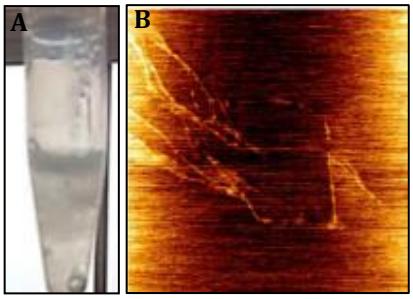
SSPWood



A : The recombinant PcSSP1 forms a gel at pH8.0
B: This gel is composed of fibers as shown by Atomic Force Microscopy imaging.

Functional characterization of fungal small secreted proteins in relation to wood degradation

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Context — Wood degradation mechanisms of saprophytic fungi are currently the focus of many research projects, especially because of the potential of these microorganisms in biomass valorization. While most of the studies concern the extracellular enzymes directly involved in wood degradation, very few concern the associated mechanisms, which enable fungi to resist to the oxidative and toxic environment arising from the degradation process. In particular, the first step of wood breakdown is the release of aromatic compounds known as extractives, which could be toxic for the cells. Interestingly, beside the classical genes involved in stress responses, small secreted proteins (SSP) seem to be involved in the cell response to oak molecules. Their exact role has not been determined yet however, first results clearly showed a relationship between wood specificity and secretion of SSP.

Objectives — The main objective of this project was to precise the role of SSP in fungal physiology (wood modification, cell signaling, stress response or detoxification....) during wood decomposition.

Approach — To determine the role of SSP in wood degradation processes various approaches have been used: comparative genomics, transcriptomics and proteomics to have an overview of the occurrence and the involvement of these proteins during wood degradation, and a more targeted approach, which consists in the production, the purification and the characterization of a recombinant SSP from *Phanerochaete chrysosporium* (PcSSP1) in heterologous bacterial system. Additionally, a physiological approach was used to characterize the protein at a functional level by generating mutants both in *Podospora anserina* and *P. chrysosporium.*

Key results — The secretion pattern of SSP in various Aspergillli species revealed that specificity exists according to the species, and the occurrence of these SSP within the secretomes, and in particular HsbA, could be related to the degradative process.

- Some of the SSP coding genes in *P. chrysosporium* are induced in presence of oak extractives suggesting a putative role either in the degradative process of these toxic molecules, or in the stress response that is associated to this process.
- The recombinant SSP1 from *P. chrysosporium* exhibits atypical biochemical features. The protein contains 4 cysteines that are fully oxidized leading to high stability.
- *P. chrysosporium* SSP1 exhibits alpha helix and beta sheet structures at pH 8.0. Moreover, SSP1 proteins organize as fibers as seen using Atomic Force Microscopy technique, and self-assemble to form a king of macroscopic gel. This organization is due to the presence of an alanine/glycine-rich extension at the C-terminal part of the sequence and is dependent on the ionic force of the buffer used.
- PcSSP1 exhibits a β-glucuronidase activity only under fiber-aggregated form.

- *P. anserina* knock-out mutants of SSP1 orthologues show a growth defect in presence of congo red, suggesting that these proteins could be involved in maintaining the integrity of the cell wall in stress condition.
- The development of a new technique for *P. chrysoporium* genetic transformation allowed obtaining mutants overexpressing GFP alone or in fusion with SSP1.

Main conclusions including key points of discussion — These proteins could have an important role in wood degradation processes since many SSP coding genes are present in the genomes of lignolytic fungi. Moreover, some of them are induced in presence of wood-derived molecules such as extractives. Our first results suggest that, because of its aggregation properties, SSP1 from *P. chrysosporium* could accumulate within the cell wall and scavenge the toxic molecules that are released during wood degradation to protect fungal cells and improve degradative activities.

Future perspectives — The perspectives of this project are to validate the role of *P. chrysosporium* SSP by generating fungal mutants in this organism and characterizing them. Moreover, some knock-out mutants have been obtained in *P. anserina* in collaboration with Philippe Silar (Université Paris Diderot) and are still under characterization. The corresponding recombinant proteins will be produced and characterized to evaluate their ability to form fibers. These *P. anserina* proteins exhibit sequence homologies with proteins involved in cell wall organization in yeast and appressoria development in pathogenic fungi; we will thus characterize them more deeply. Finally, the protein gel obtained could have biotechnological applications; a biophysical analysis will help evaluating its potential in valorization.

Valorisation —

Publications

Valette N, Benoit-Gelber I, Falco MD, Wiebenga A, de Vries RP, Gelhaye E, Morel-Rouhier M. (2016) Secretion of small proteins is species-specific within Aspergillus sp. Microb Biotechnol. 2016 May 7. doi: 10.1111/1751-7915.12361.

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

Valette N, Fernández-González AJ, Cuenot S, Gelhaye E, Morel-Rouhier M. Self-assembly of the small secreted protein SSP1 from *Phanerochaete chrysosporium* is required for β-glucuronidase activity. In preparation for submission to Biochimica and Biophysica Acta - General Subjects.

Fernández-González AJ, Valette N, Kohler A, Sormani R, Gelhaye E and Morel-Rouhier M. The early responses of *Phanerochaete chrysoporium* to oak extractives reveal the involvement of new detoxification enzymes. In preparation for submission to Environmental Microbiology.

Oral presentation at conferences and meetings

Morel-Rouhier M, Valette N, Sormani R and Gelhaye E. The various strategies developed by lignolytic fungi to cope with wood extracts. Trinational minisymposium "Environmental microbiology and biomass" Strasbourg 2015, April 10th.

Valette N, Gelhaye E and Morel-Rouhier M. Characterization of small proteins secreted by lignolytic fungi. Journées *Jean-Chevaugeon* Aussois 2016, January 25-29th.

Poster presentations at conferences:

Valette N, Gelhaye E and Morel-Rouhier M. Characterization of small proteins secreted by lignolytic fungi. ECFG Paris 2016, April 3-6th.

Valette N, Cuenot S, Gelhaye E and Morel-Rouhier M. Characterization of small proteins secreted by lignolytic fungi. 2nd MPIterMic-ABRE Workshop on Plant-Fungus Interaction Marburg (Germany) 2016, October 26-29th.

Fernandez Gonzalez AJ, Valette N, Perrot T, Sormani R, Gelhaye E and Morel-Rouhier M. Antifungal activity of wood extractives. 29th Fungal genetics Conference Asilomar California (USA) 2017, March 14-19th.

Valette N, Fernandez-Gonzalez AJ, Cuenot S, Sormani R, Gelhaye E and Morel-Rouhier M. Characterization of a *Phanerochaete chrysosporium* new protein putatively involved in biomass degradation. 29th Fungal genetics Conference Asilomar California (USA) 2017, March 14-19th.

Posters:

Valette N, Gelhaye E and Morel-Rouhier M. Caractérisation fonctionnelle de petites protéines sécrétées chez les champignons lignolytiques. Doctoral school seminar, 2015, January 15th.