



## 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 —**

- A bioinformatic pipeline to identify miRNAs and their loci in genomes has been developed. Our miRNA database contains 1145 sequences, extracted from scientific articles and sRNA databases.
- Culture conditions were optimized to test stress responses and evaluate the roles of sterols in the physiology of ligninolytic fungi.
- Total RNA was extracted for 4 cultures of the fungus under control conditions and 4 under extractable stress conditions. The quality and quantity were sufficient to prepare NGS-type libraries for sRNA.
- The 8 libraries were sequenced and for each between 9 and 12 million high quality reads were obtained, having more than 99% of the bases sequenced with a quality factor (QV) greater than 20.
- The pre-processing steps of sequencing data consist of removing low quality reads or reads containing adapters, removing reads that have similarities with tRNAs, rRNAs and other ncRNAs that are not miRNAs. The last step of pre-processing is the comparison of the reads with each other, to keep only the unique sequences. Ultimately, approximately 500,000 to 800,000 unique reads were obtained per library.
- For the prediction of miRNAs from sequencing data, all the reads were collected and analyzed by the miRdeep2 tools. Thus 684 miRNAs were predicted, including 257 sequences presenting homologies with fungal miRNAs, identified in other species.
- Expression analysis shows that 43% of microRNAs are expressed on average at more than 100 reads and 7% at more than 1000 reads. The differential expression test between the two conditions demonstrates that 40 microRNAs are significantly upregulated in the stress condition and 19 downregulated.
- Prediction of the targets of miRNAs downregulated under stress conditions indicates that potentially 607 transcripts are regulated by this mechanism. The Gene Ontology (GO) term enrichment analysis shows that the most represented categories are linked to metabolic processes (amino acids and carbohydrates) and response to stress. The target transcripts of the upregulated microRNAs numbered 2994 and in GO categories, such as transcription regulation, vesicular transport, endoplasmic reticulum and lipid biosynthesis.

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

During the first year, in silico prediction made it possible to predict functional miRNAs in the ligninolytic fungus *P. chrysosporium*. Sequencing data from sRNA libraries made it possible to predict 684 miRNAs, including 427 new miRNAs with no homology in other fungi. This prediction shows many miRNAs because only 168 miRNAs were identified in *Ganoderma lucidum*, 364 *Fusarium oxysporum* f. sp. *cupense*, for example. For comparison, the yeast *Saccharomyces cerevisiae* does not have a functional microRNA. A total of 1917 microRNAs are counted for the human genome and 327 for that of *Arabidopsis*, two model organisms for regulatory RNAs. Analysis of the expression of these miRNAs shows significant variations under stress conditions. This result indicates that the profile of the miRnome varies depending on the conditions and that miRNAs have a regulatory function, involved in the stress response of this fungus. The other element supporting the role of central regulator of miRNAs is the fact that the transcripts targeted by regulation are specific to the stress response to exposure to phytosterols, lipophilic extractables from wood. Indeed, overexpressed miRNAs, which degrade targeted transcripts or inhibit their translation, recognize transcripts encoding lipid biosynthesis, ergosterol and vesicular transport. This seems to suggest that miRNAs can regulate lipid homeostasis, more particularly biosynthesis and uptake from the external environment.

### **Perspectives —**

The identification of the presence of the miRNA regulatory mechanism in the fungus *P. chrysosporium*, particularly under stress, opens new perspectives. Thus, these results confirm that the silencing technique can be used for the characterization of genes using reverse genetics approaches. Furthermore, the involvement of miRNAs as central regulators of the stress response is an avenue to explore for biotechnological applications, not only in ligninolytic fungi. Pioneering work shows that microRNAs can act as regulators to increase resistance to solvents (ethanol) during fermentation, with significant repercussions on bioreactor yield.

### **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 evaluated but the preparation of a short communication type article is underway.



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

Following the miR-2-woodex project, a structuring research project was funded by the A2F center of the University of Lorraine to study ergosterol biosynthesis and stress tolerance in ligninolytic fungi. A thesis began in 2023 to understand the mechanisms of lipid homeostasis, particularly for sterols, in a ligninolytic fungus *Ophiostoma piceae*. *O. piceae* is an exosymbiont of the bark beetle *Ips typographus*, which is auxotrophic to sterols for its reproduction and development. The characterization of sterol metabolism and the role of miRNAs in lipid homeostasis can lead to biotechnological applications, such as the fight against insect pests.