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Study of extracellular functional system in soil fungi: Enzymes as exposure biomarkers to metals

Jérémie Lebrun^{1,2}, Nathalie Demont-Caulet², Isabelle Gattin³, Karine Laval³ and Christian Mougin¹

¹ INRA, UR251: PESSAC - Physicochemistry and Ecotoxicology of Soils from Contaminated Agrosystems, Versailles, FRANCE;

² INRA, UR501: Cellular Biology, Versailles, FRANCE; ³ ESITPA, BioSol, Mont-St-Aignan, FRANCE

1- INTRODUCTION

The release of metals into soil has led to their important accumulation in biota and often alters biological systems. By their ability to secrete enzymes and adapt their metabolism to various sources of nutriment, fungi are key colonizers in terrestrial ecosystems. They play a major role in the biogeochemical cycles and soil functioning. Since fungi represent one of the largest biomass in soils, the response of their extracellular functional systems to metals offers promising perspectives for ecotoxicological assessment. However, the functional diversity of fungi and the metal effect on their functional systems are not well known, limiting the predictive interest of such fungal biomarkers.

3- METHODS

SOIL FUNGI and CULTURE CONDITIONS

- Culture of 12 fungi isolated from an agricultural soil in liquid medium for one week (**MIAE collection**, INRA, Dijon)
- Addition of Cu or Cd at 0.1, 1 and 3 mM in the culture medium

FUNGAL RESPONSES

A. PHYSIOLOGICAL RESPONSES

- Determination of dry biomass used as a indicator of toxicity
- Quantification of total secreted proteins

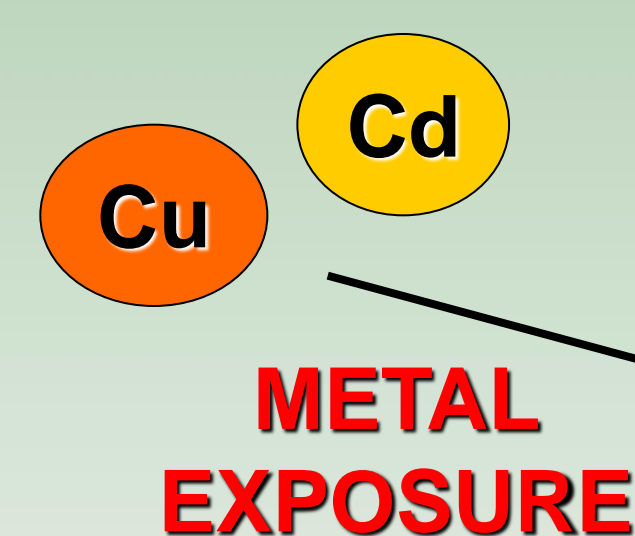
B. FUNCTIONAL RESPONSES

- Measurements of extracellular activities in liquid media
- Analysis of secretomes by electrophoresis (SDS-PAGE)
- Characterization of the laccase secretion by Western-blot and by mass spectrometry (LC-MS/MS)

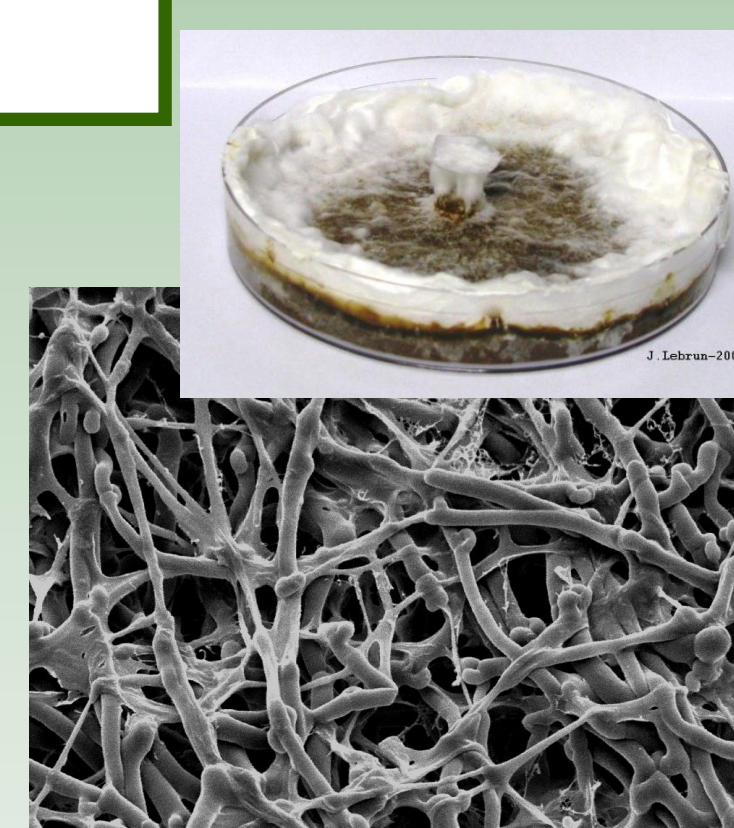
2- OBJECTIVE

The present study aims at investigating the impact of Cu and Cd on the functional system at enzymatic and protein levels in soil fungi.

For that purpose, two main families of enzymes involved in soil functioning (hydrolases and oxidases) were monitored to assess the functional diversity in 12 soil fungi in the absence of metal. Then, we assessed the response of functional systems after metal exposure.



METAL EXPOSURE



12 FUNGI ISOLATED FROM AN AGRICULTURAL SOIL

BASIDIOMYCETES	ASCOMYCETES	ZYGOMYCETES
<i>Trametes versicolor</i>	<i>Fusarium solani</i>	<i>Mucor hemialis</i>
<i>Irpex lacteus</i>	<i>Fusarium oxysporum</i>	<i>Mucor racemosus</i>
<i>Thanatephorus cucumeris</i>	<i>Penicillium brevicompactum</i>	<i>Mortierella alpina</i>
<i>Stereum rugosum</i>	<i>Trichoderma atroviride</i>	
	<i>Verticillium albo-atrum</i>	

FUNGAL RESPONSES

At physiological level

- Biomass production
- Protein secretion

At enzymatic level

- Extracellular activities

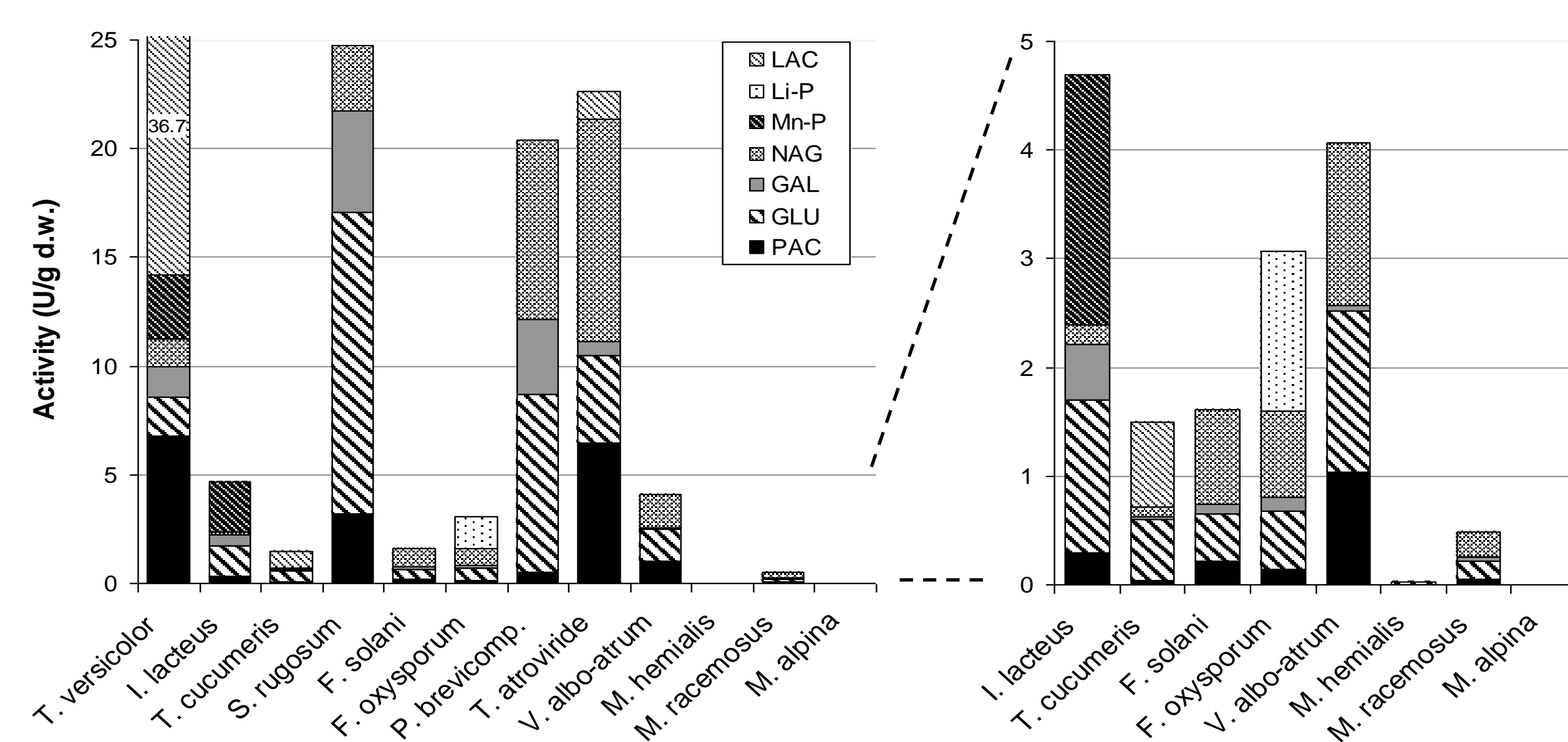
HYDROLASES
Acid phosphatase
β-glucosidase
β-galactosidase
N-acetyl-β-glucosaminidase

OXIDASES
Laccase
Mn-peroxidase
Lignin-peroxidase

At protein level

- Secretion profiles
- Laccase characterization

I. FONCTIONAL DIVERSITY IN SOIL FUNGI



- Soil fungi secreted almost all tested hydrolases (except *M. alpina*) but in variable amounts according to the fungus
- 50 % of fungi had an extracellular oxidative system

Soil fungi have extracellular systems involved in the nutriment cycles and degradation of natural polymers

II. PHYSIOLOGICAL TRAITS AS AFFECTED BY METALS

Average effect of metal on physiological traits in 12 soil fungi (%)

Physiological traits	Exposure to Cu			Exposure to Cd		
	0.1 mM	1 mM	3 mM	0.1 mM	1 mM	3 mM
Biomass production	101.4	86.7	53.7	89.6	57.2	26.2
Protein secretion	116.8	144.1	241.7	173.6	365.1	510.4

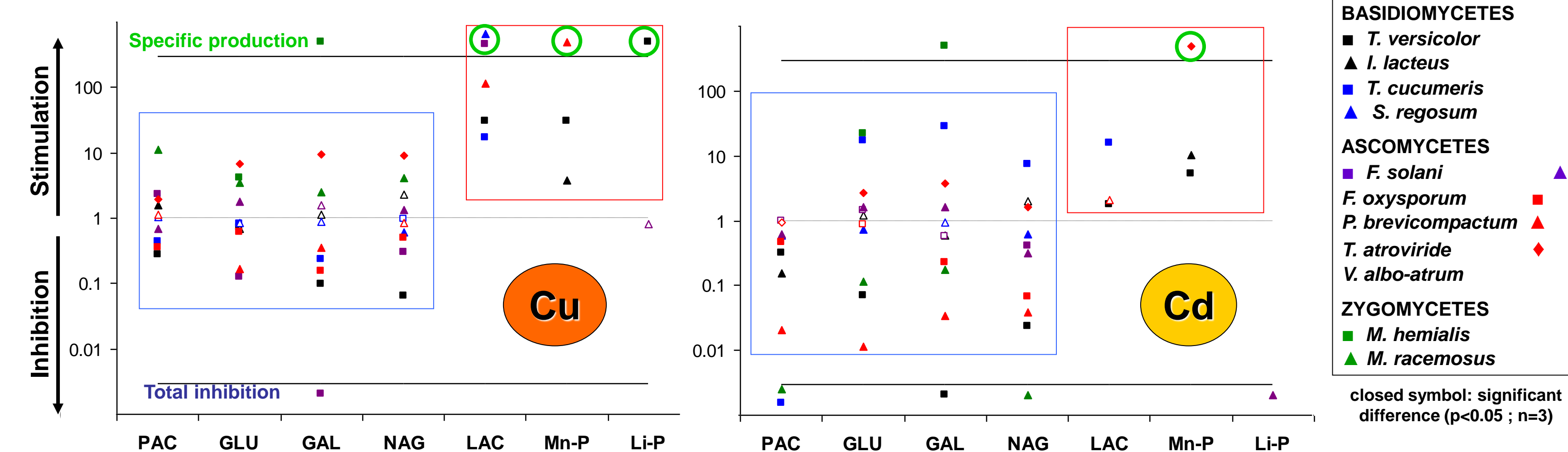
(n=3 : the protein secretion was related to dry biomass)

- In all fungi, the average production of fungal biomass is affected by Cu from 1 mM and by Cd from 0.1 mM
- Inversely, the average secretion of proteins is stimulated by Cu from 1 mM and by Cd from 0.1 mM

Despite metal toxicity, protein secretion is stimulated by metals in soil fungi: Cd > Cu

4- RESULTS

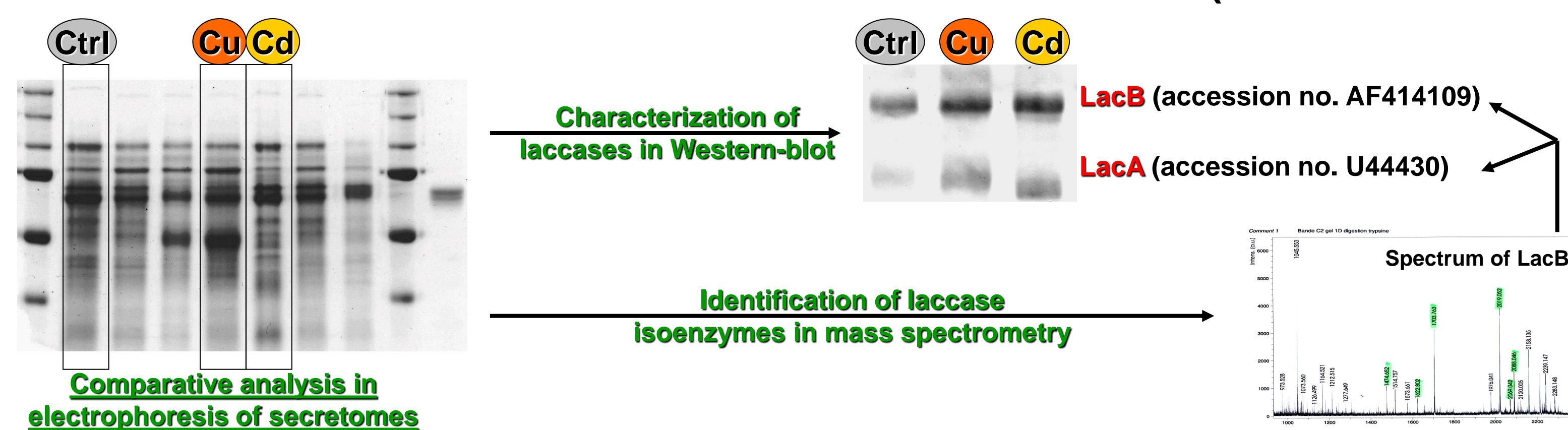
III. RESPONSES OF EXTRACELLULAR ACTIVITIES TO METALS (1 mM)



- In the presence of metals, a great variability of hydrolase activities was observed: the enzymatic responses depended on metal, enzyme and fungus considered
- Oxidase activities were stimulated by Cu and Cd whatever the strain
- In some fungi, oxidases were specifically produced in the presence of metal

Metals modify the expression of extracellular activities in soil fungi

IV. SECRETION PROFILES AS AFFECTED BY METALS (Case of *T. versicolor*)



- In the presence of metals, the electrophoretic profiles of secretomes were modified by Cu and Cd compared to the control
- 2 laccase isoenzymes were characterized in western-blot and identified by mass spectrometry: LacA (50 kDa) and LacB (59 kDa)
- The stimulation of laccase activity by metals was correlated with the increase in the amount of secreted laccase isoenzymes

Metals modify the secretion profiles at protein level

5- CONCLUSION

Soil fungi produce both extracellular hydrolases and oxidases involved in the nutriment cycles and degradation of cellulose, chitin or lignin. This functional system is modified by Cu and Cd at enzymatic and protein level. The responses of hydrolases to metals are complex and contrasted. By contrast, metals stimulate

the production of oxidases whatever the considered fungus. Our results confirm that functional traits of fungi can be used as tools for metal ecotoxicity assessment. Thus, enzymatic actors of soil functioning as fungal oxidases can be exposure biomarkers to metals in soil. Studies are now necessary to validate these functional tools in soil.