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Influence of Metal Speciation on the Sensitivity of Functional and Morphological Responses to Cu and Zn in *Trametes versicolor*

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1- INTRODUCTION

Filamentous fungi are key actors of soil functioning because of their ability to produce extracellular enzymes. Soil contamination by metals can alter these extracellular activities as well as fungal morphology. The impact will depend on metal bioavailability, which is linked to metal speciation. But the way the metal speciation influences the sensitivity of fungal responses is not well known, in particular in the case of essential metals Cu or Zn. Knowledge of metal speciation and its relationships with biological traits are important for the development of tools for metal ecotoxicity assessment, and could enhance the predictive interest in such fungal biomarkers.

2- OBJECTIVE

The present study aims at establishing the influence of metal speciation on both specificity and sensitivity of fungal responses to essential metals.

For that purpose, *Trametes versicolor* was cultured in liquid media exhibiting different levels of metal complexation. Three functional and two physiological traits were compared for their pertinence as metal exposure biomarkers.

3- METHODS

I. CULTURE MEDIA and METAL SPECIATION

- Use of three liquid media performed from Kirk medium (pH 5.5) differing only by their content in organic ligands :

Organic ligands	Complexing properties	Liquid medium		
		Lesa	Aba5	Aba2.5
Tartric acids	Highly	50 mM	-	-
Yeast extracts	Potentially	1 g/l	5 g/l	2.5 g/l

- Metal speciation was determined theoretically using a thermodynamic program (SOILCHEM) and validated experimentally using a Cu^{2+}-specific electrode
- Addition of copper in the different media yielded a good relationship: experimental vs theoretical $[Cu^{2+}]$ values ; $R^2 > 0.96$

II. FUNGAL RESPONSES

- Cultures of *Trametes versicolor* in the different media
- Addition of Cu or Zn from 1 nM to 1 mM the 3th day of culture
- Exposure during one week to essential metals

A. PHYSIOLOGICAL RESPONSES

- Determination of dry biomass as a toxicity/tolerance indicator
- Observations by scanning electron microscopy to characterize morphological impacts

B. FUNCTIONAL RESPONSES

- Measurements of 3 extracellular oxidase activities in liquid media: lignin-peroxidase ; Mn-peroxidase ; laccase

4- RESULTS

I. CULTURE MEDIA and METAL SPECIATION

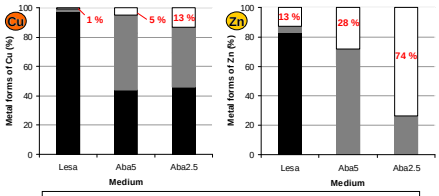

Fig.1: ■ organic-complexed, ■ inorganic-complexed and □ free forms

- Metals are mainly complexed by organic ligands in Lesa (>80%)
- The presence of free metal species are favored in Aba5 and Aba2.5 (until 13% for Cu^{2+} and 74% for Zn^{2+}, in red Fig. 1)

→ Free forms of metals: Lesa < Aba5 < Aba2.5

II.A. PHYSIOLOGICAL RESPONSES (Growth and Morphology)

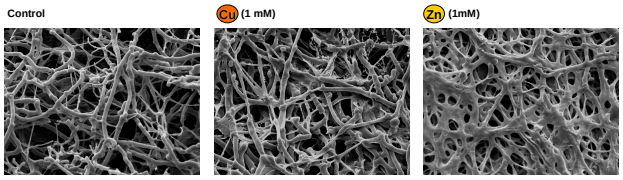

Fig. 2: Scanning electron microscopy of *T.versicolor* mycelia cultured in the medium, Aba2.5 (Magnification: x 2000)

- The growth of *T.versicolor* is not significantly affected by the essential metals whatever the medium, except with Cu at 1 mM and in Aba2.5 (data not shown)
- The fungal morphology is not affected by Cu while Zn seems to induce a coalescence of mycelia but only at 1 mM and in low complexing media Aba5 and Aba2.5 (Fig. 2)

→ Despite some physiological alterations, *T. versicolor* is tolerant to Cu and Zn

II.B. FUNCTIONAL RESPONSES

a. Oxidase responses to essential metals (1 mM)

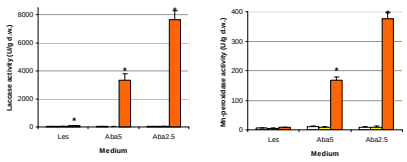

Fig. 3: □ Control ■ Zn at 1 mM ■ Cu at 1 mM ; values ± SEM (n=9 ; *p<0.05)

- Zn has no effect on the oxidase activities, whatever the complexation level of the used medium
- Laccase and Mn-peroxidase activities are highly increased by Cu in low complexing media Aba5 and Aba2.5

→ The intensity of enzymatic responses is increased when free forms of Cu are favored; it is not the case for Zn

b. Dose-response curves to Cu in low complexing media

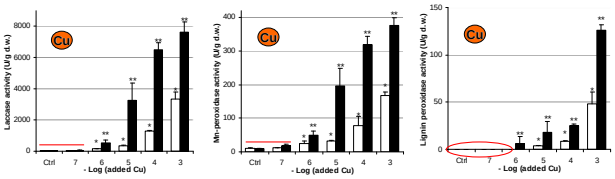

Fig. 4: Culture of *T. versicolor* in Aba5 □ and Aba2.5 ■ ; values ± SEM (n=9 ; *p<0.05)

- The threshold appearance of oxidase stimulation is lowered in the media Aba5 and Aba2.5 from 1 mM (in Lesa, Fig. 3) to 1µM (Fig. 4)
- Lignin peroxidase is specifically expressed in the presence of Cu only in these low complexing media

→ The sensitivity of enzymatic responses to Cu is increased when the metal is more bioavailable

5- CONCLUSION

The response of *Trametes versicolor* is specific to one essential metal and depends on metal speciation. We found that Zn leads to some morphological alterations at high concentration levels while Cu highly stimulates extracellular oxidase activities at environmental levels of metal contamination.

These enzymatic actors of soil functioning can be biomarkers of fungal exposure to Cu in soils. Furthermore, the specificity and sensitivity of fungal responses to metals confirm that fungal traits can be used as tools for metal ecotoxicity assessment. Studies are now engaged to improve a quantitative extraction of enzymes from soils.

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