Title

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DISCLAIMER

Functional and Comparative Genomics of Lignocellulose Degradation by the Fungus *Schizophyllum commune*

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US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

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Introduction

Wood rot fungi are among the most important wood decayers in nature. Although more than 75 genomes of Basidiomycete wood rots have been sequenced by the Joint Genome Institute, there is still a lot to learn about how these fungi degrade the tough polymers in wood. In particular, very little is known about how these fungi regulate the expression of genes involved in lignocellulose degradation. Here, we used comparative genomics, comparative transcriptomics, and promoter analysis in an effort to gain insight into the process of lignocellulose degradation.

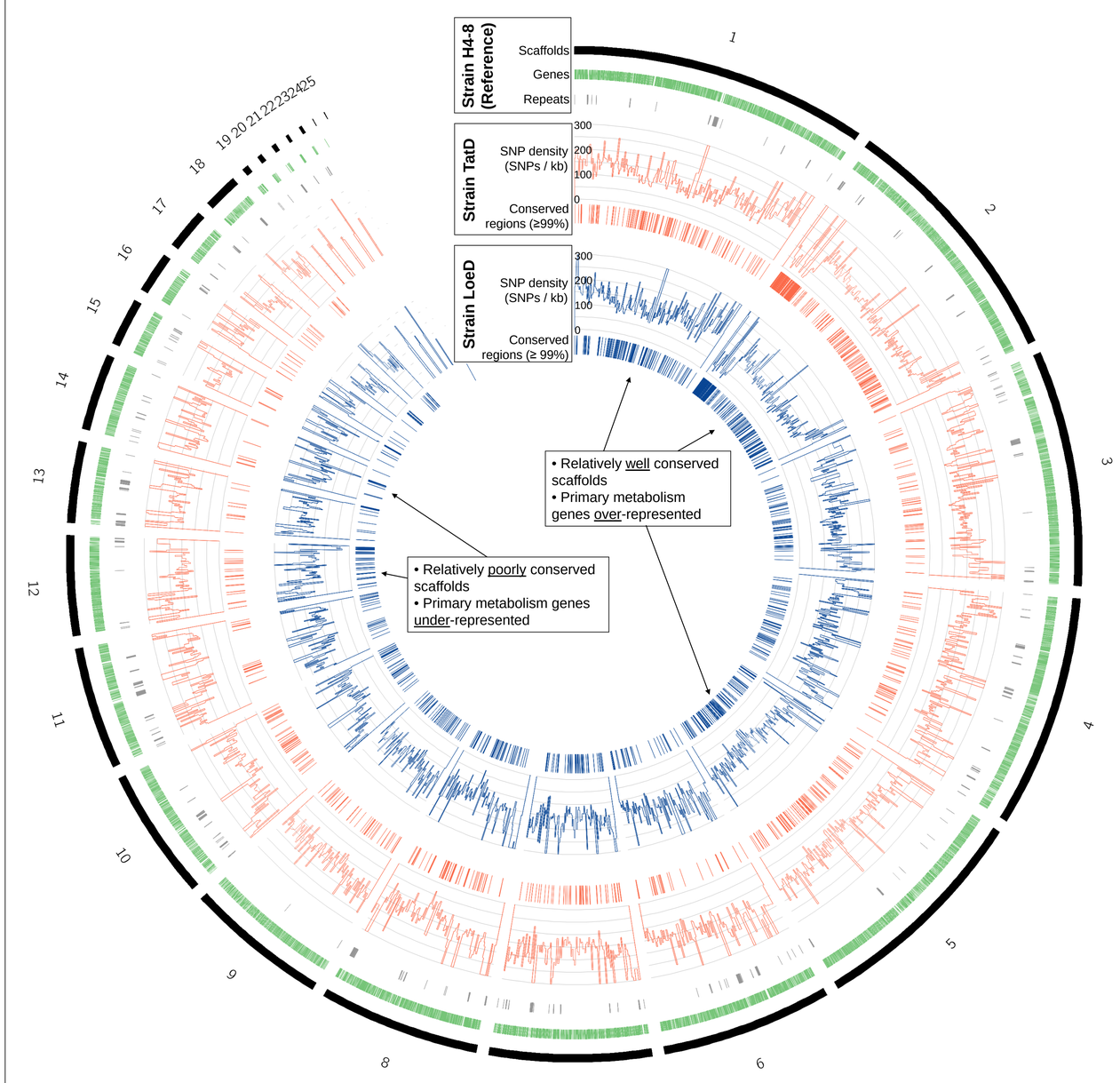
Schizophyllum commune as a Basidiomycete model system

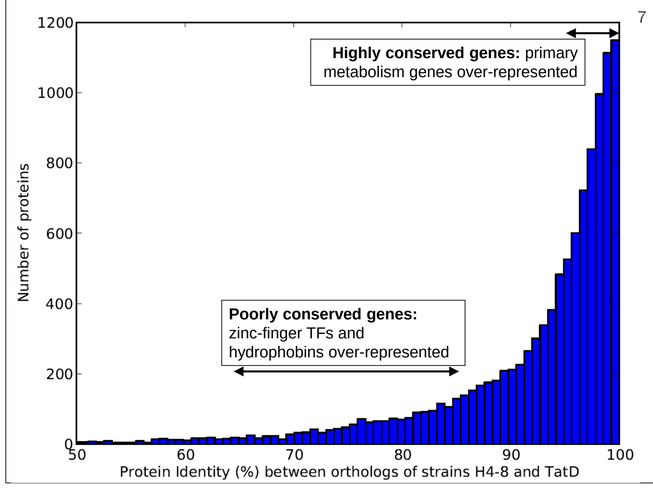
Few Basidiomycete wood rot fungi are genetically amenable, hindering a functional genomics approach to the study of lignocellulose degradation. A notable exception is *Schizophyllum commune*, for which numerous genetic tools are available:

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

High sequence diversity between strains of *S. commune*

Two additional *S. commune* strains with different wood-decay and mushroom properties were sequenced: strain TatD (from Tattone, France) and strain LoeD (from Loenen, The Netherlands). Sequence comparison shows remarkably high sequence diversity between the strains. The overall SNP rate of > 100 SNPs/kb is among the highest rates of within-species polymorphisms. For comparison: in some other fungi (mostly Ascomycota) this is <10 SNPs/kb, and in humans it is < 1 SNPs/kb.

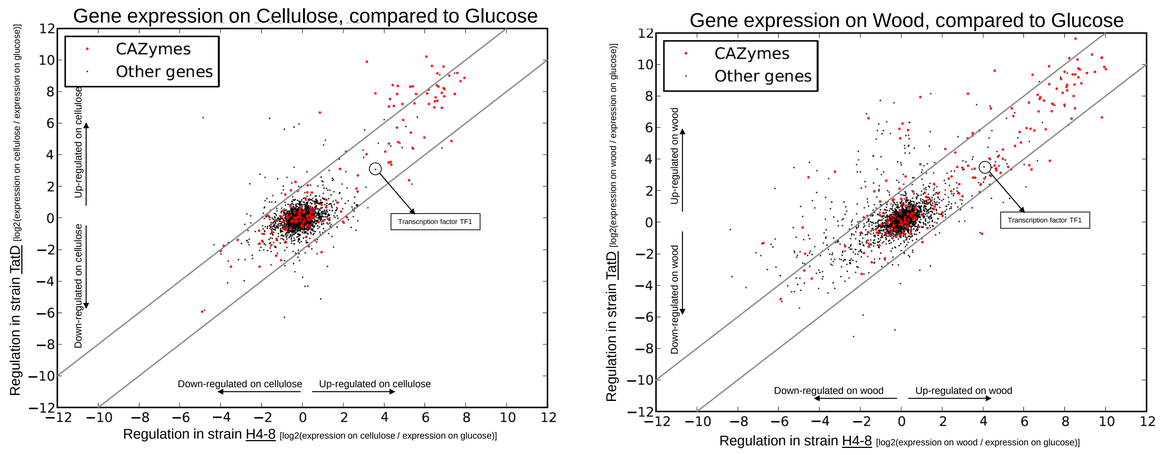




- The overall SNP-rate is very high, but some scaffolds are better conserved than others.
- Some well-described genes like transcription factors and hydrophobins have relatively low (≤ 70%) sequence identity among the strains of *S. commune*.
- This high diversity may explain the wide range of phenotypes of the various strains, as well as the wide distribution of *S. commune* across the world.

Comparative transcriptomics

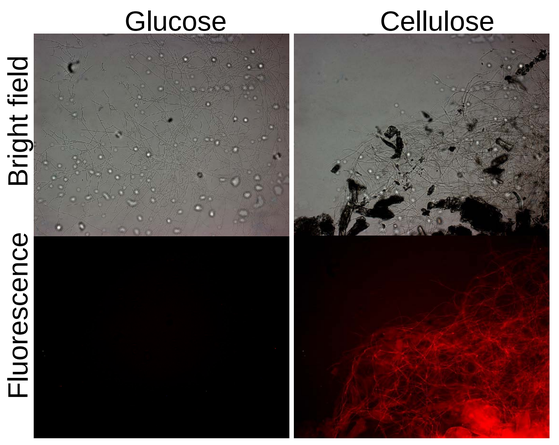
Gene expression in strains H4-8 and TatD was analyzed during growth on glucose, cellulose, and wood. This allows for a comparative transcriptomics approach.



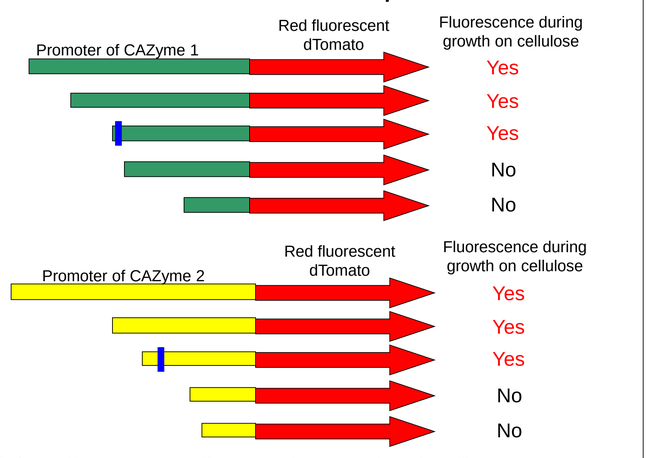
- In both strains the carbohydrate-active enzymes (CAZymes) are strongly up-regulated on both cellulose and wood, when compared to glucose.
- Several genes with unknown function are also strongly up-regulated: novel biotech enzymes?
- Many genes are more strongly up-regulated in strain TatD than in strain H4-8.
- Transcription factor TF1 is strongly up-regulated on both cellulose and wood, and in both strains. This makes it a potential candidate as a CAZyme regulator.

Promoter analysis of strongly up-regulated CAZymes

Transcriptomics and proteomics identified several carbohydrate-active enzymes (CAZymes) that are strongly up-regulated during growth on cellulose and wood. A promoter analysis of two CAZymes revealed potential conserved transcription factor binding sites. The red fluorescent protein dTomato was used as a reporter of promoter activity.



The promoter of CAZyme 1 strongly induces dTomato fluorescence when grown on cellulose, but not on glucose.



Schematic summary of the promoter analysis of two CAZyme genes. Various promoter lengths were tested for activity using the reporter dTomato. The shortest active promoters have a conserved domain (blue bar). This domain is also over-represented in other up-regulated CAZymes (not shown).

Conclusions

- The is a remarkably high sequence diversity between different strains of *S. commune*.
- Transcriptomics, comparative genomics and proteomics analyses have identified enzymes, regulators and other proteins that are likely involved in lignocellulose degradation. These genes will be studied on more detail.
- A promoter analysis revealed a conserved motif in promoters of CAZymes.

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