



Understanding the molecular mechanisms of fungal-bacterial-mediated detoxification of preservative treated wood.

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Thematic action(s) concerned : *WP1*

Context —

The wood industry extensively exploits wood preservatives, leading to the generation of approximately 1.4 million tons of treated wood waste annually in France (ADEME, CODIFAB/FCBA). According to European and French regulations (Decree No. 2002-540), treated timbers are classified as hazardous waste. Currently, incinerating wood waste poses environmental pollution and risks to public health. In response to this issue, we have created a microbial consortium of bacteria and fungi through the ANR project "WOODWASTE" (Project-ANR-18-CE04-0012). This consortium can detoxify wood treated with copper/azole compounds in just a few days. Additionally, the DETOXOMICS project aims to identify the molecular mechanisms underlying the interactions between microorganisms involved in the detoxification process, ensuring their survival.

Objectives —

The overarching goal of the project is to unravel the efficiency of microorganisms, including fungi and soil bacteria, whether acting independently or in collaboration, as potent biocatalysts for detoxifying wood treated with azoles and copper (Tanalith E3474). To achieve this, we outline two specific objectives for the project:

- 1) Understanding the molecular mechanisms used by the microorganisms against toxic preservatives
- 2) Investigating the cooperative mechanisms between fungi-bacteria during wood detoxification

Approaches —

To achieve these goals, we utilized a system biological approach “multi-Omics” to identify of new genes and pathways that are involved in the detoxification of different recalcitrant compounds.

The combination of these techniques will allow us to identify specific gene regulation (transcriptomics), protein production (proteomics), and the community metabolism (metabolism) of the microbial partners. In addition, this will provide a path for self-validating findings through a combined parallel ‘omics’ approach and will ultimately result in the accelerated understanding of these complex interaction and processes, leading to better bioremediation strategies.

Key results —

We were able to obtain the proteomes and metabolomes of the two consortia studied (white rot/bacteria, brown rot/bacteria) and the transcriptomes of the white rot/bacteria consortium. We have thus generated a massive dataset that is still being analyzed. However, some important conclusions can already be drawn.

- The massive production of oxalate by the brown rot fungus leads to the biological leaching of copper and its immobilization in the liquid phase. Conversely, biosorption of copper by the white rot fungus allows it to develop on the treated wood. The two fungi thus allow bacteria to survive through very different mechanisms of action, leading to the development of bacterial communities whose composition varies greatly depending on the fungus present. Certain strains dominate in the presence of the brown rot fungus, while others are favored by the presence of the white rot fungus. In addition, although the bacterial strains in the consortium are closely related, only 4 of the 10 strains inoculated at T0 developed in the presence of the fungi and Tanalith.
- Over 12,000 peptides were identified in the metaproteome of bacteria-fungus consortia grown on Tanalith-treated wood vs. untreated wood. The presence of Tanalith induced a strong response at the proteomic level, with around 25% of proteins varying in level depending on the conditions. Bacteria whose growth increased in the presence of Tanalith were characterized by a high level of over-expressed proteins, in contrast to bacteria whose growth was reduced. Among the proteins highly expressed in the presence of Tanalith are numerous metal transporters, as well as sugar transporters and elements of the DNA repair machinery. However, the levels of expression of these proteins varied according to the type of fungus present in the microcosm, suggesting that the level of toxicity differed according to the fungus.
- Conversely, the study of the fungal transcriptome showed a low rate of gene over-expression in the presence of bacteria on Tanalith and a massive under-expression of fungal transcripts.
- Cross-analysis of the transcriptomes, proteomes and metabolomes will provide an integrative view of the response at cellular level of the various micro-organisms (in progress). These results will contribute to our understanding of the bacterial communities that survive in the toxic microcosm, both in the presence and absence of fungi.

Main conclusions including key points of discussion —

In summary, our findings indicate that fungi and bacteria can coexist and cooperate, despite competing for a common resource in the toxic microcosm. This collaboration is feasible as long as one of them engages in the detoxification process, and the toxicity level remains low enough for at least one detoxifier to thrive. There is speculation that fungi may serve as gatekeepers, influencing the survival of specific bacteria in a toxic microcosm.

Perspectives —

Analyses of data are still on going. Overall, the high throughput dataset generated during the DETOXOMICS project will lead us to understand the molecular dialogues involved in the wood detoxification process and will have the potential to identify new bioactive compounds. Furthermore, it will allow us characterize enzymes or metabolites involved in the detoxification. Finally, it will help us optimize the detoxification process, which could further help us develop at a large-scale process required for industry to valorize their wood wastes.



Valorization —

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

18th International Symposium on Microbial Ecology Lausanne, Switzerland – Presented my work

Labex Doc-post doc day and UMR internal seminars – Presented my work

Pandharikar, G.; Claudien, K.; Rose, C.; Billet, D.; Pollier, B.; Deveau, A.; Besserer, A.; Morel-Rouhier, M. Comparative Copper Resistance Strategies of *Rhodonia placenta* and *Phanerochaete chrysosporium* in a Copper/Azole-Treated Wood Microcosm. *J. Fungi* 2022, 8, 706. Visualization of Fungi During Wood Colonization and Decomposition by Microscopy: From Light to Electron Microscopy. *A Besserer, C Rose, A Deveau Microbial Environmental Genomics (MEG)*, 337-361

Leveraging effect of the project—

We have submitted funding requests to Lorraine Université d'Excellence (LUE), and we are pleased to announce that we have successfully secured a grant for recruiting a post-doctoral candidate. The appointed post-doctoral candidate will play a crucial role in conducting in-depth analyses of the data acquired during the detoxomics project.