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Exocellular oxidases can be sensitive and selective biomarkers of fungal exposure to metals

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INTRODUCTION

Soil pollution by heavy metals is a worldwide problem. Only a few tests are available to assess the effect of pollutant on soil microorganisms, and more widely on this ecosystem. Metals can modulate the expression of enzymes secreted by microorganisms and involved in soil functioning. Methods based on enzymatic activity measurements may offer promising perspectives for ecotoxicological risk assessment in soils. Because of their ecological pertinence, we retain filamentous fungi for the development of enzymatic methods.

OBJECTIVES

The present study aims at assessing both selectivity and sensitivity of the response of exocellular oxidases secreted by the basidiomycete *Trametes versicolor* exposed to metals.

For that purpose, the activities are monitored in the presence of metals and in media exhibiting different levels of metal complexation. Exposures with mixtures of metals are designed to reflect soil multiple contaminations. Threshold metal toxicity to this fungus were determined.

METHODS

I- METAL TOXICITY AND MORPHOLOGICAL RESPONSES

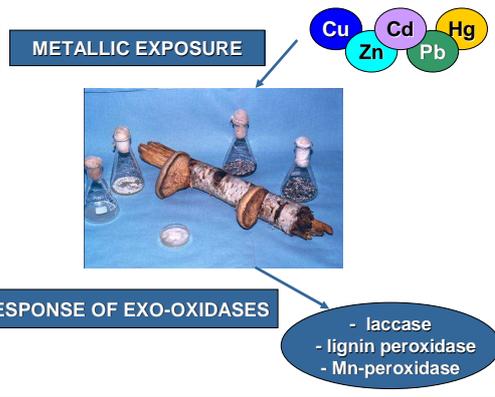
- Cultures of *Trametes versicolor* in basic liquid medium during two weeks
- Addition of Cu, Zn, Cd, Pb or Hg solutions during the cultivation (3th day)
- Determination of dry biomass used as toxicity/tolerance indicator at the end of cultivation
- Observations in scanning electron microscopy to characterize the morphological responses

II- RESPONSES OF EXOCELLULAR OXIDASES

- Each metal is added in basic medium at three concentrations ($Cu_{tot} = 0.01, 0.1$ and 1 mM)
- Measurements of oxidase activities in culture media during the cultivation (after three days, one or two weeks exposure)

III- SENSITIVITY OF ENZYMATIC RESPONSES

- Use of a low complexing liquid medium (suppression/diminution of some nutritive elements known as metal ligands) to raise the bioavailability
- Addition of Cu (from 1 nM to 1 mM) or mixtures of metals (0.1 mM for each metal)
- Measurements of activities in culture media at defined times



RESULTS

I- METAL TOXICITY AND MORPHOLOGICAL RESPONSES

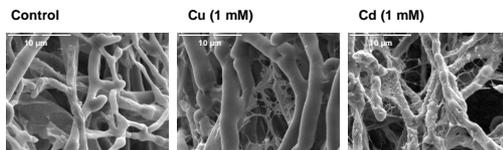


Fig. 1. Scanning electron microscopy (magnification, x 5000) of *T. versicolor* mycelia exposed for two weeks to Cu or Cd

- Metals have no significant effect on the biomass of *T. versicolor* except mercury at the total concentration of 1 mM.
- However, metals cause some morphological alterations of mycelia and the synthesis of extracellular matrix (Fig. 1).

→ In spite of morphological impacts, *T. versicolor* is tolerant of heavy metals

II- RESPONSES OF EXOCELLULAR OXIDASES (exposure for two weeks)

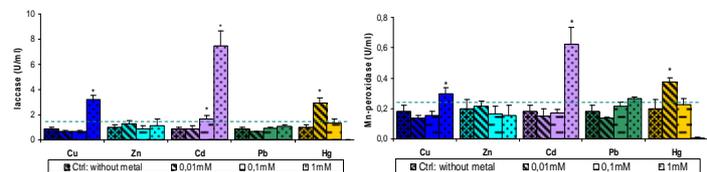


Fig. 2a. The values means \pm SE (n=9); significant differences $^*p < 0.05$

Fig. 2b. The values means \pm SE (n=9); significant differences $^*p < 0.05$

- Laccase and Mn-peroxidase activities are increased by copper and cadmium at the total dose of 1 mM (Fig. 2a and 2b).
- The response to mercury is more sensitive with an increase in activities from 0.01 mM (At 1 mM, the fungus doesn't grow).
- Zinc and lead are without effects on these activities.
- Lignin peroxidase is specifically expressed in the presence of copper.

→ Expression of exocellular oxidases is modulated by heavy metals

III- SENSITIVITY OF ENZYMATIC RESPONSES (exposure for two weeks)

1- Comparison of media

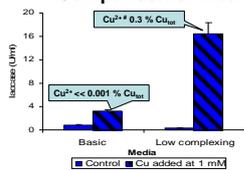


Fig. 3. The values means \pm SE (n=9).

- In the low complexing medium, the presence of free copper species is favoured (determination of Cu^{2+}).
- The production of oxidases increases with the free copper content (Fig. 3).

2- Dose-responses to copper

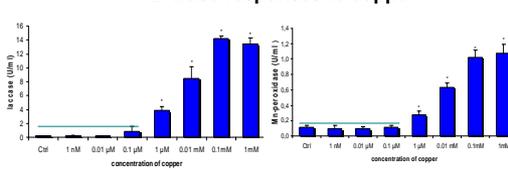


Fig. 4a, and 4b. The values means \pm SE (n=9); significant differences $^*p < 0.05$

- In the low complexing medium, the oxidase activities are significantly increased by copper from 1 μ M (Fig. 4a and 4b).
- This response of exo-oxidases is more sensitive than in the basic medium where the threshold is 1 mM (Fig. 4 vs Fig. 2).

3- Mixtures of metals

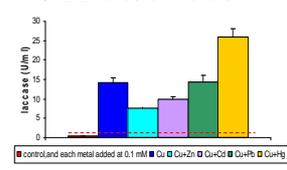


Fig. 5. The values means \pm SE (n=9).

- Mixtures of metals provided contrasted results.
- In all cases, the oxidase activities increase compared to control (Fig. 5).

→ The sensitivity and the intensity of enzymatic responses to copper are increased when metal is more bioavailable

CONCLUSION

Trametes versicolor responds to heavy metals by morphological modifications and modulations of enzymatic expression. Cadmium, copper and mercury are able to induce the production of exocellular oxidases. Since the enzymatic responses carry out at environmental levels of metals, these enzymes can be biomarkers of fungal exposure to metals in contaminated soils. Further studies are now necessary to improve enzyme extraction from soils.