



Exocellular oxidases: tools to assess the exposure of soil fungi to xenobiotics?

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Exocellular oxidases

Tools to assess the exposure of soil fungi to xenobiotics?

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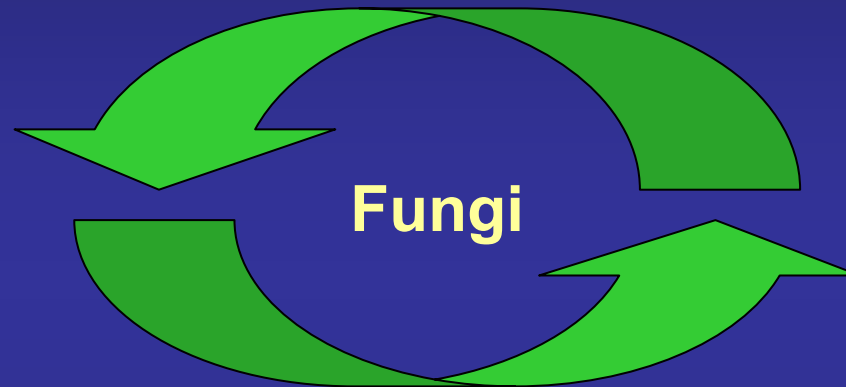
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Xenobiotics and Environment, INRA Versailles



Research areas of the team Xenobiotics and Environment

Structural and metabolic biochemistry
structural data, regulation of expression, enzyme evolution



Biocatalysis,
bioconversion, biotransformation

Fate and impacts of
xenobiotics in soils

► ► bioremediation, ecotoxicology

The aims of the present project

- 1 - Improvement of the knowledge on fungal laccases**
- 2 - Development of tools to assess the adverse effects of pollutants on soil ecosystems**



- Effect of xenobiotics on laccase expression and activity in liquid cultures (identification of biomarkers)**
- Effect of xenobiotics on laccase expression and activity in soils inoculated with fungi (validation of biomarkers)**
- Effect of xenobiotics on endogenous laccases of soils**

Rationale for filamentous fungi as models

Ecological relevance

- Provide one of the highest biomass in the soil
- Initiate numerous trophic chains

Functional relevance

- Responsible of the breakdown and mineralization of organic matter
- Catalyze the biotransformation of numerous xenobiotics

Practical relevance

- Secrete exocellular oxidases: peroxidases, laccases



The main characteristics of laccases

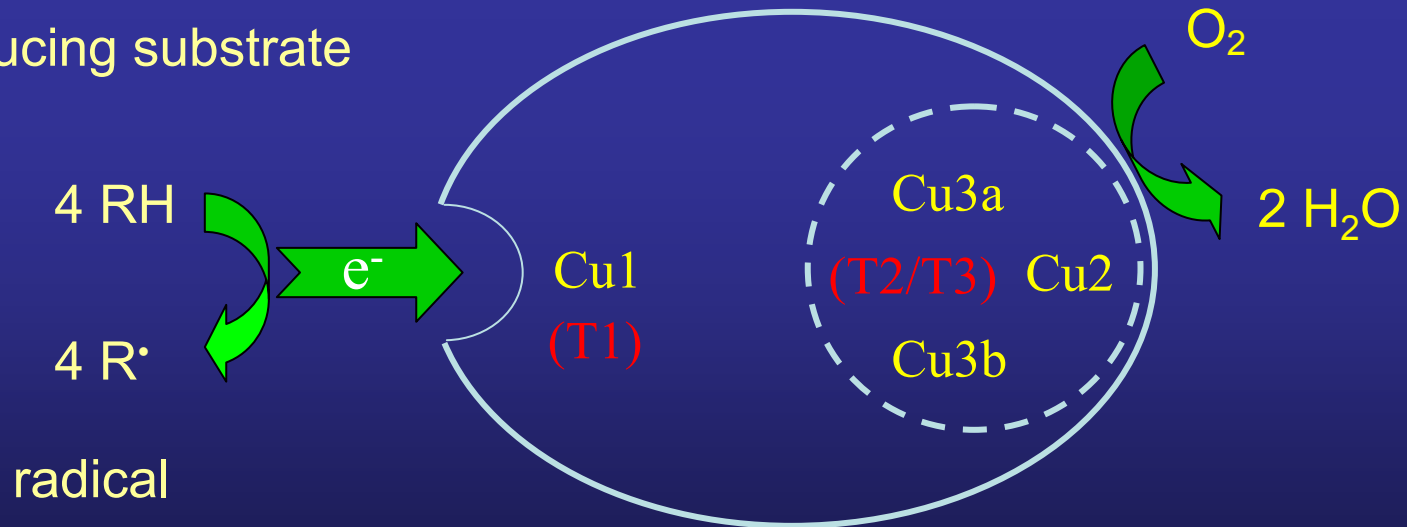
oxidases mainly produced by fungi (*Trametes versicolor*)
and also by higher plants (*Rhus vernicifera*)
insects (*Bombyx*)
bacteria (*Azospirillum lipoferum*)



extracellular glycoproteins containing copper
520 to 550 aa, 60 to 80 kD

high redox potential: 0.7 V, very robust

reducing substrate



radical

Functions and uses of fungal laccases

1 - Natural

- biodegradation of ligno-cellulosic materials
- morphogenesis, pathogenesis....

2 - Industrial

- textile and food industry
- pulp paper bleaching

3 - Environmental

- biotransformation of xenobiotics, bioremediation
- biomarkers for the assessment of adverse effects of chemicals ???

The laccases of *T. versicolor*

2 families of isozymes

- type A: N-terminale sequence GIGP
constitutive and induced
gene AF 414109
 - ▶ lac A
- type B: N-terminale sequence AIGP
constitutive, could be induced ???
gene U44430
 - ▶ lac B

Effect of xenobiotics on laccase activity

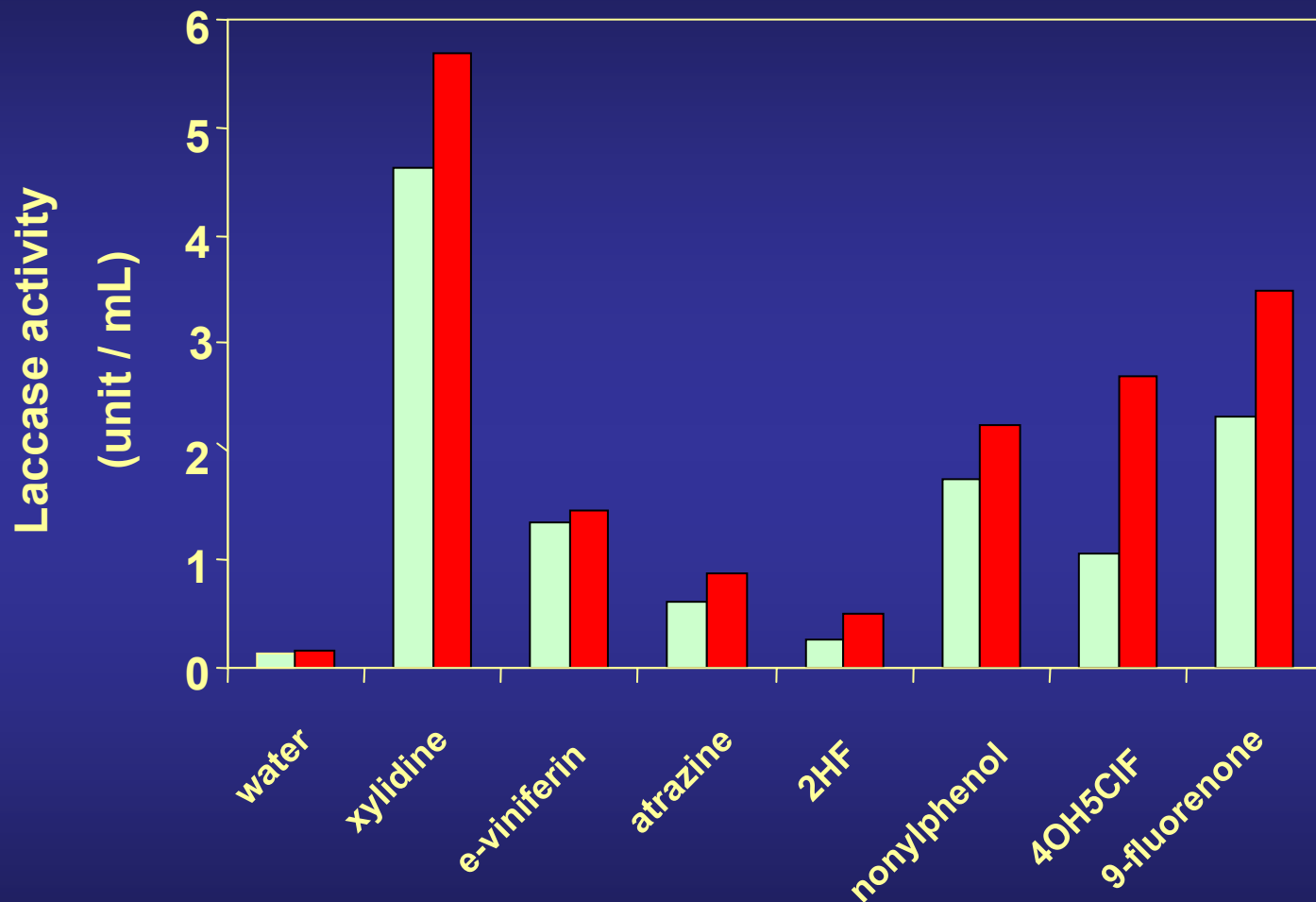
- Treatment of 4-day liquid cultures of *T. versicolor* by xenobiotics
- Measurement of laccase activity after 2 and/or 3 days (ABTS)



Screening for chemicals efficient in increasing laccase activity

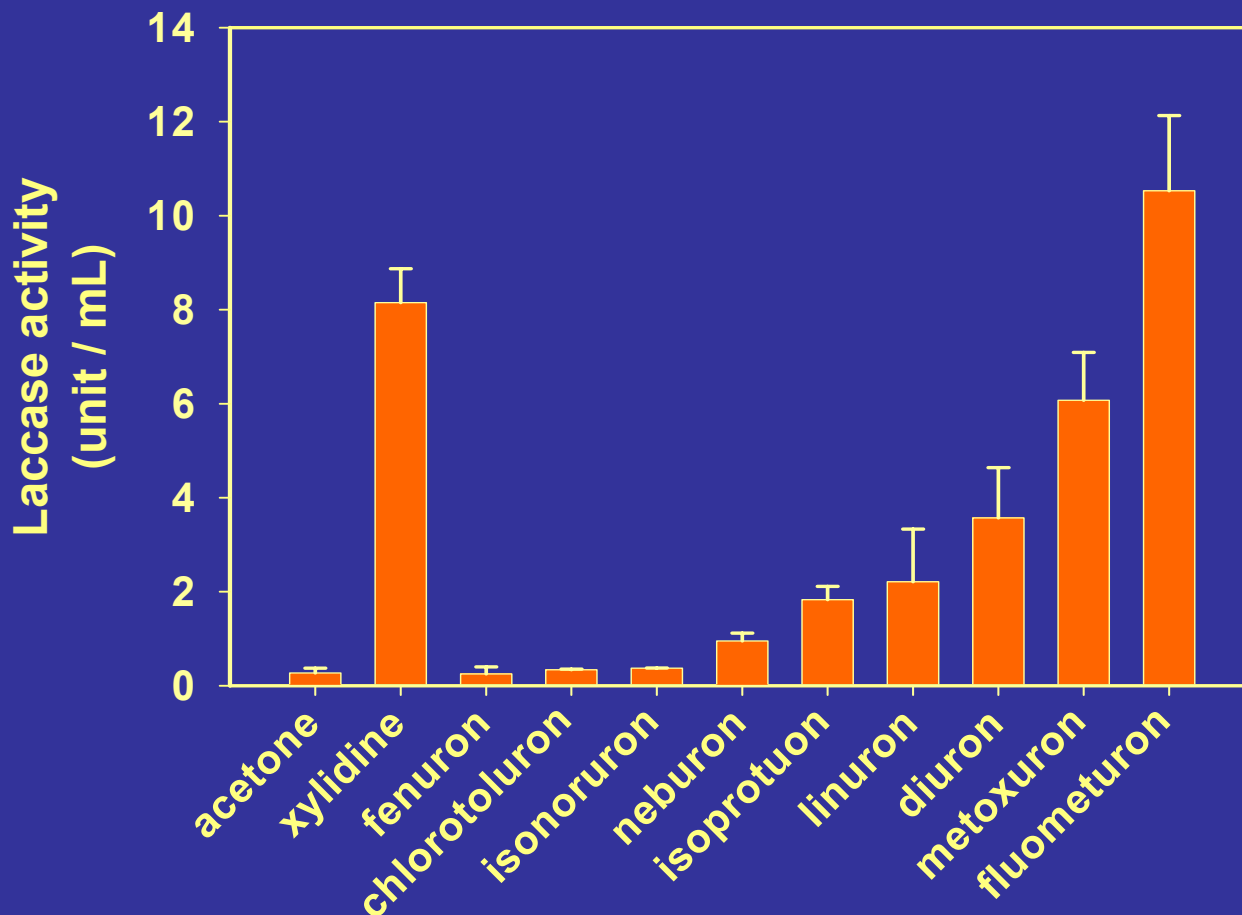
Dose/response relationships

Laccase activity after 2 (●) and 3 (●) days of treatment



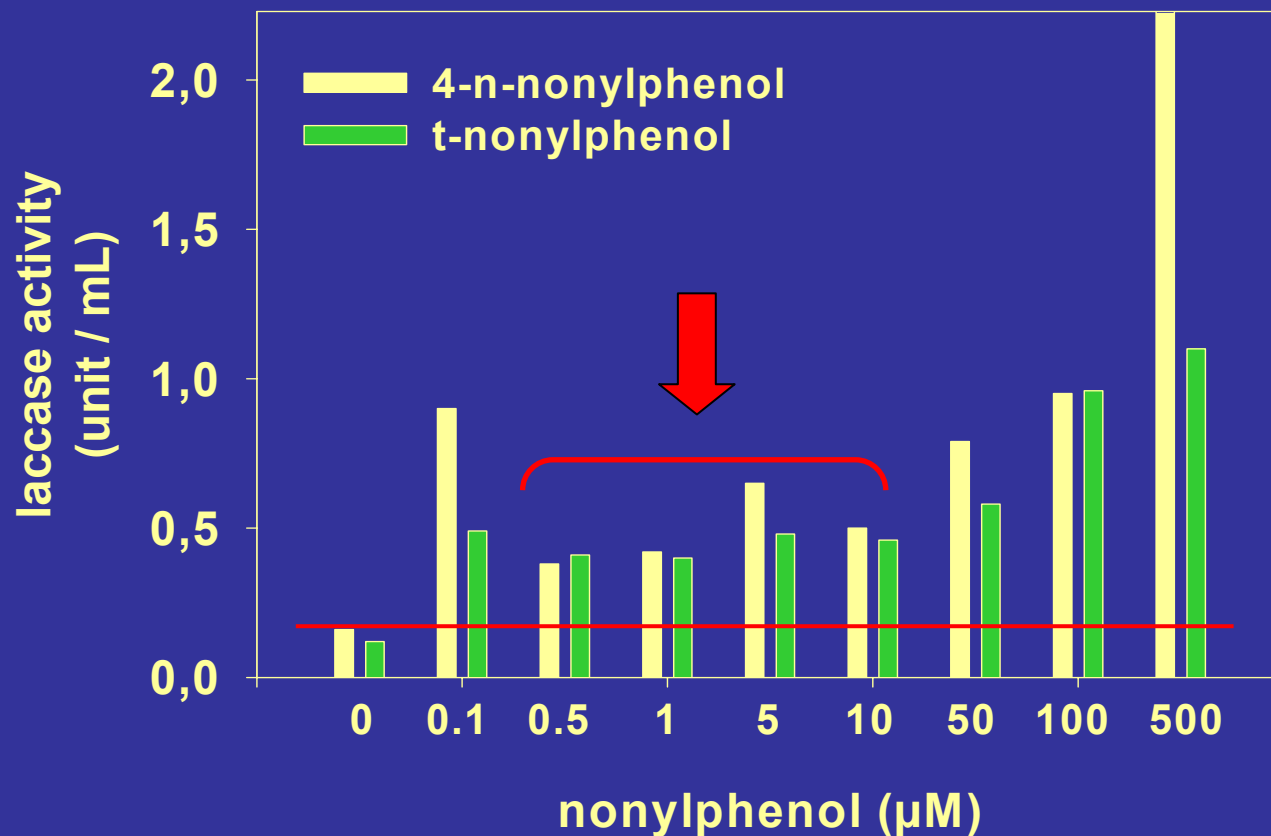
Chemical concentration: 500 μ M

Laccase activity after 3 days of treatment with phenylureas



Chemical concentration: 200 µM

Laccase activity after 3 days of treatment with nonylphenols



Effect of xenobiotics on the expression of laccase genes

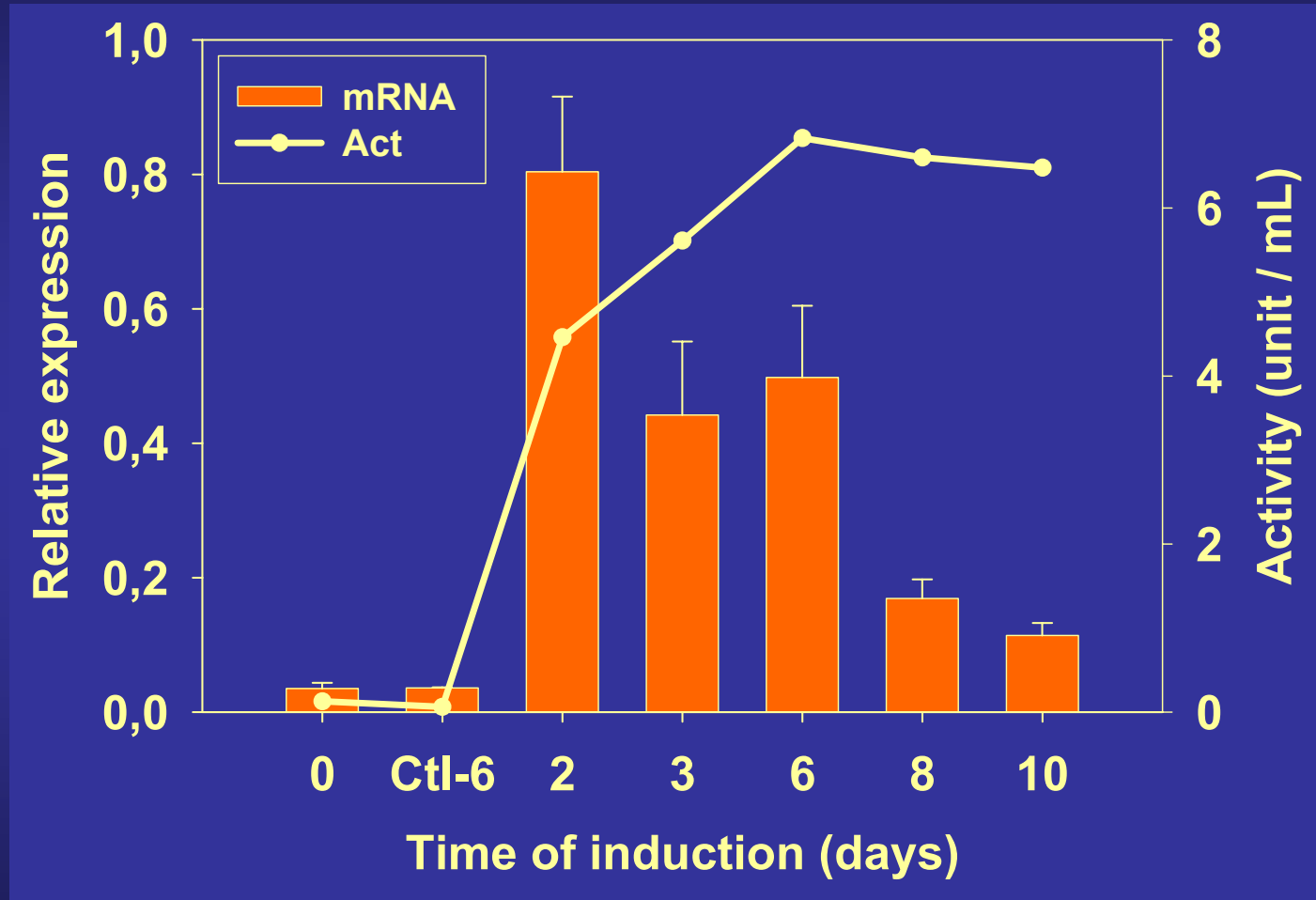
- Treatment of 4-day liquid cultures of *T. versicolor* with xenobiotics
- Measurement of laccase activity (ABTS)
- Extraction of mRNAs (RNeasy Mini Kit Qiagen)
- Synthesis of cDNAs by RT-PCR (Advantage RT Clontech)
- relative quantification of genes by real time PCR (Thermocycler Roche, ref: TEF1, gene X94913)
- detection by fluorescence (SYBR Green I)



quantification of gene/activity expression

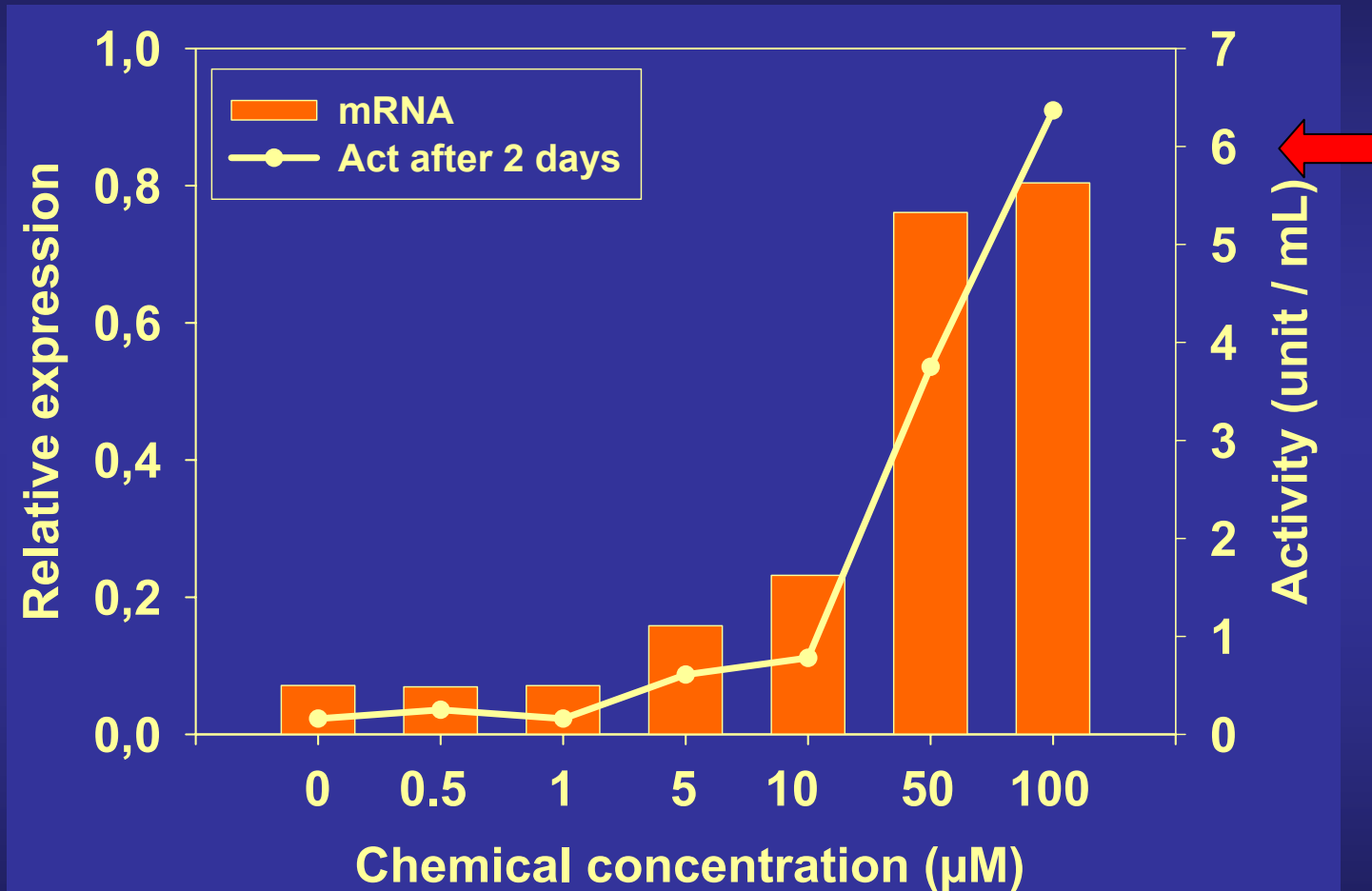


High level of activity: xyloidine, lac A

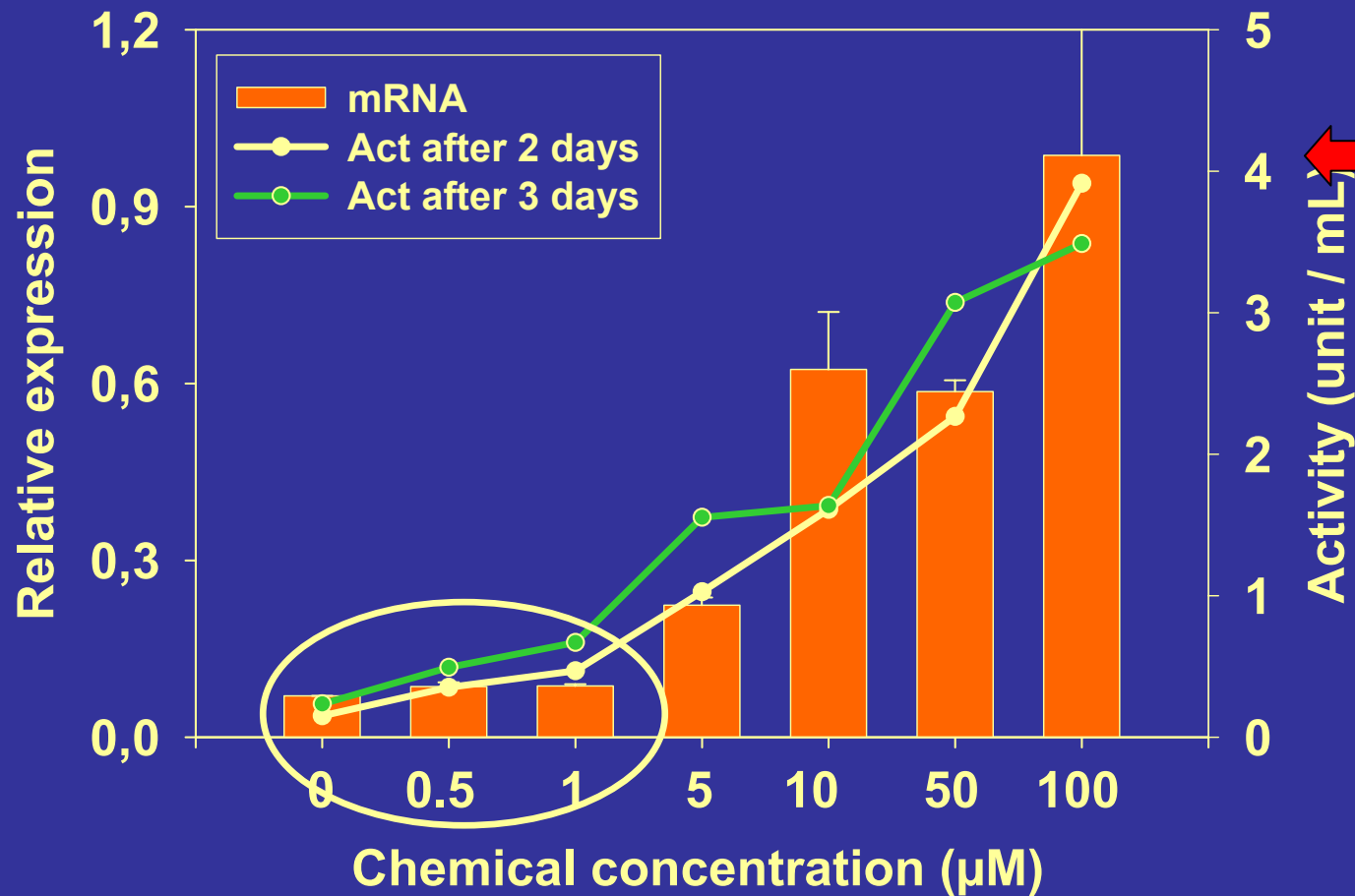


Chemical concentration: 100 μ M

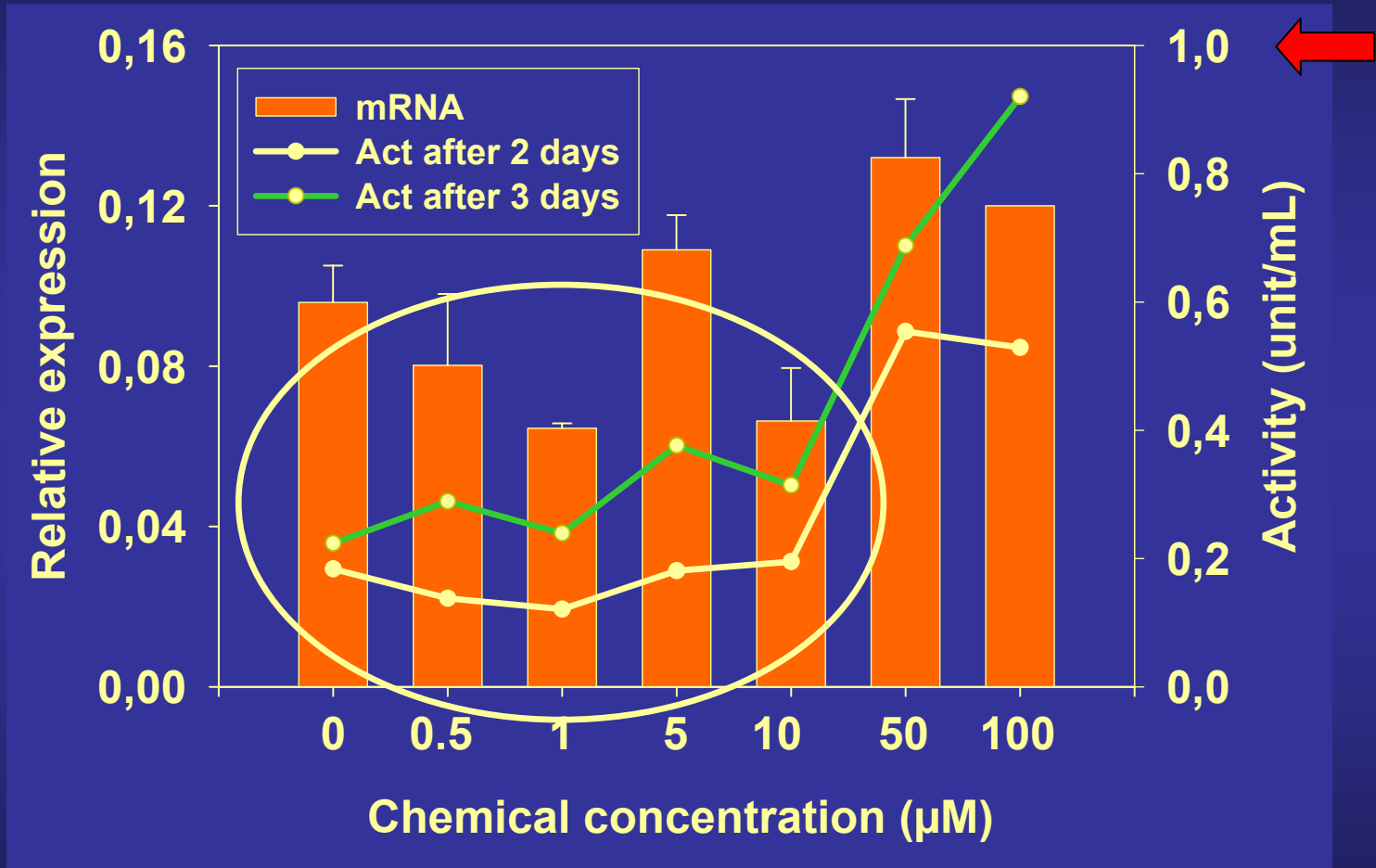
Xylidine: dose-response relationship, lac A



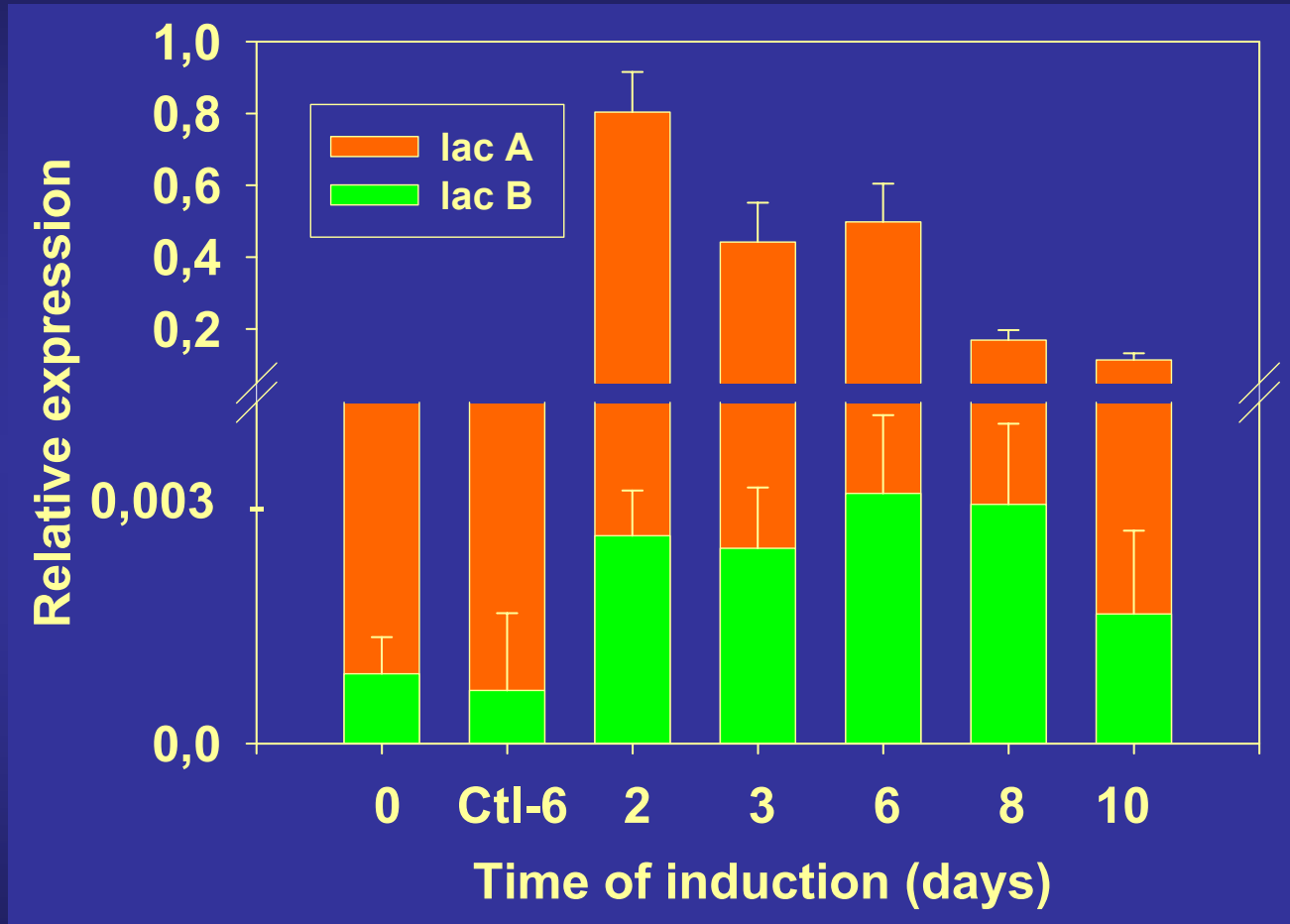
Moderate level of activity: diuron, lac A



Low level of activity: 4-n-nonylphenol, lac A



Can lac B be induced by xenobiotics?



Chemical concentration: 100 μ M

Effect of xenobiotics on the production of laccase proteins

- Treatment of 4-day liquid cultures of *T. versicolor* with xenobiotics
- Separation of the extracellular fluid
- Precipitation of polysaccharidic compounds with 10% acetone
- Diafiltration of the protein solution (cut off 10kD, Amicon)
- Purification on Concanavalin A-Sepharose 4B column (Sigma-Aldrich)
elution with 0.2M Tris Hcl in the presence 0.5M NaCl and 0.2M o-methyl glucose)
- separation by SDS-Page (12%)
- Identification of proteins by Maldi-TOF (Bruker)

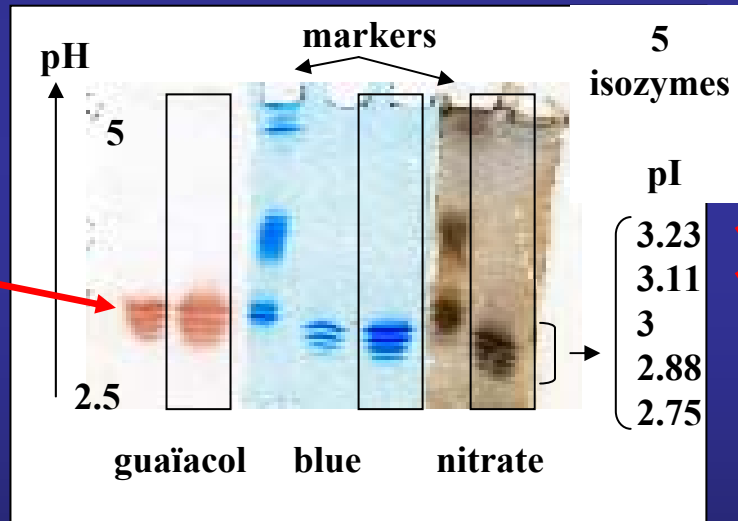


profiles of proteins



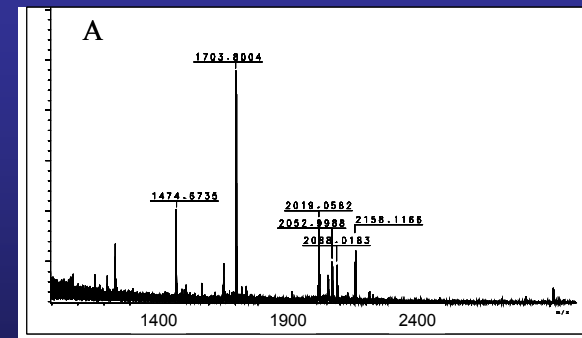
Separation and identification of lac A isoforms

1st migration mass → 2nd migration pI → Maldi-TOF



MW Da

58463
59776



Conclusions

- Laccase activity can be increased following fungal exposure to environmental pollutants
- Lac A (but not lac B) are induced by chemicals
- mRNA measurement is highly specific and well related to enzymatic activities, but the response is not sensitive enough to be useful (induction by 15-20)
- the proteomic approach should be developed



Could fungal laccases be used for point contamination (heavy pollution) assessment?

Perspectives

- Understand the effects of xenobiotics at the transcriptional, traductional and post-traductional levels
- Assess the effects of metals
- Develop tools to inoculate fungi into soils and perform assays in soil

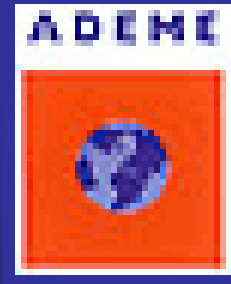


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