

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

Robin A. Ohm*¹, Hanbyul Lee^{1,2}, Hongjae Park^{1,2}, Heather M. Brewer³, Akiko Carver¹, Alex Copeland¹, Jane Grimwood⁴, Erika Lindquist¹, Anna Lipzen¹, Joel Martin¹, Samuel O. Purvine³, Wendy Schackwitz¹, Martin Tegelaar⁵, Andrew Tritt¹, Scott Baker³, In-Geol Choi^{1,2}, Luis G. Lugones⁵, Han A. B. Wösten⁵, Igor V. Grigoriev¹

1. DOE Joint Genome Institute, Walnut Creek, California, USA
2. Korea University, Seoul, South Korea
3. Pacific Northwest National Laboratory, Richland, Washington, USA
4. Hudson Alpha Institute for Biotechnology, Huntsville, Alabama, USA
5. Utrecht University, Utrecht, The Netherlands

* Presenting author. Email: raohm@lbl.gov

March 2014

The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor The Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or The Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or The Regents of the University of California.

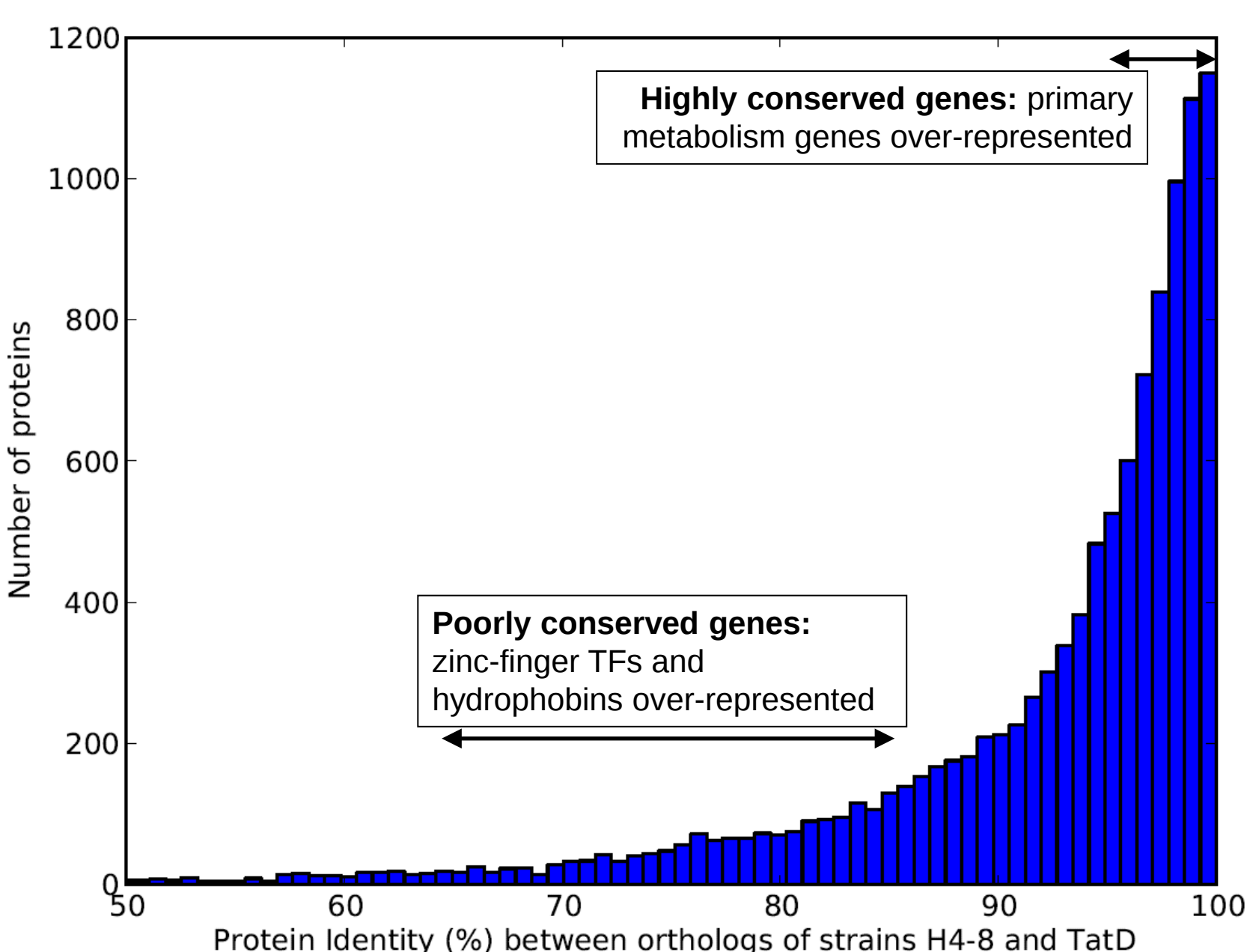
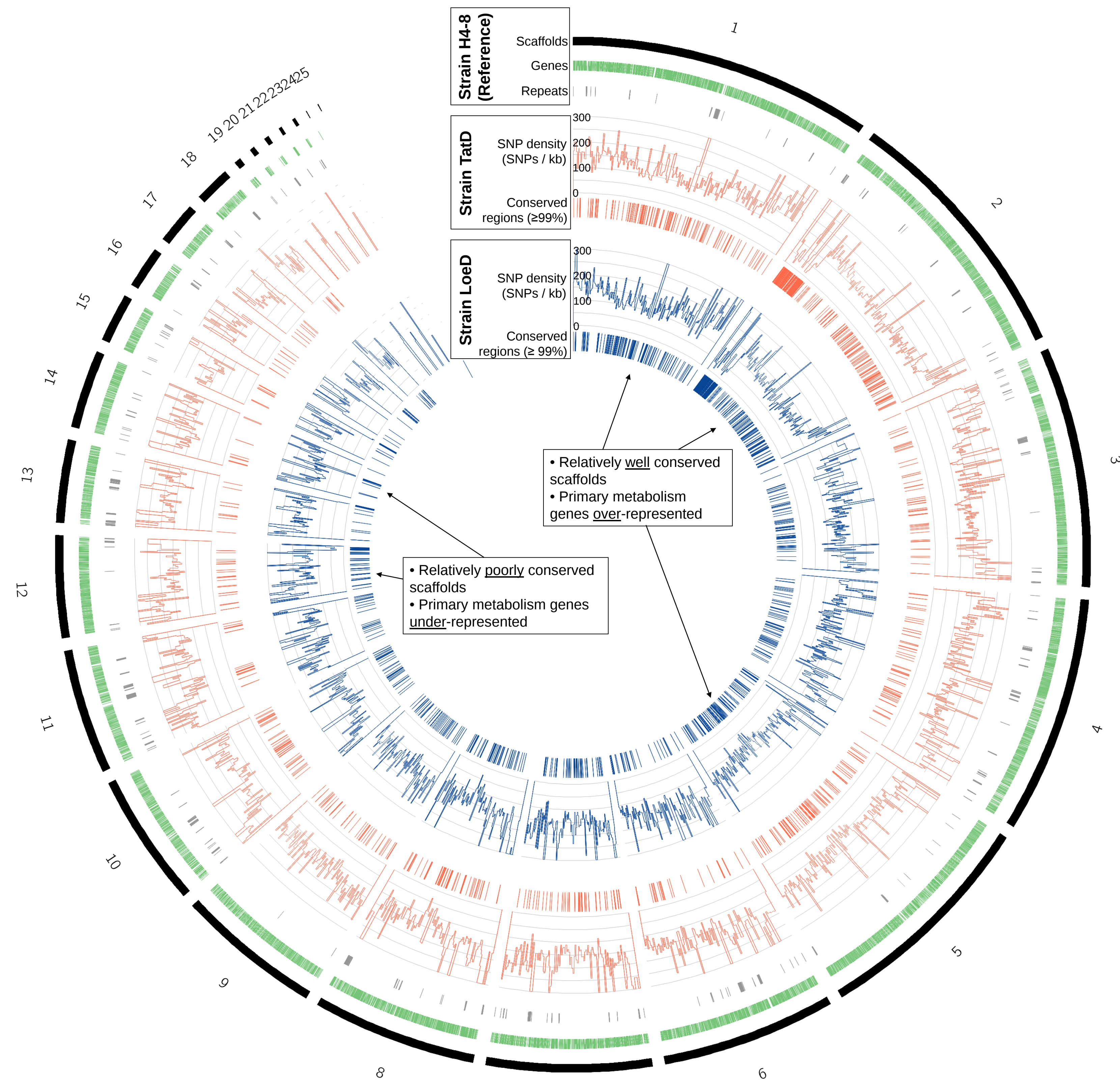


Introduction

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.

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 ($\leq 70\%$) 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.

Schizophyllum commune as a Basidiomycete model system

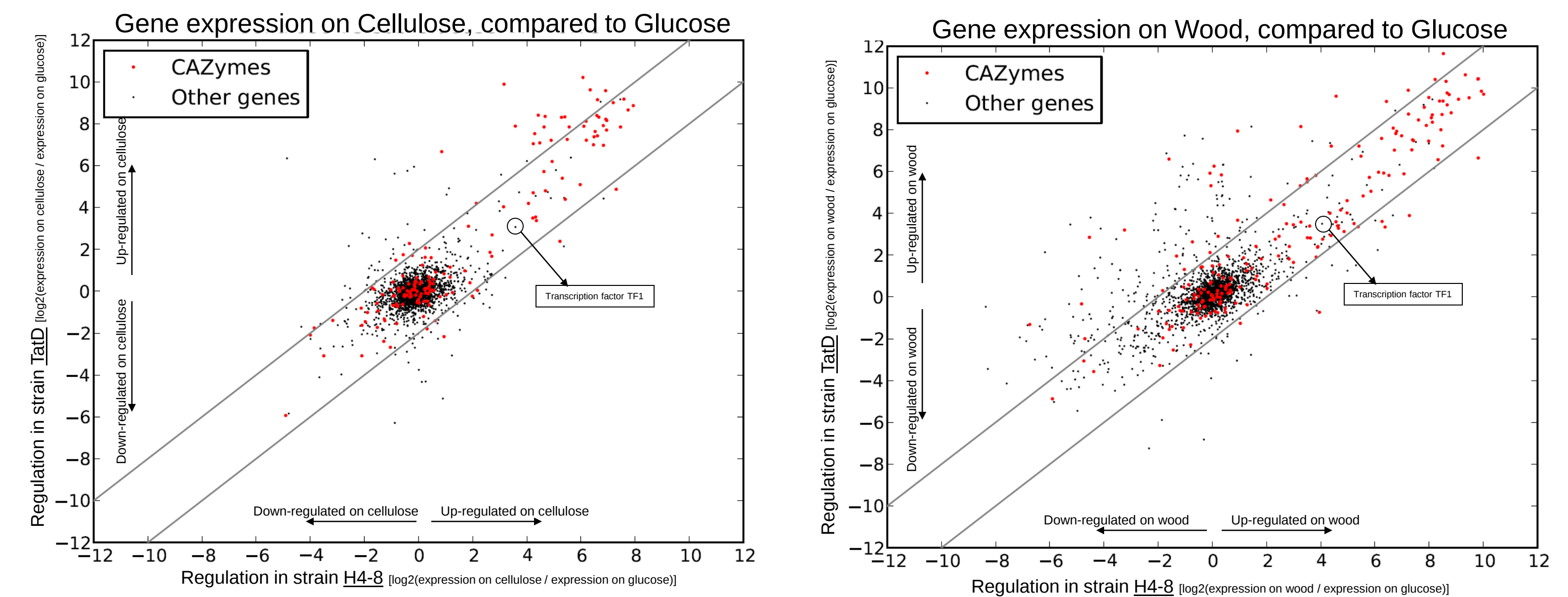
Few Basidiomycete wood rot fungi are genetically amenable, hindering a functional genomics approach to the study of lignocellulose degradation. A notable exception is *Schizophyllum commune*, for which numerous genetic tools are available:

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



Comparative transcriptomics

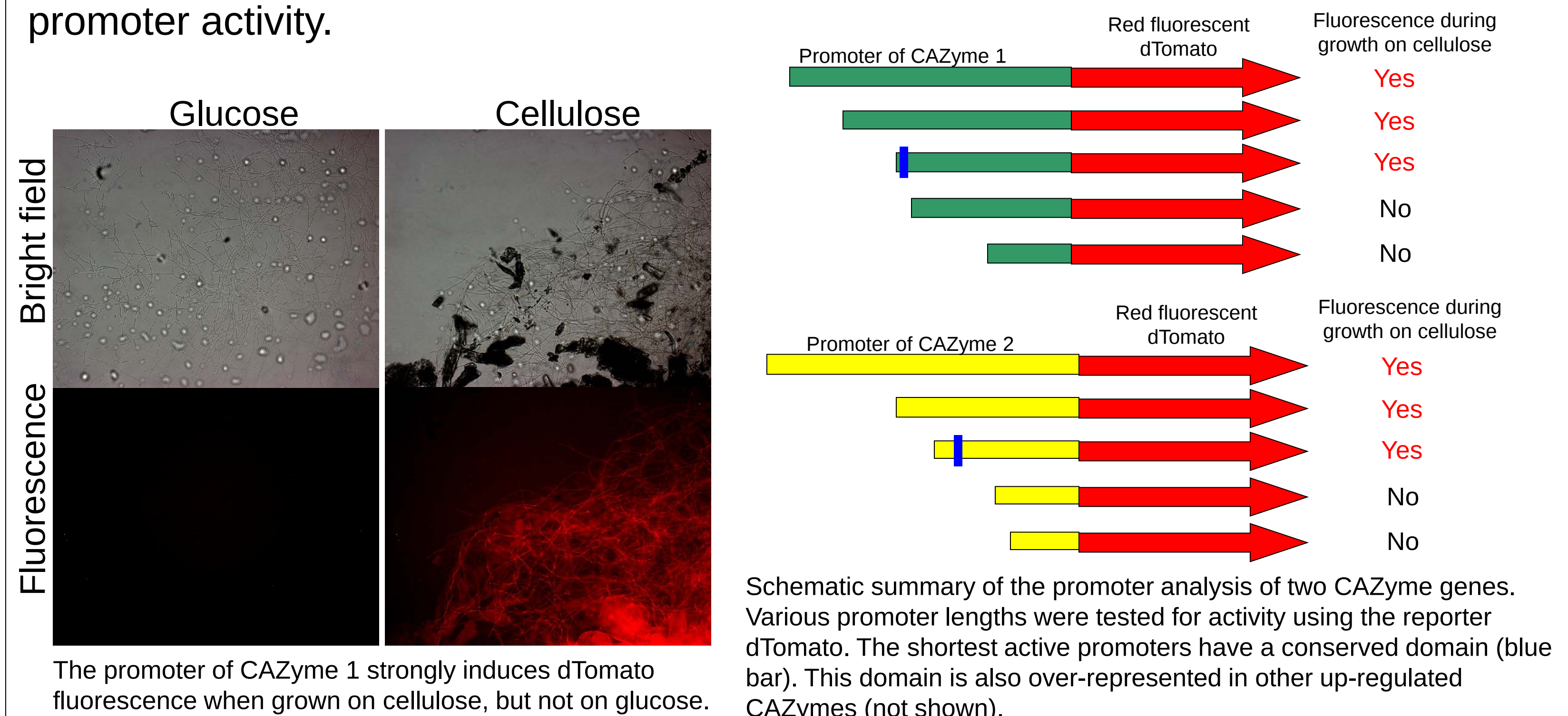
Gene expression in strains H4-8 and TatD was analyzed during growth on glucose, cellulose, and wood. This allows for a comparative transcriptomics approach.



- 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.



Conclusions

- There 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.