Effect of cultivation conditions on the bioconversion of 4-hydroxybenzoic acid in two white-rot fungi

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Introduction

White-rot fungi (WRF) are the most efficient organisms for lignin degradation in nature. A recent study has also shown the ability of WRF in funneling lignin-derived aromatics, including 4-hydroxybenzoic acid (4HBA) and vanillic acid, to central metabolism[1]. This is achieved by several types of enzymes such as hydroxylases, dioxygenases, and decarboxylases. However, it is unknown how the cultivation conditions affect the conversion of lignin-derived aromatic compounds. To address this, we performed multi-omic analyses and tracked the conversion of 4HBA in different cultivation conditions in two white-rot fungi: *Trametes versicolor* and *Ceriporiopsis subvermispora*. Specifically, we evaluated the effect of static and agitation cultivation conditions in the absence and the presence of antioxidants on fungal performance.

Rationale

- Two white-rot fungi
  - → Tv: *Trametes versicolor*[2] (degrade cellulose and lignin simultaneously)
  - → Cs: *Ceriporiopsis subvermispora*[3] (preferentially degrade lignin)
- Cultivation conditions
  - → **Static/Agitation**
    - Difference in cell morphology, and potentially cell wall/membrane structure could affect transport of aromatics (Figure 1).
    - Agitation enhances oxygenation in the system and potentially increases the oxidation.
  - → **Antioxidant/Without Antioxidant**
    - Ascorbic acid and α-tocopherol: a synergistic effect
    - Antioxidant hampers oxidative stress for the fungi.
    - Antioxidants are likely to reduce the repolymerization of monomeric aromatic compounds (which ultimately may enhance the uptake).

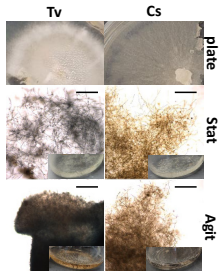


Figure 1 Culture and cell morphologies of *Trametes versicolor* (Tv) and *Ceriporiopsis subvermispora* (Cs). Top: fungal hyphae on YMPG plates. Middle: cell and culture morphologies of fungi under static condition (Stat). Bottom cell and culture morphologies of fungi under agitation condition (Agit). Bars: 100 µm.

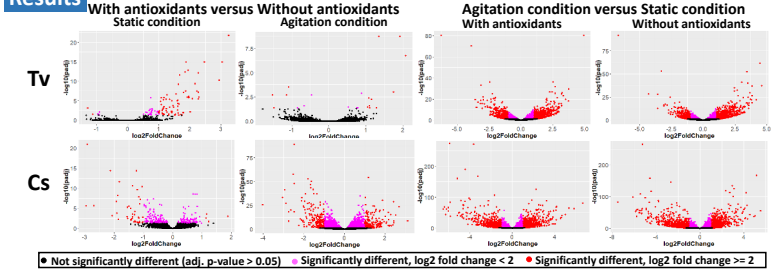
Methodology

2 WRF (Tv and Cs), **4HBA and Cellobiose** as C source
Cultivation conditions: Static/Agitation (Stat/Agit) and Antioxidant/Without Antioxidant (AO/WO)
Combination: StatAO, AgitAO, StatWO, AgitWO

Carbon conversion experiments
- 3.5 mM Cellobiose and 7.5 mM 4HBA
- Sample collection at 2, 4, 6 and 8 days
- Track conversion of 4HBA to 4-hydroxybenzaldehyde (4HBal), protocatechuate (PCA), hydroquinone (HQ) in supernatant

Transcriptomics and proteomics analyses
- 5 g/L cellobiose as a carbon source
- 2 mM 4HBA as an inducer
- Sample harvest after 24 hours

Results



The comparison between agitation and static cultivation condition shows a higher number of differentially expressed transcripts than the comparison between cultivations in the presence or the absence of antioxidants

Figure 2 Volcano plots depicting transcriptomic results for pairwise comparisons among all cultivation conditions: static with antioxidants versus static without antioxidants, agitation with antioxidants versus agitation without antioxidants, agitation with antioxidants versus static with antioxidants, agitation without antioxidants versus static without antioxidants. Tv: *Trametes versicolor*, Cs: *Ceriporiopsis subvermispora*.

The comparison between agitation and static cultivation conditions has more concordant changes in intracellular proteins than the comparison between cultivations in the presence and the absence of antioxidants

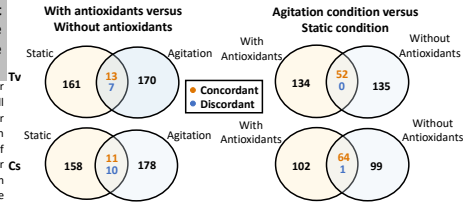


Figure 3 Venn diagrams depicting the number of intracellular proteins that show significant abundance differences among all cultivation conditions. Overlapping regions indicate the number of proteins that show significant abundance differences in both pairwise comparisons. Orange text indicate the number of proteins with concordant patterns (significant higher or lower abundances in both conditions). Blue texts indicate protein numbers with discordant patterns (higher abundance in one case but lower in the other case, and vice-versa). Tv: *Trametes versicolor*, Cs: *Ceriporiopsis subvermispora*.

Cultivations in agitation cause upregulation of several oxidase genes in the proposed 4HBA catabolic pathway such as aldehyde dehydrogenases, aldehyde oxidases, and hydroxylases in both fungi.

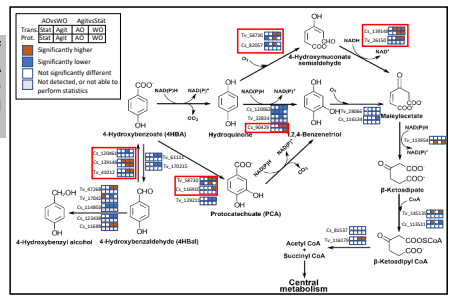
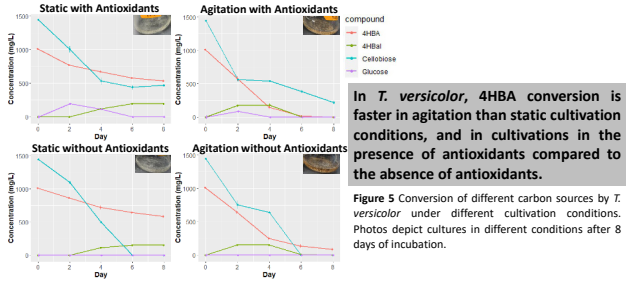
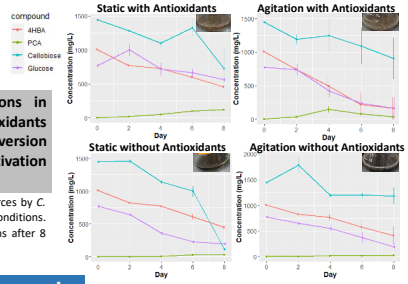


Figure 4 Gene mapping on the enzymatic steps of the proposed 4HBA conversion pathway[1]. Gene ids of *T. versicolor* and *C. subvermispora* in each proposed enzymatic step are indicated. Transcriptomic and proteomic results of each gene/enzyme are labeled. Highlights are oxidase genes that are overexpressed under agitation conditions. Abbreviations: Trans., transcriptomics; Prot., proteomics; AO, with antioxidant treatment; WO, without antioxidant treatment; Stat, static condition; Agit, agitation condition; vs, versus.



In *T. versicolor*, 4HBA conversion is faster in agitation than static cultivation conditions, and in cultivations in the presence of antioxidants compared to the absence of antioxidants.

Figure 5 Conversion of different carbon sources by *T. versicolor* under different cultivation conditions. Photos depict cultures in different conditions after 8 days of incubation.



In *C. subvermispora*, cultivations in agitation with additional antioxidants have the fastest 4HBA conversion compared to other cultivation conditions.

Figure 6 Conversion of different carbon sources by *C. subvermispora* under different cultivation conditions. Photos depict cultures in different conditions after 8 days of incubation.

Conclusions and future work

- Different cultivation conditions have greater effects on 4HBA conversion in *T. versicolor* than *C. subvermispora*.
- Trends of carbon conversion in *C. subvermispora* suggest different carbon metabolism compared to *T. versicolor*.
- Intracellular metabolomics will be further conducted to determine the effect of cultivation conditions on catabolic intermediates belonging to the 4HBA conversion pathway.

References

1. Del Cerro, Carlos, et al. "Intracellular pathways for lignin catabolism in white-rot fungi." *Proceedings of the National Academy of Sciences* 118.9 (2021).
2. Floudas, Dimitrios, et al. "The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes." *Science* 336.6089 (2012): 1715-1719.
3. Fernández-Fueyo, Elena, et al. "Lignin-degrading peroxidases from genome of selective ligninolytic fungus *Ceriporiopsis subvermispora*." *Journal of Biological Chemistry* 287.20 (2012): 16903-16916.

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