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Abstract Book



56T Selective cleavage of β -O-4 lignin bond by novel β -etherase of the white-rot fungus *Dichomitus squalens*. M. Marinovic¹, M.R. Mäkelä¹, P. Nousiainen⁴, A. Dilokpimol², R. Moore⁴, J. Sipilä⁴, R.P. de Vries^{2,3}, K. Hildén¹ 1) Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, University of Helsinki, Finland; 2) Fungal Physiology, CBS-KNAW, Utrecht, The Netherlands; 3) Fungal Molecular Physiology, Utrecht University, The Netherlands; 4) Department of Chemistry, Laboratory of Organic Chemistry, University of Helsinki, Finland.

Lignin is a recalcitrant aromatic polymer present in woody plant cell walls. It is depolymerized by classical lignin-modifying oxidoreductases (laccases and class II heme peroxidases) produced by wood decaying fungi, which catalyze non-selective cleavage of different types of bonds present in lignin. Novel enzymes catalyzing aromatic conversions are needed to unlock the potential of lignin as a major renewable source of bulk and fine chemicals.

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) constitute a complex and diverse superfamily of multifunctional enzymes with essential roles in cellular detoxification. These proteins are widespread in animals, plants, bacteria and fungi. Some of the bacterial GSTs possess β -etherase activity that can catalyze selective cleavage of β -O-4 aryl ether linkages present in lignin. However, only few fungal GSTs have been characterized so far, and little is known about these enzymes. In this work, putative GST-encoding genes from the lignocellulose degrading basidiomycete *Dichomitus squalens* were cloned and heterologously expressed in *Escherichia coli*. One of the enzymes that showed a weak amino acid level homology to bacterial β -etherase ligE was shown to cleave β -O-4 bond in α -oxo erol lignin model compound. To our knowledge, this is the first report of eukaryotic β -etherase.

57F Expanding feruloyl esterase gene family of *Aspergillus niger*: characterization of a new feruloyl esterase, FaeC. A. Dilokpimol^{1,2}, M. R. Mäkelä^{1,2,3}, S. Mansouri³, O. Belova^{1,2}, M. Waterstraat⁴, M. Bunzel⁴, R. P. de Vries^{1,2,3}, K. S. Hildén³ 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; 2) Fungal Molecular Physiology, Utrecht University, Utrecht, the Netherlands; 3) Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki, Finland; 4) Department of Food Chemistry and Phytochemistry, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Germany.

Ferulic acid (FA) and to a lesser extent other hydroxycinnamic acids (e.g. *p*-coumaric acid) are ester-linked to plant cell wall polymers, mainly to (hetero)xylans and pectins, forming polysaccharide-polysaccharide and polysaccharide-lignin cross-links. Phenolic cross-links increase the physical strength and integrity of plant cell walls and reduce their biodegradability by microorganisms. Feruloyl esterases (FAEs) [E.C. 3.1.1.73] are able to release FA and other phenolic acids from natural plant sources and agro-industrial by-products and are therefore widely used in food, feed, pulp-paper, bioethanol and pharmaceutical industries.

Two FAEs from *Aspergillus niger* (FaeA and FaeB) have been previously characterized and are among the best-studied FAEs. Phylogenetic analysis of fungal FAEs revealed numerous FAE candidates, including one from *A. niger* (FaeC).^{1,2} Recombinantly produced FaeC was most active at pH 7.0 and 50°C and showed broad substrate specificity. The enzyme released both ferulic acid and *p*-coumaric acid from wheat arabinoxylans and sugar beet pectins, and acted synergistically with a commercial xylanase. The expression profile of *faeC* on a set of phenolic compounds differed from those of *faeA* and *faeB*, indicating that these FAE isoenzymes may target different substrates in a complementary manner. FaeC showed promising potential for applications, particularly because of its broad substrate profile and its maximum activity at neutral pH.³

Acknowledgement

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Reference

- 1 Benoit, I., Danchin, E.G.J., Bleichrodt, R.J., de Vries R.P. 2008. Biotechnological applications and potential of fungal feruloyl esterases based on prevalence, classification and biochemical diversity *Biotechnol Lett.* 30:387-396.
- 2 Dilokpimol, A., Mäkelä, M.R., Aguilar-Pontes, M.V., Benoit-Gelber, I., Hildén, K.S., de Vries R.P. 2016. Diversity of fungal feruloyl esterases: updated phylogenetic classification, properties and industrial applications. *Biotechnol Biofuels.* 9:1-18.
- 3 Dilokpimol, A., Mäkelä, M.R., Mansouri, S., Belova, O., Waterstraat, M., Bunzel, M., de Vries, R.P., Hildén, K.S. Expanding the feruloyl esterase gene family of *Aspergillus niger* by characterization of a feruloyl esterase, FaeC. *In revision.*

58W Disruption of the glucose repressor gene *cre1* in *Trichoderma harzianum* CFAM-422 and its effect on plant cell wall degrading enzymes production. M.S. Tamietti¹, G.E.O. Midorikawa¹, T.D. Mendes¹, M.C.T. Damaso¹, E.P.S. Bon², A.S. Silva³, L.M.F. Gottschalk⁴, E.F. Noronha⁵, L.C.L. Fávaro¹ 1) Embrapa Agroenergia, Brazilian Agricultural Research Corporation (Embrapa), Brasília, DF, BR; 2) Biochemistry, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, BR; 3) Catalysis and Biochemical Processes, National Institute of Technology (INT), Rio de Janeiro, RJ, BR; 4) Food Agroindustry, Brazilian Agricultural Research Corporation (Embrapa), Rio de Janeiro, RJ, BR; 5) Molecular Biology, University of Brasilia (UnB), Brasilia, DF, BR.

The Carbon Catabolite Repression (CCR) is a mechanism by which saprophytic fungi and bacteria can utilize preferably highly energetic compounds over compounds of difficult degradation. For *Trichoderma reesei*, the protein that acts as the repressor in the presence of glucose is CRE1. In this project, we aim to disrupt the *cre1* gene in the strain CFAM-T422 of *Trichoderma harzianum*, obtaining mutants with enhanced plant cell wall degrading enzymes production. The disruption of *cre1* in *T. harzianum* CFAM-422 was performed by transforming protoplast cells with a cassette containing a hygromycin B phosphotransferase gene (*hph*) flanked by *cre1* gene sequence, favoring its integration in the fungal genome by homologous recombination. A stability test was performed by successive transfers to nonselective medium (without hygromycin B) for ten generations, the last one being transferred to medium containing hygromycin B (200 μ g/mL). Mutants genetically stable and the parental strain were tested for enzyme production in both inducing and repressing conditions with four different carbon sources (carboxymethylcellulose, pectin, starch and xylan). Their enzymatic indexes (e.i.), the ratio between the degradation halo diameter over the colony diameter, were determined. The glucose content for the test under repressing condition was determined as the minimal concentration that triggers CCR in CFAM-T422. The totality of the stable transformants showed increased enzymatic index in all carbon sources tested. For the xylan containing media, which showed the most expressive differences between wild and mutant strains, the i.e. for the parental was 1 and transformants varied from 2,0 to 2,9 regardless of the presence of glucose. These

findings indicate that the *cre1* gene in *T. harzianum* regulates the expression of carbohydrate active enzymes and its deletion can result into mutant strains with enhanced cellulase, hemicellulase and pectinase production with lower glucose inhibition.

59T Comparing Fungal Capacity for Bioremediation of Human Solid Waste. Alexander Mehreteab, Victor Manzanilla, *Michael Waters* Dept Biol, Valparaiso Univ, Valparaiso, IN.

Neurospora crassa can be used to reduce human solid waste while converting it into fungal biomass which has the potential to be used as a dietary supplement. Typically portrayed as an environmental hazard and source of undesirable odor, solid waste represents an untapped resource. Filamentous fungi are natural decomposers with the ability to use this resource and reduce its environmental impact.

We report here on experiments to determine the conditions which maximize yield of fungal biomass. We compared the effect of the length of incubation and alternative methods of aeration as well as choice of fungal species on fungal growth. Rates of conversion were variable, with *Neurospora* producing the highest average rate of conversion (grams of dry fungal biomass produced from grams of dry waste in the media) of fungi tested. Additionally fungal growth reduced the characteristic odor of the media. Although this project was initially proposed to address problems inherent to long-term space flight: food storage & waste management, it holds potential benefit in diverse situations including livestock confinement operations.

60F Genome analysis and gene silencing of the citrus pathogen *Colletotrichum abscissum*. Eduardo Goulin^{1,2}, Marco Takita¹, Marcos Machado¹ 1) Biotechnology, Centro de Citricultura "Sylvio Moreira", Cordeirópolis, Cordeirópolis, BR; 2) State University of Campinas.

The citrus crop is a worldwide important crop. But, it is constantly affected by several pathogens, and fungi is an economically relevant class of citrus pathogens. New technologies are being applied every year to better understand the pathogens biology, and it can contribute to plant diseases control. The whole genome sequencing become a popular technology making possible and fast many knowledge advances. Therewith, we used NGS to investigate the RNA interference canonical machinery genes in the Post-Bloom Fruit Drop causal agent, *Colletotrichum abscissum*, as well as targets to RNAi silencing. The genome sequencing was performed in Illumina HiSeq platform, followed by *de novo* assembly using CLC genomics workbench software, and ORFs prediction by Augustus. The Local Blast were used for gene sequences investigation. The genome of *C. abscissum* is well coverage (169 times), which allowed the gene prediction, checked by RNAi machinery proteins search and confirmed by sanger sequencing of Argonaute, Dicer and RdRp. The RNAi functionality was proved after RT-qPCR of several genes and also transforming the fungus with a hairpin construction to induce the gene silencing. The fungus had a previously inserted report gene silenced after transformation attesting the machinery functionality. The genome provided a range of genes that can become a target to RNAi. Therefore, the genome of *Colletotrichum abscissum* was efficiently sequenced, assembly and used to a relevant investigation, providing information about the fungus biology, and also a tool to gene function studies and pathogen control.

61W Parasitism of *Trichoderma* on closely related fungi (adelphoparasitism) is linked to intensive interfungal DNA exchange. Irina S. Druzhinina Institute of Chemical Engineering, TU Wien, Vienna, Austria.

The remarkable cellulolytic activity of the initially fungicolous fungus *Trichoderma* (Hypocreales, Pezizomycotina, Ascomycota) arose as a result of unprecedented intensive lateral transfer of genes (LGT) that are required for the degradation of plant biomass. The evolutionary analysis of all lignocellulose degrading carbohydrate active enzymes (lclCAZymes) of *Trichoderma* showed that one half of more than 120 genes was obtained through LGT from herbivore Pezizomycotina fungi (such as Eurotiomycetes, Sordariomycetes incl. Hypocreales, Leotiomycetes and others) but none from other fungi or prokaryotes. Here we review the ecology of *Trichoderma* and present details on ultrastructure of its parasitism on different fungi. We provide evidence that the high frequency of LGT of lclCAZymes is linked to the unique ability of *Trichoderma* to parasitize on genetically closely related fungi that is extended up to adelphoparasitism in its strict sense. The data suggests that the taxonomically and functionally biased genome enrichment of lclCAZome by LGT allowed *Trichoderma* to expand its diet from plesiomorphic fungivory/carnivory up to apomorphic phytosaprotrophy, thus resulting in the emergence of its outstanding ecological versatility referred as environmental opportunism.

The case study on *Trichoderma* allows us to assume that intracellular adelphoparasitism may be a prerequisite for the lateral transfer of genes also in other organisms. Although such parasitic interactions are rare in nature, potential systems in other low eukaryotes will be discussed.

62T Marker recycling through CRIME. A.P. Mitchell, M. Huang Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

We describe here a new approach to marker recycling, a controlled sequence of manipulations in which a genetic marker is selected, then lost, then selected again. Our work was carried out with the fungal pathogen *Candida albicans*, which is typically a diploid and has no complete sexual cycle. The approach makes use of the RNA-guided DNA cleavage activity of CRISPR-Cas9, which was recently engineered for use in *C. albicans* by Valmik Vyas and colleagues (PMID: 25977940). We used the high-speed adaptation for gene deletion described by Kayden Min in our lab (PMID: 27340698) for the specific studies that will be presented. Because the CRISPR-Cas9 system has been implemented in diverse fungi, we believe that CRIME may be applicable to diverse fungi.

To use CRIME, we first created marker cassettes flanked by direct repeats. We used one repeat-flanked marker to create a homozygous mutation in our favorite gene, *OFG1*, by CRISPR-Cas9 transformation. Then, in the *ofg1/ofg1* strain, we made a homozygous mutation in a second gene, *OFG2*, by use of a different repeat-flanked marker. In that second transformation, we also included a guide RNA gene to direct cleavage of the marker that lay in the *ofg1* mutant alleles. The idea was that either single-strand annealing or homology-directed repair would then excise the marker we cleaved and leave behind only a copy of the flanking repeats. The idea proved correct: we recovered *ofg1/ofg1 ofg2/ofg2* double mutants from the transformation that had only the marker in the *ofg2* alleles; they had lost both copies of the marker that had been in the *ofg1* alleles. In fact, we have been able to create successive homozygous mutations in three genes by use of two markers, and still ended up with one marker available for further selection in the triple homozygote. That sequence of steps and analyses required 3 weeks total. Our findings illustrate that CRIME pays, at least in this one context.