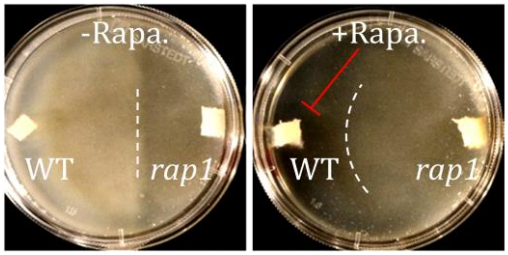
L4REX



Looking for REX (looking for *Phanerochaete chrysosporium* mutants Resistant to EXtractives)

Principle investigator: Rodnay Sormani

Partners: EA 4370 LERMAB

Collaboration: S. Dumarçay

Context — White-rot fungi are responsible for wood degradation. Those organisms are able to decay and use cellulose, hemicelluloses, and lignin as sources of carbon and energy for their growth. However, the oxidative processes used by those fungi to decay wood generate a myriad of potentially toxic molecules, including extractives. Extractives are non-structural components of wood that can be removed with a neutral to polar solvent. The composition of these extractives can vary depending on considered tissue, culture conditions and is species-specific. The extractives are responsible for the wood properties such as color and durability, as they are part of the tree's defense system.

To adapt to this toxic environment, rot fungi have developed various detoxification strategies. Comparative genomics approaches have shown extensions of families of genes coding for detoxification systems in wood-decaying fungi compared to other fungi (mainly cytochromes P450 and glutathione transferases (GST)). Transcriptomic analysis of *Phanerochaete chrysosporium* grown in the presence of oak-wood extractives revealed the induction of the expression of some of these genes involved in detoxification. Finally, at the functional level, interesting results concerning the biochemical characterization of GST and their interaction with extractives were obtained. However, although very informative, these data are not sufficient to determine the physiological role of these proteins in fungal cells during wood degradation. An unbiased approach is therefore essential to identify the actors involved in the detoxification process.

Objectives — The lack of physiological data on extractives detoxification pathways in wood-decaying fungi is mainly due to the lack of genetic tools available for those organisms. To circumvent this problem, we have developed a direct genetic strategy in the white-rot fungus *P. Chrysosporium*. This fungus has been chosen by the community as a model for wood decay studies. The first part of the project I developed aimed to prove the feasibility and relevance of such an approach. Two proof-of-concept studies were carried out, the first using an antifungal drug called itraconazole, the second using rapamycin. Wood extractives with antifungal activity from three different species were then used.

Approach — Description of the direct genetic strategy implemented:

- Identification of toxic molecules.
- Production of P. chrysosporium mutants
- Screening for mutants able to resist to the toxic molecule.
- Identification of causal mutations in resistant mutants.
- Understanding how the mutation enables resistance.

Key results —

- The "looking for *rex*" project has produced 5 collections of mutants which are resisting to toxic molecules.
- The *rit* mutants (*resistant to itraconazole*) carry mutations in a gene coding for CYP51. This cytochrome P450 is involved in ergosterol biosynthesis. *rit* mutants can produce ergosterol even in presence of itraconazole.
- The phenotype of *rap* (*rapamycin-resistant*) mutants is linked to mutations in at least one of the 3 genes coding for FKBP12, TOR 1 and TOR 2 (FK506 Binding Protein 12, Target Of Rapamycin respectively).
- Three collections of wood extractable resistant (rex) mutants were generated, with the causal mutation identified in 1 of the cases. Study of the mutated gene function is underway.