the dot correlates with cellulolytic activity. A-H represent increasing inhibitor concentration. 1-4 are examples of enzyme sample from different strains, showing variable inhibitor tolerance.

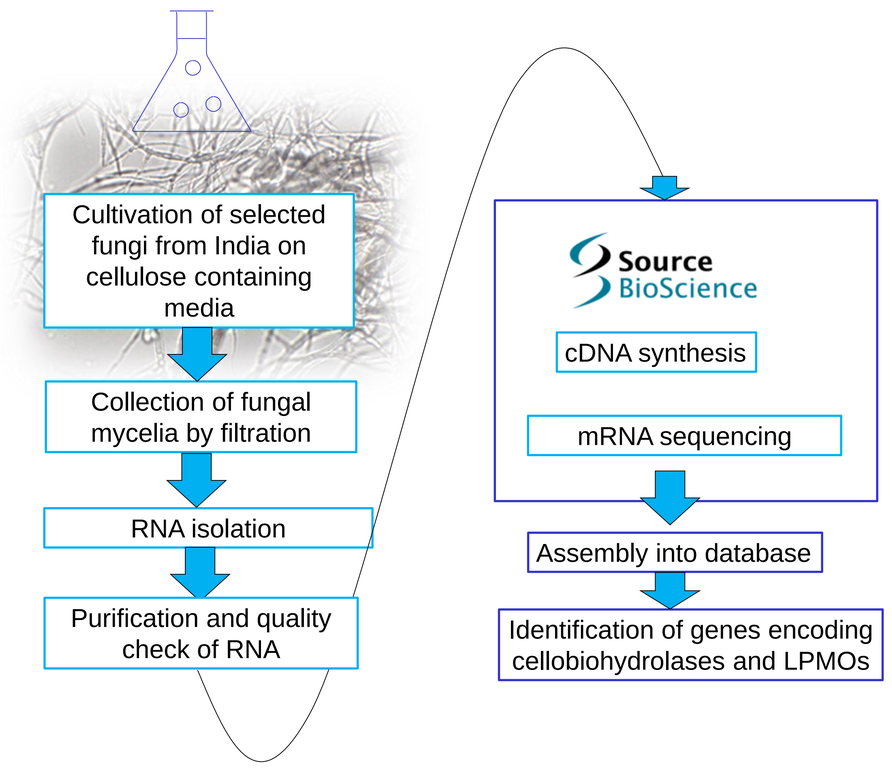

Figure 4. Schematic illustration for the workflow for isolation of novel genes encoding for cellobiohydrolases and LPMOs from selected fungi.

Research partners

- VTT Technical Research Centre of Finland, Ltd
- Vivekananda Institute of Tropical Mycology, India
- RWTH Aachen University, Germany
- University of Tartu, Estonia

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Project website https://www.vtt.fi/sites/indzyme

Acknowledgements to the national funding organisations

Academy of Finland
Research funding and expertise

सत्यमेव जयते
Department of Biotechnology

Eesti Teadusagentuur
Estonian Research Council

Bundesministerium für Bildung und Forschung