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DISCLAIMER

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OFFICE OF SCIENCE

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Introduction

White and brown rot fungi are among the most important wood decayers in nature. Although more than 50 genomes of Basidiomycete white and brown rots have been sequenced by the Joint Genome Institute, there is still a lot to learn about how these fungi degrade the tough polymers present in wood.

In particular, very little is known about how these fungi regulate the expression of genes involved in lignocellulose degradation. Here, we used transcriptomics, proteomics, and promoter analysis in an effort to gain insight into the process of lignocellulose degradation.

Schizophyllum commune as a Basidiomycete model system

Few Basidiomycete white or brown rots are genetically amenable, hindering a functional genomics approach to the study of lignocellulose degradation. A notable exception is *Schizophyllum commune*, for which numerous genetic tools are available:

- Most importantly: an efficient gene deletion protocol
- Sequenced genome
- Transformation is routine
- Three antibiotic resistance markers
- Gene expression analysis (RNAseq) is routine

Ascomycete regulators are poorly conserved in Basidiomycetes

In Ascomycetes, several conserved regulators of complex carbon source degradation have been identified. However, these are poorly conserved in Basidiomycetes. It would seem that Basidiomycetes use different transcription factors to regulate genes involved in complex carbon source degradation. What are they?

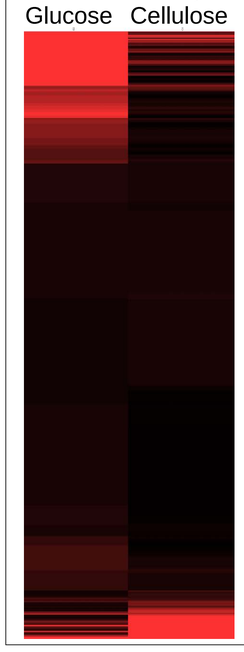
Presence of putative orthologs of Ascomycete regulators, as determined by sharing a cluster in a homology-based MCL clustering analysis.

* Homology restricted to DNA binding domain

			Ascomycetes			Basidiomycetes		
Regulator	**Description**	**Origin**	***Aspergillus nidulans***	***Neurospora crassa***	***Saccharomyces cerevisiae***	***Schizophyllum commune***	***Phanerochaete chrysosporium***	***Postia placenta***
CreA	Carbon catabolite repressor	*Aspergillus nidulans*	Yes	Yes	Yes	Yes*	No	No
XlnR	(Hemi-)cellulolytic regulator	*Aspergillus nidulans*	Yes	Yes	No	No	No	No
Gal4	Regulator of galactose-induced genes	*Saccharomyces cerevisiae*	Yes	Yes	Yes	No	No	No
Clr1	Cellulolytic regulator 1	*Neurospora crassa*	Yes	Yes	Yes	No	No	No
Clr2	Cellulolytic regulator 2	*Neurospora crassa*	Yes	Yes	Yes	No	No	No

Proteomics

The secretome during growth on glucose and cellulose was analyzed using Mass Spectrometry.



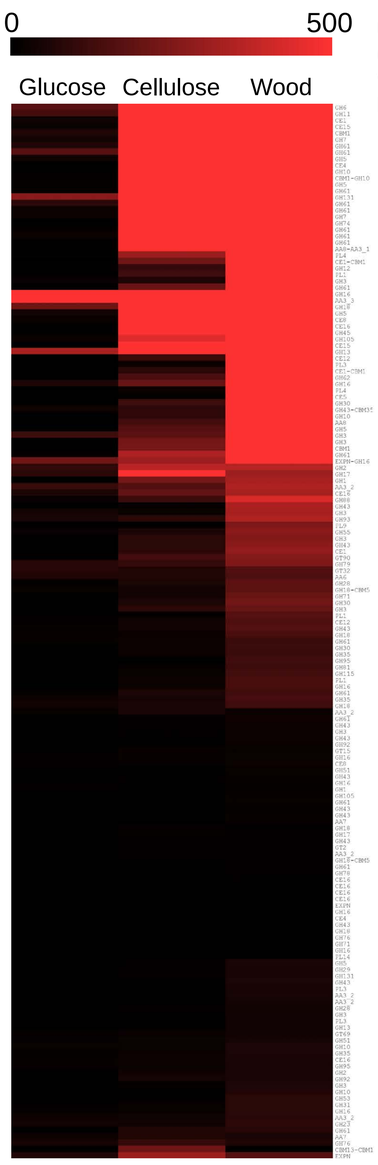
Abundance of proteins in the secretome, when grown on glucose or cellulose. Cultures were grown for 5 days as liquid shaken cultures. The extracellular medium was isolated and analyzed.

Statistics of the identified proteins. Although 672 proteins are identified, only a small part is abundantly present. Many are CAZymes or FOLymes, which likely play a role in lignocellulose degradation. As expected, a large percentage of the proteins have a secretion signal peptide. Members of the GH61 family are strongly over-represented in the identified proteins.

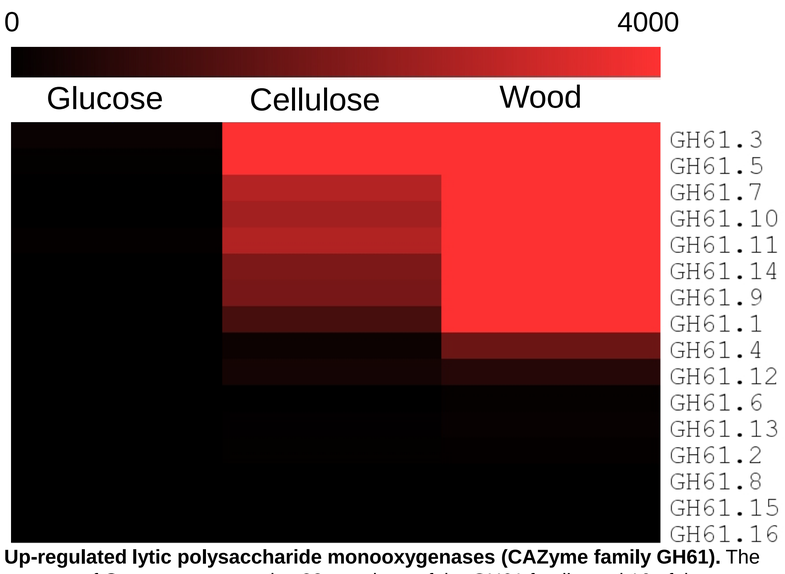
	Protein count	**CAZyme/ FOLyme**	**Signal peptide**	**GH61s (out of 22 in the genome)**
All identified proteins (on glucose, cellulose, or both)	672	26.30%	49.70%	16
Abundant proteins (on glucose, cellulose, or both)	84	60%	60.00%	5
More abundant on glucose	56	37.50%	82.10%	0
More abundant on cellulose	22	100%	84.50%	3

Transcriptomics

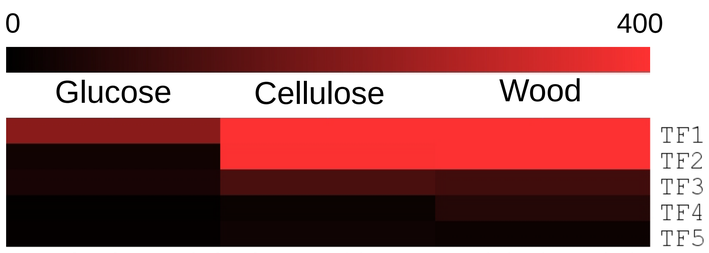
Gene expression is determined (using RNAseq) during growth on glucose, cellulose, and wood as carbon source. Glucose is a monosaccharide and can be easily transported into the cell. In contrast, cellulose and wood are complex carbon sources that require a large set of enzymes to be degraded, before transportation into the cell.



Up-regulated carbohydrate-active enzymes (CAZymes) and fungal oxidative enzymes (FOLymes). There are 164 CAZymes and FOLymes that are up-regulated on either cellulose or wood, compared to glucose. These are thought to encode enzymes that break down the tough polymers of cellulose and wood, and are interesting targets for further analysis.



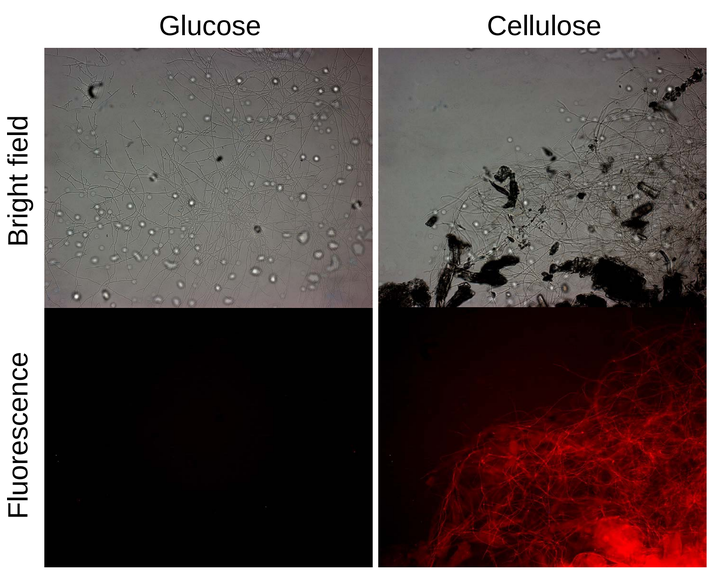
Up-regulated lytic polysaccharide monooxygenases (CAZyme family GH61). The genome of *S. commune* encodes 22 members of the GH61 family, and 16 of these are (strongly) up-regulated during growth on either glucose or wood, compared to glucose. This is a strong indication that they are involved in breaking down these polymers. (Note the different color scale compared to the other two heat maps.)



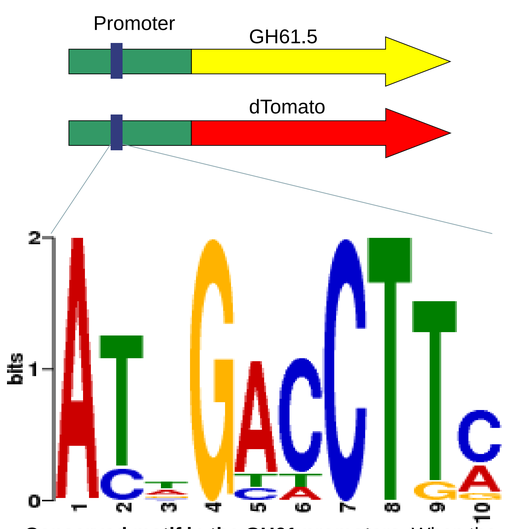
Up-regulated transcription factors. Of the 450 transcription factors in the genome of *S. commune*, 5 are up-regulated on both cellulose and wood. These regulators may be involved in up-regulating CAZymes and FOLymes during growth on these carbon sources.

Promoter analysis of the strongly up-regulated GH61.5

From the transcriptomics and proteomics data it is clear that GH61.5 is strongly up-regulated during growth on cellulose and wood (see other panels on this poster). It would be interesting to know how this gene is regulated. To identify regulatory elements, we did a promoter analysis. The 700 bp promoter of GH61.5 was used to express dTomato, which encodes a red fluorescent protein.



The 700 bp promoter of GH61.5 strongly induces dTomato fluorescence when grown on cellulose, but not on glucose. This confirms the RNAseq and proteomics data. This promoter can be used as a marker for GH61.5 expression, or to express genes during growth on cellulose.



Conserved motif in the GH61 promoters. When the promoters of the 16 up-regulated GH61 genes are compared, a conserved motif is found. This motif is present in all 16 promoters, and strongly under-represented in all other promoters ($p < 1e\text{-}8$). Using the fluorescence assay, we will test if this motif is indeed a regulatory element.

Conclusions

- Transcriptomics and proteomics analyses have identified enzymes, regulators and other proteins that are likely involved in lignocellulose degradation. In particular, members of the GH61 family appear to play an important role.
- These genes will be studied on more detail. This is possible in *S. commune*, since there are many molecular tools available (in contrast to most other wood rots).

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