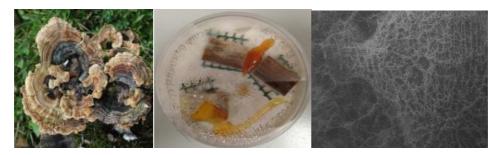
SYMWOOD



Comparative Genomics and Biochemistry of Symbiosis and Wood Decay Machineries in Fungi

Principle investigator: Francis Martin

Partners: E. Gelhaye (IAM), P. Gérardin (LERMAB)

Context — Lignocellulosic plant materials comprise the most abundant biopolymers in terrestrial ecosystems. Fungal-mediated degradation of lignocellulose is thus a critical link in the carbon cycle, and is of great interest for its potential applications in the production of biomaterials, including biofuels, enzymes, and other commodities. Saprotrophic Agaricomycotina are of particular interest as they are active and abundant degraders of all classes of plant tissues. Two principal modes of decay occur in the Agaricomycotina, termed white rot and brown rot. Ectomycorrhizal (ECM) fungi, which play important ecological roles as tree symbionts, are also polyphyletically derived within the Agaricomycotina.

Objectives — We propose to explore the genomes of a diverse suite of saprotrophic Agaricomycotina, including representatives of several independent origins of wood decayers and ectomycorrhizal symbionts, to identify the key gene networks controlling fungal adaptation to their ecological niches. We also aim to access the impact of intraspecific genomic polymorphisms on the adaptation of a wood-decay Agaricomycotina fungus, Trametes versicolor, to its lignocellulolytic environment.

Approach — We have focused this study on the mechanisms of lignocellulose decomposition in wood decayers and acquisition of symbiotic features (i.e., carbohydrate-active enzymes (CAZy), lignin oxidoreductases, secreted effectors). Working with Bernard Henrissat (Head of the CAZy database) and his bioinformatics team, we have compiled the list of CAZy for all fungal genomes included in the Mycorrhizal Genomics Initiative and Saprotrophic Basidiomycetes Genomics Initiative dataset. This list contains module counts for Glycoside Hydrolases (GH), GlycosylTransferases (GT), Polysaccharide Lyases (PL), Carbohydrate Esterases (CE), Carbohydrate Binding Modules (CBM) and Auxiliary Activities (AA). Each of these categories was mined for modules that act on plant cell wall carbohydrates for a total of 38 families of plant cell wall degrading enzymes (PCWDE) modules.

To assess the rate of polymorphism in decay-related genes in a model saprotrophic fungus, 10 samples of T. versicolor have been obtained from different fungal collections, their genome re-sequenced and their phenotypic features characterized.

Key results —

- To elucidate the genetic bases of mycorrhizal lifestyle evolution, we sequenced new fungal genomes, including 13 ectomycorrhizal (ECM), orchid (ORM) and ericoid (ERM) species, and five saprotrophs, which we analyzed along with other fungal genomes.
- Ectomycorrhizal fungi have a reduced complement of genes encoding plant cell walldegrading enzymes (PCWDEs), as compared to their ancestral wood decayers. Nevertheless,

they have retained a unique array of PCWDEs, thus suggesting that they possess diverse abilities to decompose lignocellulose.

- Polyphyletic evolution of the ECM lifestyle is marked not only by convergent losses of different components of the ancestral saprotrophic apparatus but also by rapid genetic turnover in symbiosis-induced genes, some of which may reflect lineage-specific functional innovations, such as mycorrhiza-induced small secreted proteins (MiSSPs) involved in in planta accomodation.
- Ten Trametes versicolor strains isolated from different tree species and from various parts of the world have been phenotypically characterized (colonization rate on various media, ability to degrade various wood substrates (oak, beech, spruce), detection of different enzymatic activities involved in lignocellulose breakdown secreted during oak sapwood degradation, proteomic analysis of the same secretomes (oak sapwood degradation). Principal Component Analysis (PCA) of the obtained data suggest that the studied collection could divided into two major groups related to their origin (tree species).
- The functional analysis of the glutathione transferases superfamily has revealed their involvement in the adaptation of Trametes versicolor to its chemical environment. These results demonstrate the importance of the intracellular detoxification networks in the wood decay process mediated by white-rot fungi.
- Functional analysis revealed also that Trametes versicolor produce an exudate during oak degradation. We propose that such exudate production by pioneer wood colonizers such as Trametes versicolor is a way for facilitating extraction, concentration and degradation of wood extractives and other potential toxic by-products produced during the wood degradation oxidative process.
- These resources will facilitate field studies aiming to predict responses of communities of mycorrhizal and saprotrophic fungi to environmental shifts, such as altered forest-management practices and climate change.

Future Perspectives —

- Pursue the phylogenomic analysis of ectomycorrhizal symbionts and related wood/litter decayers.
- Pursue the functional characterization of MiSSPs.
- Pursue phenotypic/genomic analysis of the different studied Trametes strains.
- Pursue the functional characterization of the intracellular detoxification networks
- Pursue the functional characterization of the guttation phenomenon

Valorization —

Publications

Patyshakuliyeva A., Jurak E., Kohler A., Baker A., Battaglia E., De Bruijn W., Burton K.S., Challen M. P., Eastwood D. C., Gruben B. S., Mäkelä M. R., Martin F., Nadal M., Van den Brink J., Wiebenga a., Zhou M., Henrissat B., Kebal M., Gruppen H., De Vries R. P. (2013). Carbohydrate utilization and metabolism is highly differentiated in *Agaricus bisporus*. *BMC Genomics* 4: 663.

Bödeker, I. T., Clemmensen, K. E., de Boer, W., Martin, F., Olson, Å., & Lindahl, B. D. (2014). Ectomycorrhizal C ortinarius species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist*, 203(1), 245-256.

Levasseur, A., Lomascolo, A., Chabrol, O., Ruiz-Dueñas, F. J., Boukhris-Uzan, E., Piumi, F., ... & Benoit, I. (2014). The genome of the white-rot fungus Pycnoporus cinnabarinus: a basidiomycete model with a versatile arsenal for lignocellulosic biomass breakdown. *BMC genomics*, *15*(1), 486.

Riley, R., Salamov, A. A., Brown, D. W., Nagy, L. G., Floudas, D., Held, B. W., ... & Lindquist, E. A. (2014). Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. *Proceedings of the National Academy of Sciences*, *111*(27), 9923-9928.

Veneault-Fourrey, C., Kohler, A., Morin, E., Balestrini, R., Plett, J., Danchin, E., ... & Martin, F. (2014). Genomic and transcriptomic analysis of Laccaria bicolor CAZome reveals insights into polysaccharides remodelling during symbiosis establishment. *Fungal Genetics and Biology*, 72, 168-181.

Deroy, A., Saiag, F., Kebbi-Benkeder, Z., Touahri, N., Hecker, A., Morel-Rouhier, M., ... & Gelhaye, E. (2015). The GSTome reflects the chemical environment of white-rot fungi. *PLoS One*, *10*(10), e0137083.

Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., ... & Colpaert, J. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature genetics*, 47(4), 410-415.

Talbot, J. M., Martin, F., Kohler, A., Henrissat, B., & Peay, K. G. (2015). Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. *Soil Biology and Biochemistry*, 88, 441-456.

Roret, T., Thuillier, A., Favier, F., Gelhaye, E., Didierjean, C., & Morel-Rouhier, M. (2015). Evolutionary divergence of Ure2pA glutathione transferases in wood degrading fungi. *Fungal Genetics and Biology*, 83, 103-112.

Shah, F., Nicolás, C., Bentzer, J., Ellström, M., Smits, M., Rineau, F., ... & Braesel, J. (2016). Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytologist*, 209(4), 1705-1719.

Nagy, L. G., Riley, R., Tritt, A., Adam, C., Daum, C., Floudas, D., ... & Matsuura, K. (2016). Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities. *Molecular biology and evolution*, *33*(4), 959-970.

Nagy, L. G., Riley, R., Bergmann, P. J., Krizsán, K., Martin, F. M., Grigoriev, I. V., ... & Hibbett, D. S. (2017). Genetic bases of fungal white rot wood decay predicted by phylogenomic analysis of correlated gene-phenotype evolution. *Molecular biology and evolution*, *34*(1), 35-44.

Peter, M., Kohler, A., Ohm, R. A., Kuo, A., Krützmann, J., Morin, E., ... & Clum, A. (2016). Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic fungus Cenococcum geophilum. *Nature communications*, 7(1), 1-15.

Schwartz, M., Didierjean, C., Hecker, A., Girardet, J. M., Morel-Rouhier, M., Gelhaye, E., & Favier, F. (2016). Crystal structure of saccharomyces cerevisiae ECM4, a Xi-class glutathione transferase that reacts with glutathionyl-(hydro) quinones. *PloS one*, *11*(10), e0164678.

Valette, N., Perrot, T., Sormani, R., Gelhaye, E., & Morel-Rouhier, M. (2017). Antifungal activities of wood extractives. *Fungal Biology Reviews*, *31*(3), 113-123.

Posters

Aurélie Deroy : Ecole doctorale RP2E.

Others

IRG International Research Group on Wood Protection (St George, Utah, May 2014)

European Conference on Fungal Genetics, March 2014

Knudson Endowed Lectureship Award 2013, Oregon State University

Whetzel-Westcott-Dimock Endowed Lectureship 2014, Cornell University April 2014

33th New Phytologist Symposium "Networks of Power & Influence", May 2014

XVI Molecular Plant-Microbe Interactions Congress, Rhodes, July 2014

International Mycological Congress, Bangkok, August 2014

Plett et al. (2014) Proceedings of the National Academy of Sciences USA 111: 8299-8304

Kohler et al. (2015) Nature Genetics, 47, 410–415. Fungal Genomics Symposium, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 10 May 2014 Molecular Plant Microbe Interactions XVI, Rhodes, July 10th, 2014 Max Planck Institute for Plant Breeding Research, Cologne, April 15th, 2015

ICOM8, Flagstaff, August 1-7 2015

8th Utrecht PhD Summerschool on Environmental Signaling, August 24-26, 2015

SNHF 2015 "Quand les plantes se parlent", Paris, 12 février 2015

2nd International Molecular Mycorrhizal Meeting, 3-4 September 2015, Cambridge, UK

IRG International Research Group on Wood Protection (Vina del Mar, Chili, May 2015)

Kickoff Workshop of Bioasia program: "Extraction, characterization and valorization of biomolecules issued from wood first transformation by-products in Asia", (Bogor, September 2015)

Deroy et al., PloS ONE, DOI: 10.1371/journal.pone.0137083

Aurélie Deroy: PhD (defense 6th November 2015)