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SETAC Europe 2008, Warsaw





STRUCTURAL AND FUNCTIONAL TRAITS OF THE BASIDIOMYCETE TRAMETES VERSICOLOR ARE BIOMARKERS FOR THE ASSESSEMENT OF SOIL ECOTOXICITY?

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Rationale / objectives

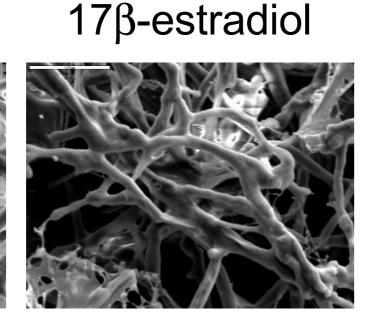
- Fungi constitute one of the largest biomass in the soil, and are responsible of key environmental functions.
- As a consequence, they may be pertinent indicators for soil ecotoxicity assessment.
- ► Our objectives are i) to understand the physiological basis of structural and functional disturbances of these organisms after exposure to pollutants, ii) to develop fungal-based tools for soil ecotoxicity assessment.

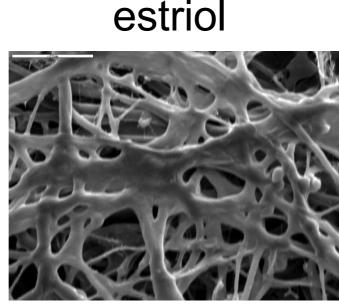
Pollutants alter the morphology of fungal hyphae

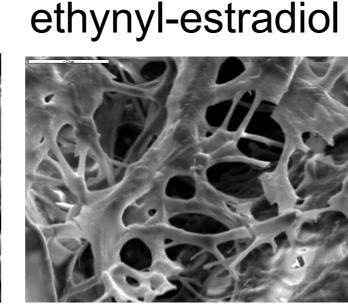
The effects on fungal morphology were assessed by treating pure cultures of *T. versicolor* with estradiol, estrone, estriol and ethynyl-estradiol. Each hormone was provided at 0.01 mM in the culture medium. The effects were assessed by SEM after 4 days of exposure.

Control









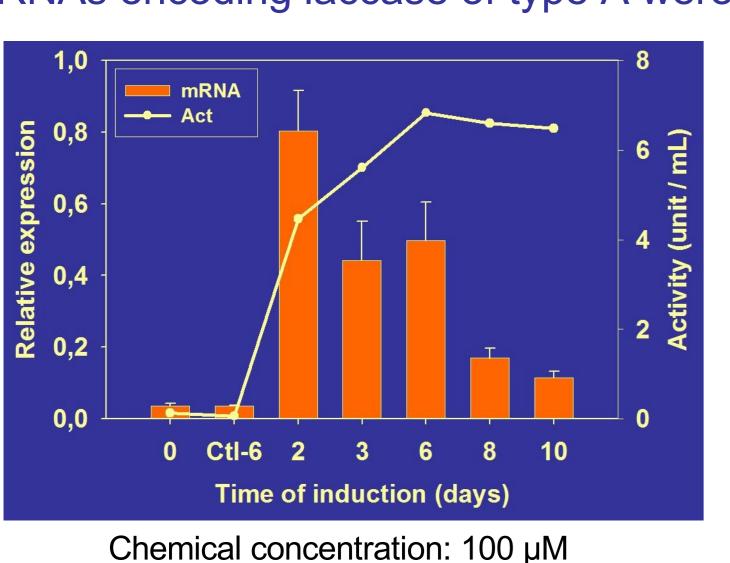
▶ Different morphological alterations arise from fungal exposure to estrogens, even at lower concentrations (10-6 mM). Ethynyl-estradiol induces an unusual hyphae morphology. 17β-estradiol seems the less active compound, because of its rapid biotransformation by the fungus.

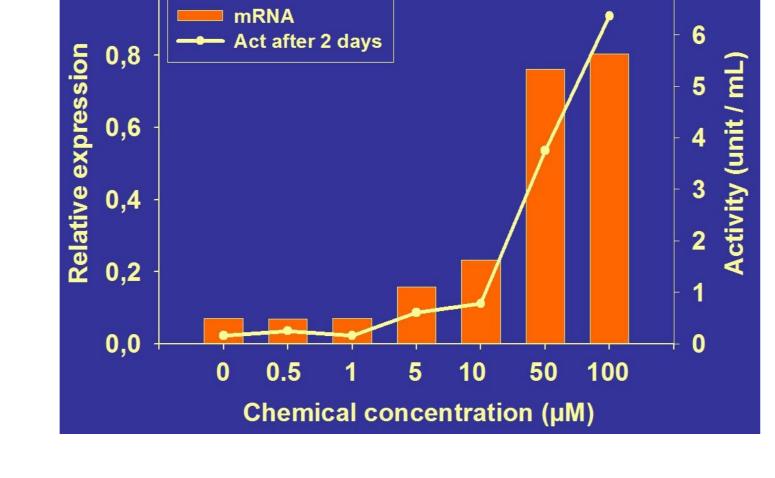
► Other effects have been previously noticed after exposure to heavy metals, including hyphae shrinkage, reduced index of ramification and roughness.

► Image analysis will be used to quantify these morphological alterations, and establish a typology by family of pollutants.

Pollutants have effects at the transcriptomic level

The effects of pollutants on mRNA production were assessed by treating pure cultures of *T. versicolor* with 2,5-dimethylaniline. After different durations of exposure, mRNAs encoding laccase of type A were extracted and quantified by Q-PCR. In parallel, enzymatic activity was measured using ABTS as a substrate.





- ► Fungal expose to 2,5-dimethylaniline results in a time-dependent and dose-dependant synthesis of mRNA encoding the laccase. That induction well corresponds to an increase of enzymatic activity.
- ► Similar effects are observed with environemental pollutants such as herbicides and endocrine disrupters.

Pollutants modify the activity of oxidases

The effects of pollutants on the activity of the oxidases secreted by *T. versicolor* were assessed by treating pure cultures with copper in media exhibiting moderate (black bars) or low (white bars) metal complexing properties. After 7 days of exposure, activities of secreted enzymes were measured using UV-Vis spectrophotometry.

- ► Laccase and Mn-peroxidase are constitutive enzymes in *T. versicolor*. Their activity is increased after expose to Cu, with a dose-response relationship. An low complexing medium, allowing a high metal bioavailability, increases the sensitivity of the response.
- ► Lignin peroxidase is not constitutive in *T. versicolor*. It is specifically expressed in the presence of copper.
- 9000 8000 7000 6000 6000 Ctrl 7 6 5 4 3 - Log [Cu added]

 140 600 Ctrl 7 6 5 4 3 - Log [Cu added]

 140 600 Ctrl 7 6 5 4 3

► Similar effects have been previously observed on laccase activity with other environmental pollutants such as herbicides, endocrine disrupters, and heavy metals.

Conclusions

- 1) In pure cultures, structural and functional traits of *T. versicolor* are affected by fungal exposure to environmental pollutants.
- 2) Additional studies are needed to assess the sensitivity and selectivity of the responses.
- 3) Methodologies have to be developed to perform studies in soils.