The response of extracellular enzymes to copper depends on the developing phase in the fungus Trametes versicolor.

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INTRODUCTION

Human activities introduce organic and metallic contaminants in the cropped soils that can alter their functioning. Environmental risk assessment needs the development of tools to quantify the ecotoxicity of the contaminants. Among soil organisms, filamentous fungi produce enzymes involved in the biogeochemical cycles. These enzymes may be used as indicators of fungal exposure to contaminants in soils. However, soils are dynamic ecosystems in which fungi are in growing or stationary phases. The fungal response to contaminants can depend on the developing phase.

OBJECTIVES

The present study aims at establishing how the developing phases of the fungus *Trametes versicolor* modulate the expression of extracellular enzymes after exposure to copper.

For that purpose, it is necessary to monitor the activities of selected enzymes during the fungal growing and the stationary phases without metal contamination, and thereafter in the presence of metal.

METHODS

I- FUNGAL DEVELOPMENT AND METAL EXPOSITION
- Cultures of *Trametes versicolor* in liquid medium during two weeks
- Determination of dry biomass during the cultivation to characterize the different phases of fungal development.

II- EXPRESSION OF EXTRACELLULAR ENZYMES WITHOUT COPPER
- Measurements of hydrolase and oxidase activities in culture media during each phase
- Activities are brought back in the dry biomass

III- RESPONSES OF ENZYMES IN THE PRESENCE OF COPPER
- Addition of copper either during the growing or stationary phases at the total concentrations of 20 p.p.m. or 200 p.p.m. (ranged in environmental or contaminated levels respectively)
- Measurements of activities in culture media during each phase

SECRETION OF ENZYMES

HYDROLASES
- aryl-sulfatase
- β-D-glucosidase
- acidic and alkaline phosphatases
- N-acetyl-β-glucosaminidase
- β-D-galactosidase
- urease

OXIDASES
- laccase
- Mn-peroxidase
- lignin peroxidase

RESULTS

I- FUNGAL DEVELOPMENT AND METAL EXPOSITION

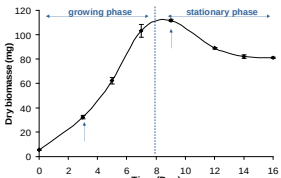

Fig. 1. The values means ± SE (n= 3).

- In our cultivating conditions, *T. versicolor* grows until on the 8th day.
- Then, the fungus is in stationary phase in which carries out a progressive diminution of the biomass.

→ **Addition of copper either on 3th or 9th days, during the growing or stationary phases respectively**

II- EXPRESSION OF EXTRACELLULAR ENZYMES WITHOUT COPPER

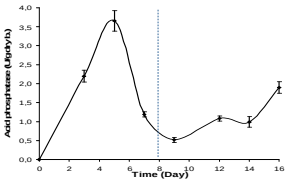

Fig. 2. The values means ± SE (n= 9).

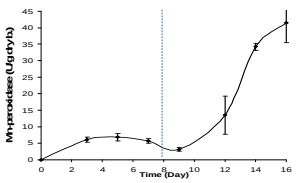

Fig. 3. The values means ± SE (n= 9).

- Aryl-sulfatase, alkaline phosphatase and lignin peroxidase are not produced.
- Acidic phosphatase, urease, β-D-glucosidase and N-acetyl-β-glucosaminidase are mainly expressed during the growing phase (Fig 2).
- Oxidase (laccase and Mn-peroxidase) and β-D-galactosidase activities increase during the stationary phase (Fig 3).

→ **Expression of extracellular enzymes depends on the developing phase**

III- RESPONSES OF ENZYMES IN THE PRESENCE OF COPPER

1- Responses of extracellular hydrolases to copper (200 p.p.m.)

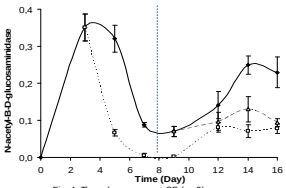

Fig. 4. The values means ± SE (n= 9).

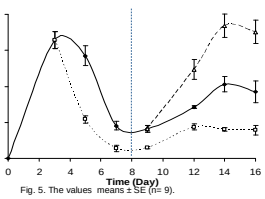

Fig. 5. The values means ± SE (n= 9).

Control ---□--- Cu added during the growing phase ---Δ--- Cu added during the stationary phase

- Hydrolase activities are decreased by copper independently of the fungal development, excepted for β-glucosidase (Fig. 4 vs Fig. 5).

2- Responses of extracellular oxidases to copper (200 p.p.m.)

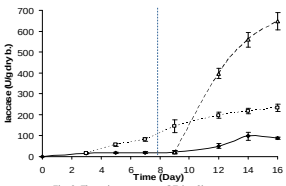

Fig. 6. The values means ± SE (n= 9).

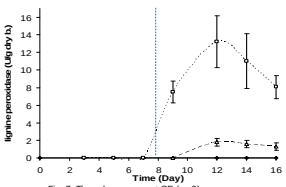

Fig. 7. The values means ± SE (n= 9).

Control ---□--- Cu added during the growing phase ---Δ--- Cu added during the stationary phase

- Oxidase activities are strongly increased by copper (Fig 6 and Fig. 7).
- Lignin peroxidase is specifically expressed in the presence of copper.
- The intensity of responses depends on the fungal development (Fig. 6 vs Fig. 7).

→ **Copper modifies the expression of extracellular enzymes (diminution of hydrolase activities / strong augmentation of oxidase activities)**
→ **The sensitivity of responses to copper depends on the fungal development and the type of secreted enzyme**

CONCLUSION

Trametes versicolor produces extracellular hydrolases and oxidases. The exposure of this fungus to copper modulates the expression of these enzymes, whatever its developing phase. The responses of these biochemical actors of soil functioning validate that they can be indicators of a metallic contamination in soils. It is now necessary to identify enzymatic profiles that could be used as tools for metal ecotoxicity assessment.

ACKNOWLEDGEMENTS : This research is supported by INRA and Conseil Régional de Haute Normandie

EMEC8, Dec. 2007