



Figure 1 : Modèle 2D d'une séquence pré-miRNA pour un microRNA-like, régulateur de l'expression génique chez *Phanerochaete chrysosporium*. La séquence du microRNA-like mature est indiquée en vert.

### Exploring the miRnome of the ligninolytic fungus *Phanerochaete chrysosporium* during wood extractive-induced stress

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Thematic action concerned: WP1

#### **Context** —

In the context of ecosystem preservation, forest management and sustainable economic development, understanding the key factors that improve wood durability has crucial importance. The wood durability is directly related to lignin/cellulose proportion, and the accumulation of specialized metabolites, known as wood extractives, prevents the degradation by fungi. Some ligninolytic fungi are more tolerant than others likely because of both an efficient non-specific extracellular enzymatic system for the degradation of complex molecules, and an advanced intracellular multistep detoxification system that neutralizes wood extractives. Deciphering the molecular mechanisms responsible for fungal tolerance to wood extractives is crucial to develop new strategies to increase wood durability and to obtain microorganisms with efficient detoxification capacities for biotechnological applications.

#### **Objectives** —

The objectives are to identify and describe the microRNA-likes (miRNAs) found in the ligninolytic fungus, *Phanerochaete chrysosporium*, and evaluate the gene regulatory network, controlled by these small RNAs, allowing the stress tolerance in wood-degrading conditions.

### **Approaches —**

Genomic and bioinformatic approaches will be used to predict and characterize the miRNAs. Based on the *in silico* predictions, the miRnome sequencing using the NGS will be done to confirm the function of miRNA in stress tolerance and the function of central regulators of physiological processes.

### **Key results —** (presented as separated bullet points)

- A bioinformatic pipeline to identify miRNAs and their loci in genomes was developed. Our miRNA database has 1145 sequences, retrieved from scientific papers and RNA databases.
- 37 miRNAs were predicted by homology in *P. chrysosporium*.
- RNA modeling has revealed a hairpin structure in 13 pre-miRNA sequences (example figure 1), confirming the presence of functional miRNAs in *P. chrysosporium*.
- About 90% of mRNA sequences are predicted as targeted by the identified miRNAs, and in stress-related gene category, the mevalonate and ergosterol pathways are one of the main targets.
- Culture conditions were optimized to test stress responses to wood extractives and evaluate the roles of sterols in the physiology of ligninolytic fungi.

### **Main conclusions including key points of discussion —**

During the first year, the *in silico* prediction has allowed to confirm the presence of functional miRNAs in the ligninolytic fungi, *P. chrysosporium*. The sequence homology of miRNAs in basidiomycota is relatively low, and a loci conservation between species is not observed. Among all predicted miRNAs, two seem to be regulators of stress tolerance, modulating the membrane sterol level and targeting several transcripts coding for key enzymes of the mevalonate and ergosterol pathways.

### **Perspectives —**

Based on the *in silico* predictions and the optimization of culture conditions for stress tolerance, the miRnome sequencing will be performed in the second year of the project to confirm the predicted miRNAs and discover new miRNAs. In parallel, the gene expression of mevalonate and ergosterol biosynthesis and its regulation by miRNA(s) will be evaluated in stress conditions.

**Valorization** — (scientific: publications, book chapter, presentation at conferences,...); economic: Soleau envelope, patent, license,...; distribution: press release, interview,...)

*To date, the miR-2-woodex project has not yet been valued.*

### **Leveraging effect of the project—**

Following the miR-2-woodex project, a structuring research project was funded by the A2F pole of the University of Lorraine to study ergosterol biosynthesis and stress tolerance in ligninolytic fungi. A project to test new ergosterol biosynthesis as wood preservatives was applied to Grand-Est Region, to fund a thesis.