**Introduction**

Feruloyl esterases (FAEs, E.C. 3.1.1.73, CAZy family CE1) and glucuronoyl esterases (GEs, E.C. 3.1.1.-, CAZy family CE15) are involved in the degradation of plant biomass by hydrolysing ester linkages in plant cell walls, and thus have potential use in biofuel production from lignocellulosic materials and in biorefinery applications with the aim of developing new wood-based compounds [1, 2]. GEs and FAEs are present in the genomes of a wide range of fungi and bacteria. Under conditions of low water content, these enzymes can also carry out (trans)esterification reactions, making them promising biocatalysts for the modification of compounds with applications in the food, cosmetic and pharmaceutical industry. Compared to the chemical process, enzymatic synthesis can be carried out under lower process temperatures (50-60°C) and results in fewer side products, thus reducing the environmental impact.

**Methods & Results**

**Hydrolitic assays**

**FAEs**

- **MFA**
- **FA**

**GEs**

- **Benz-D-GluA**
- **GluA**

For screening of key hydrolitic activity of FAEs and GEs, spectrophotometric assays were used.

**Initial screening of 19 fungal strains (mesophilic and thermophilic) isolated from soil, wood and agricultural compost in Vietnam**

**Transesterification reaction**

- **MFA**
- **BFA**

Two thermophilic fungal strains were chosen for DNA and RNA sequencing. Methanol was grown for 49h at 40°C on glucose, washed thoroughly and subsequently divided into three subsamples containing 0.008, 0.018 and 0.038% ferulic acid as the carbon source. Samples were taken at different time points, RNA extracted and sequenced.

**Selection of novel FAEs/GEs**

- **condition 1 (e.g. growth on plate)**
- **condition 2 (e.g. growth on wheat bran/extract)**

Condition specific expression patterns of genes on different inducing and non-inducing media will allow the identification of novel FAEs and GEs. These enzymes will then be cloned and overexpressed in the heterologous host Pichia pastoris.

**Conclusions**

We characterised new FAE and GE enzymes from mesophilic, thermophilic and cold-tolerant filamentous fungi produced in Pichia pastoris. The enzymes were characterised for both their hydrolitic abilities on various model substrates (methyl ferulate, pnp-ferulate) - for potential applications in deconstruction of lignocellulosic materials and extraction of valuable compounds - as well as for their biosynthetic capacities. We tested and optimised the FAEs’ transesterification capabilities on ferulate esters in a 1-butanol-buffer system, with the aim of using the most promising candidates for the production of antioxidant compounds with improved hydrophobic or hydrophilic properties, such as prenyl ferulate, prenyl caffeate, glycerol ferulate and 5-O-(trans-feruloyl)-arabinofuranose.

**Citations**
