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## **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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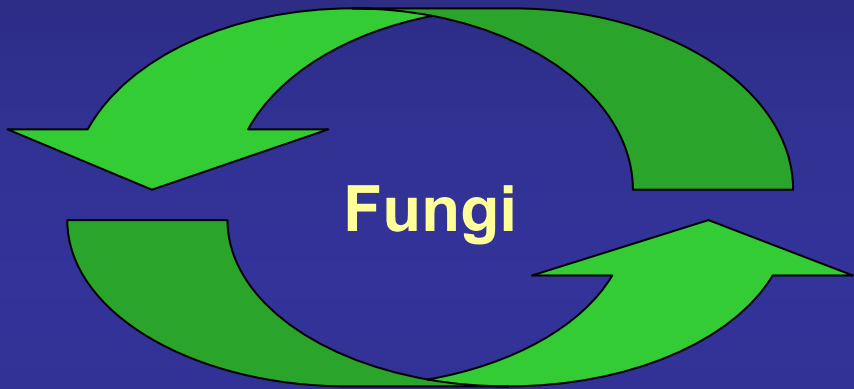
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Isabelle Touton, Agathe Brault, Albert Kollmann, Christian Mougier  
Xenobiotics and Environment, INRA Versailles



# 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

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- 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

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## 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



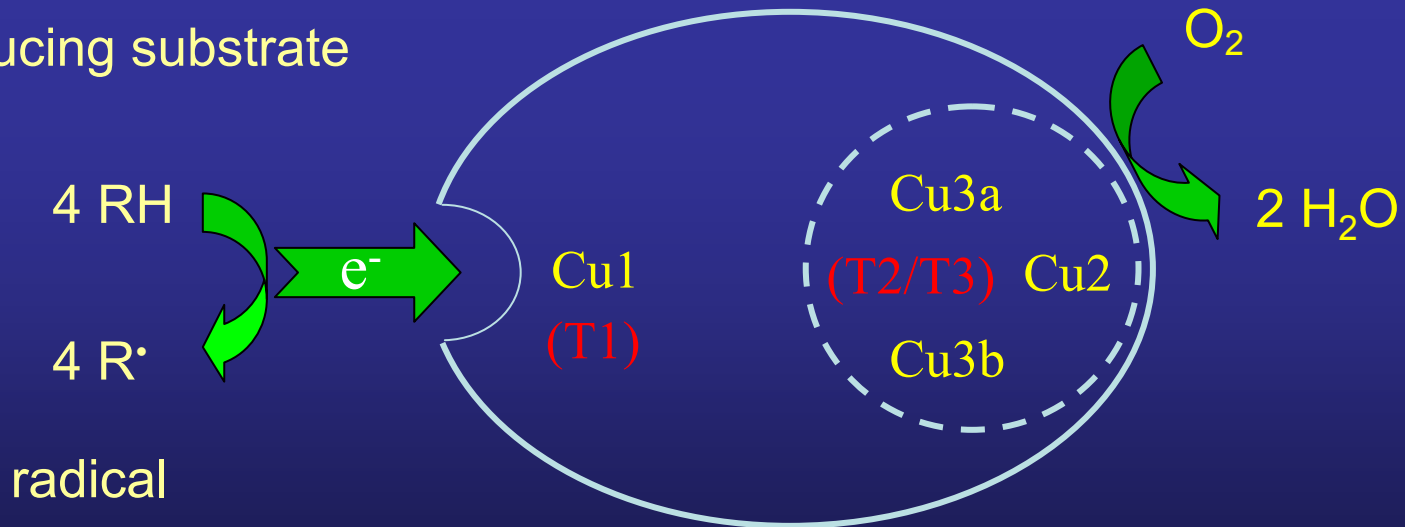
# The main characteristics of laccases

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

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## 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*

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## 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

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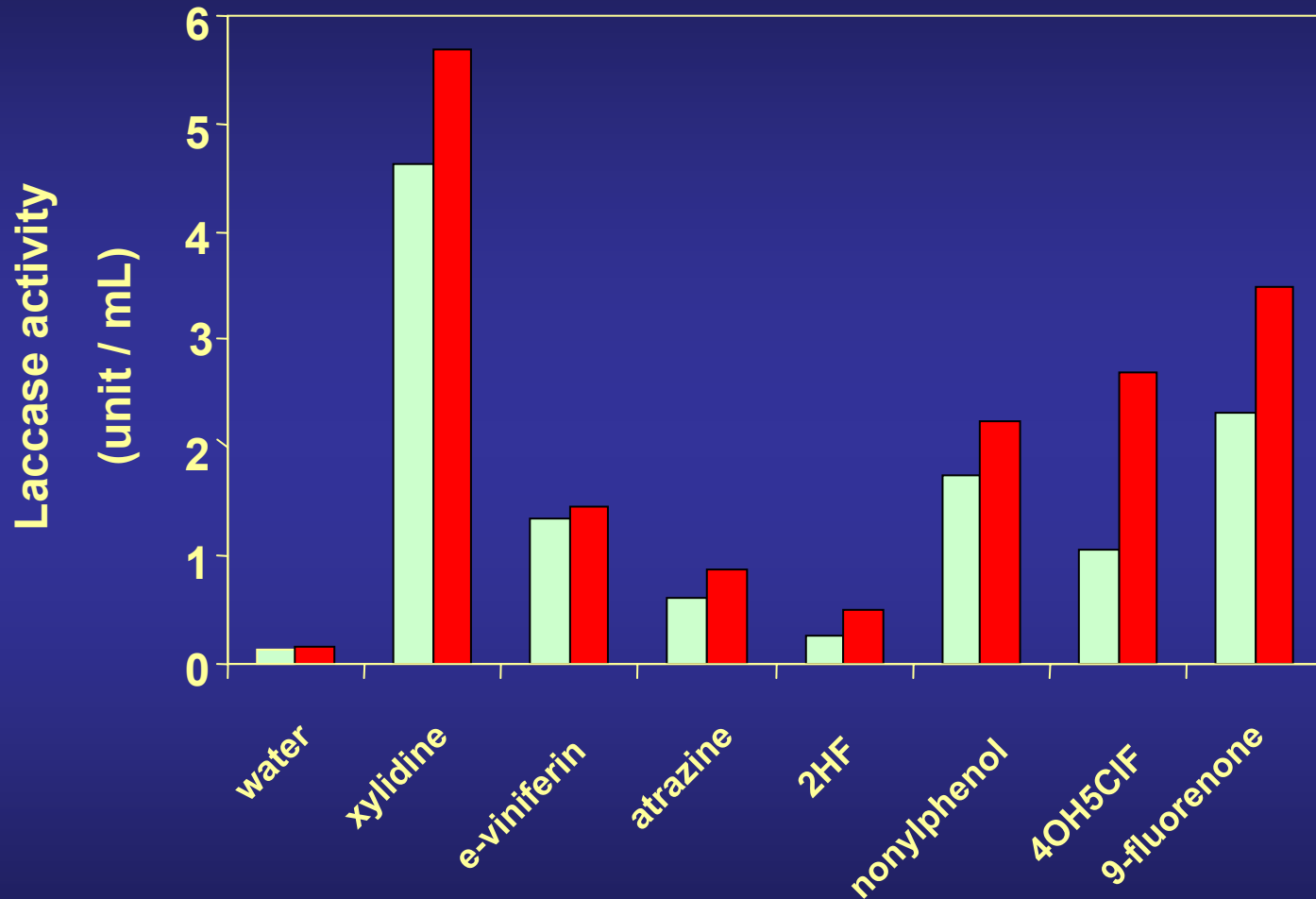
- Treatment of 4-day liquid cultures of *T. versicolor* by xenobiotics
- Measurement of laccase activity after 2 and/or 3 days (ABTS)



Screening for chemicals efficient in increasing laccase activity

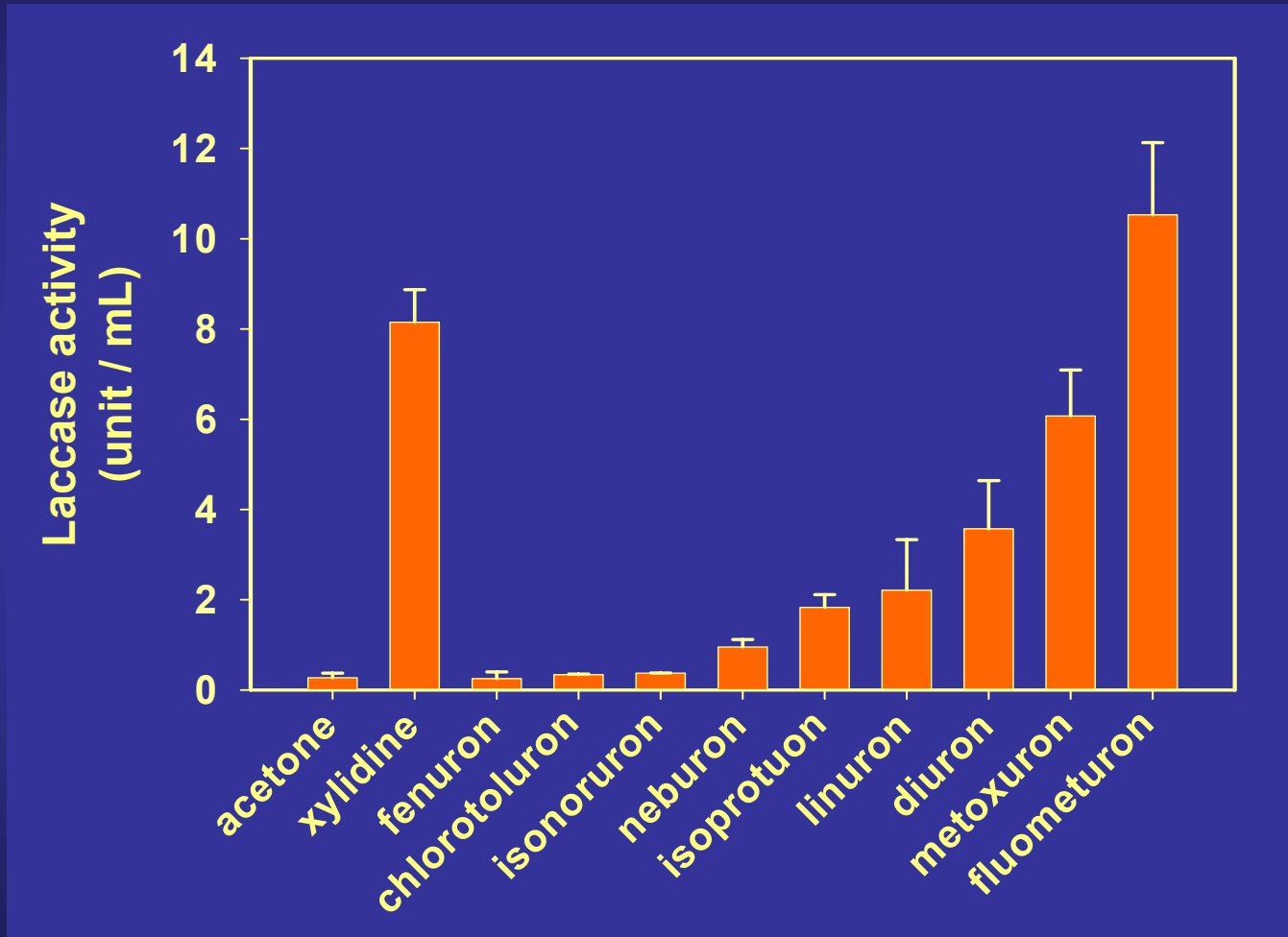
Dose/response relationships

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



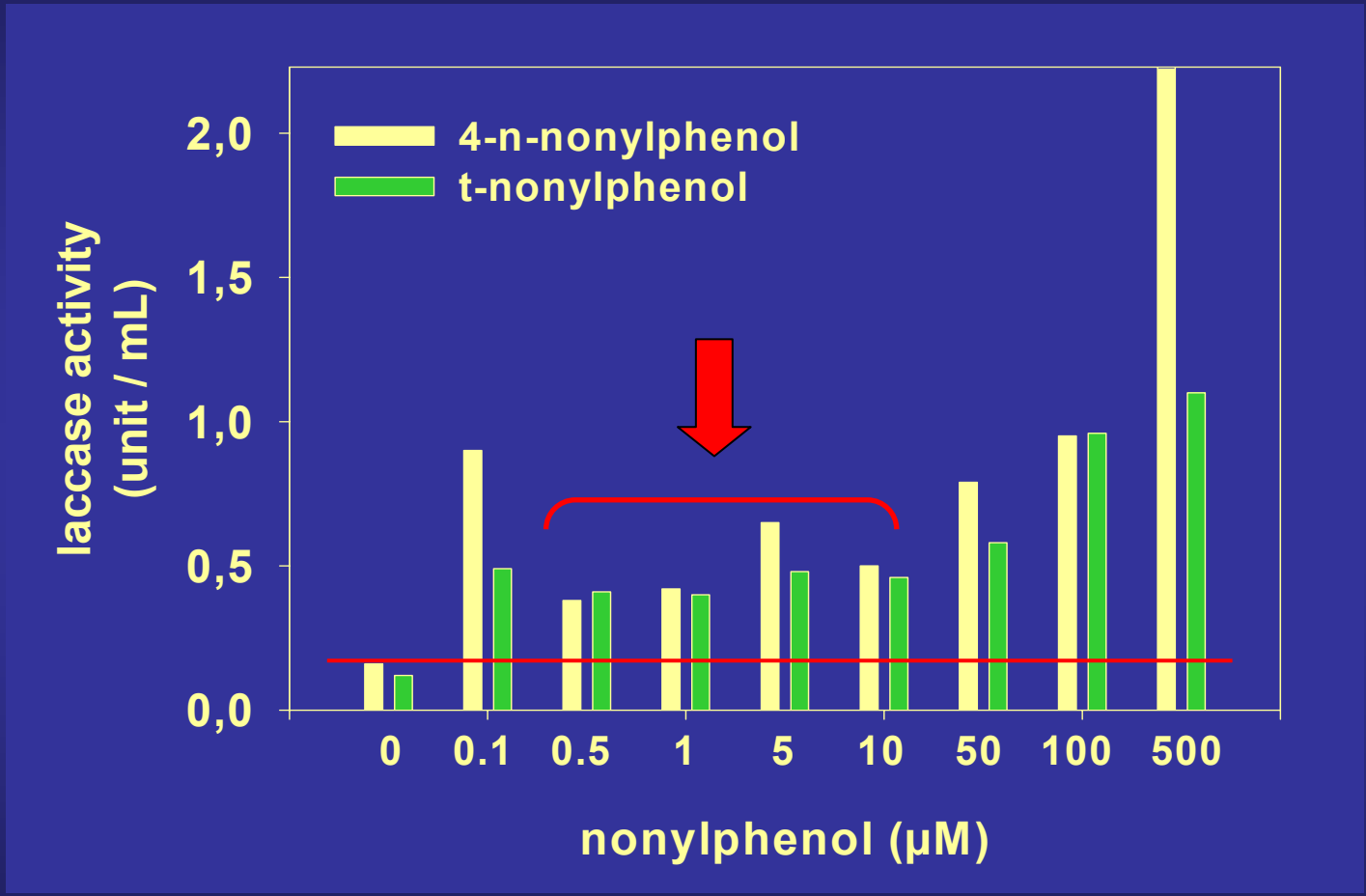
Chemical concentration: 500  $\mu$ M

# Laccase activity after 3 days of treatment with phenylureas



Chemical concentration: 200  $\mu$ M

# Laccase activity after 3 days of treatment with nonylphenols



# Effect of xenobiotics on the expression of laccase genes

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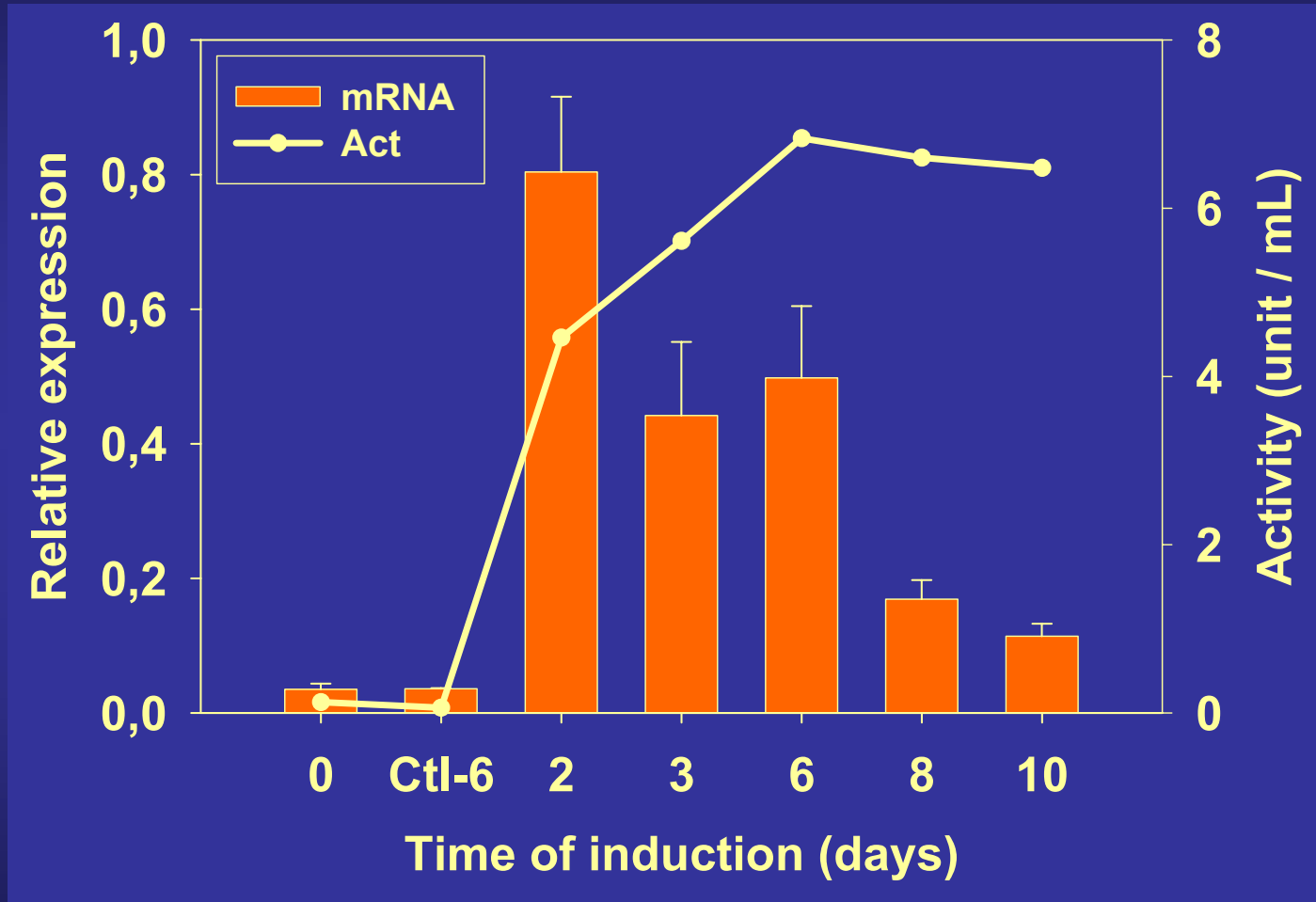
- Treatment of 4-day liquid cultures of *T. versicolor* with xenobiotics
- Measurement of laccase activity (ABTS)
- Extraction of mRNAs (RNeasy Mini Kit Qiagen)
- Synthesis of cDNAs by RT-PCR (Advantage RT Clontech)
- relative quantification of genes by real time PCR (Thermocycler Roche, ref: TEF1, gene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



quantification of gene/activity expression

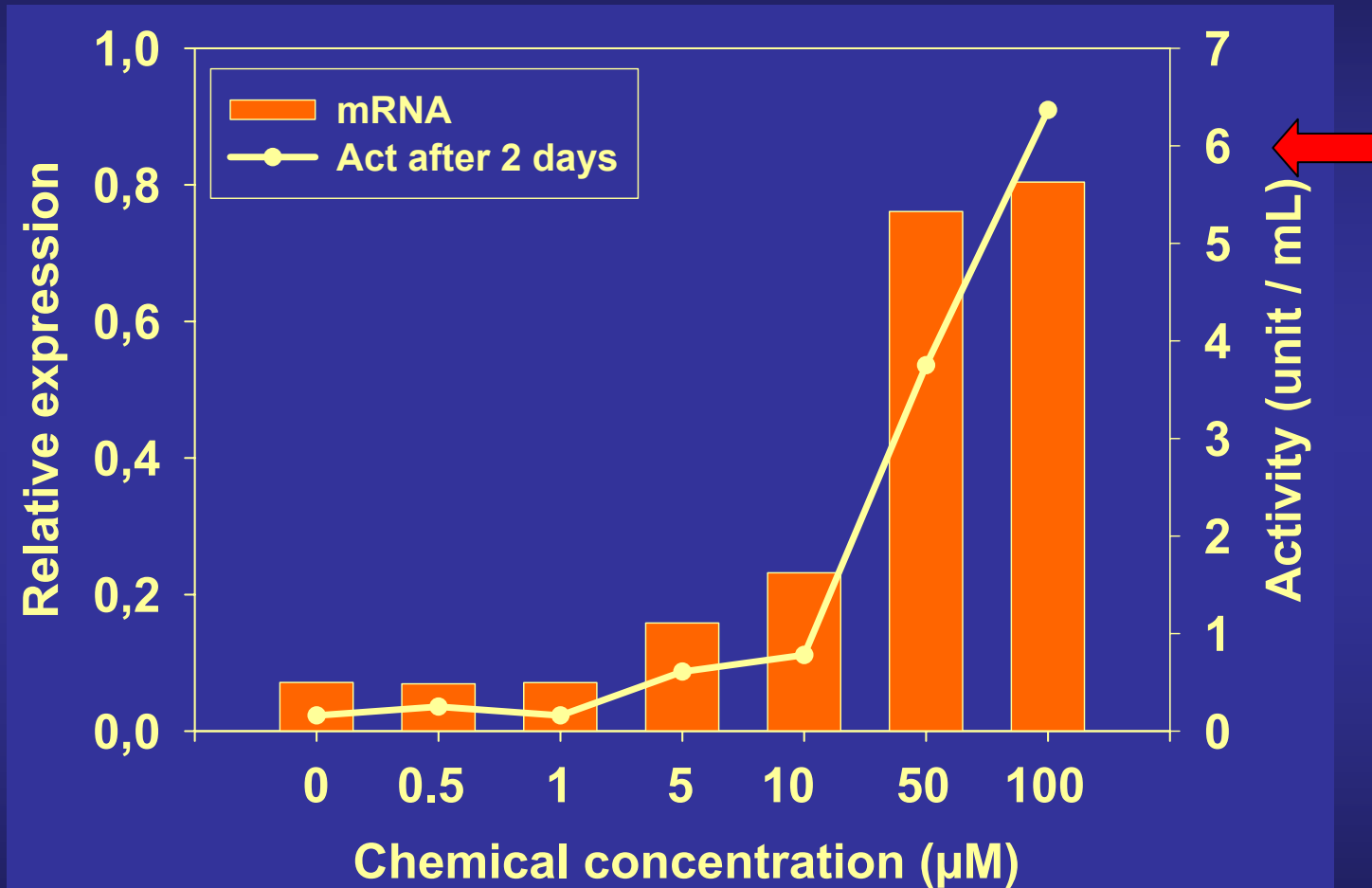
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# High level of activity: xyloidine, lac A

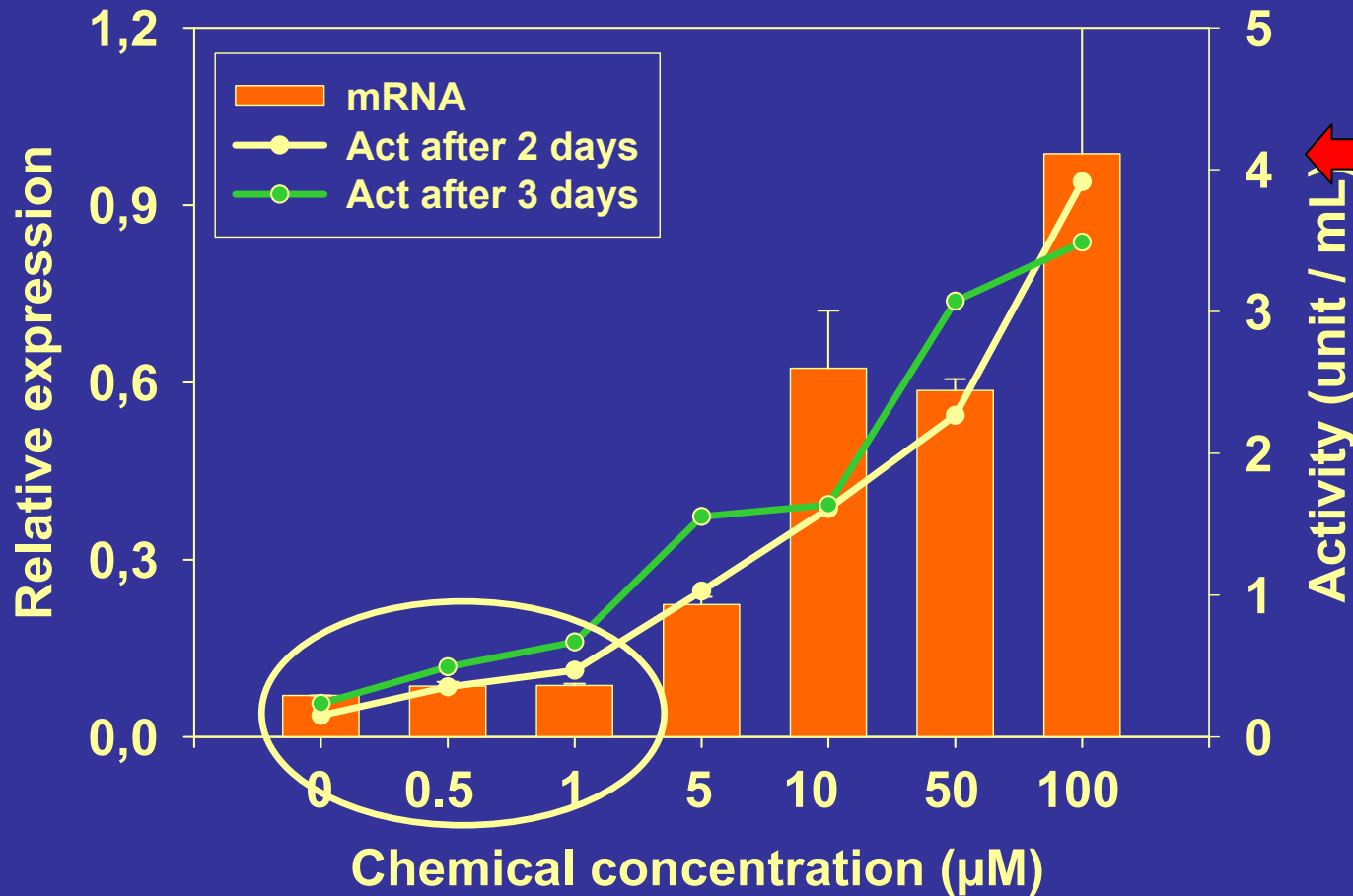


Chemical concentration: 100  $\mu$ M

# Xylidine: dose-response relationship, lac A

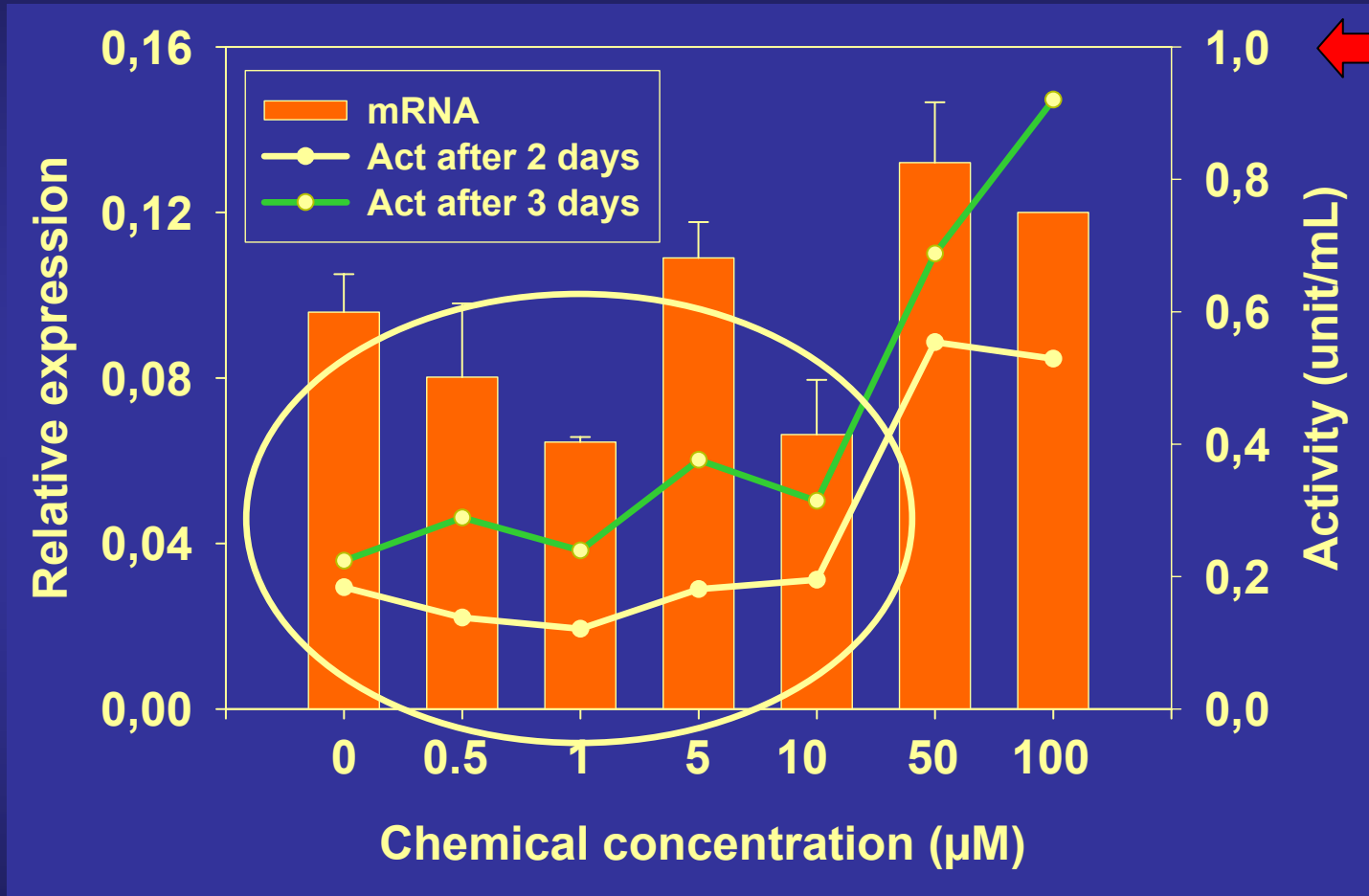


# Moderate level of activity: diuron, lac A

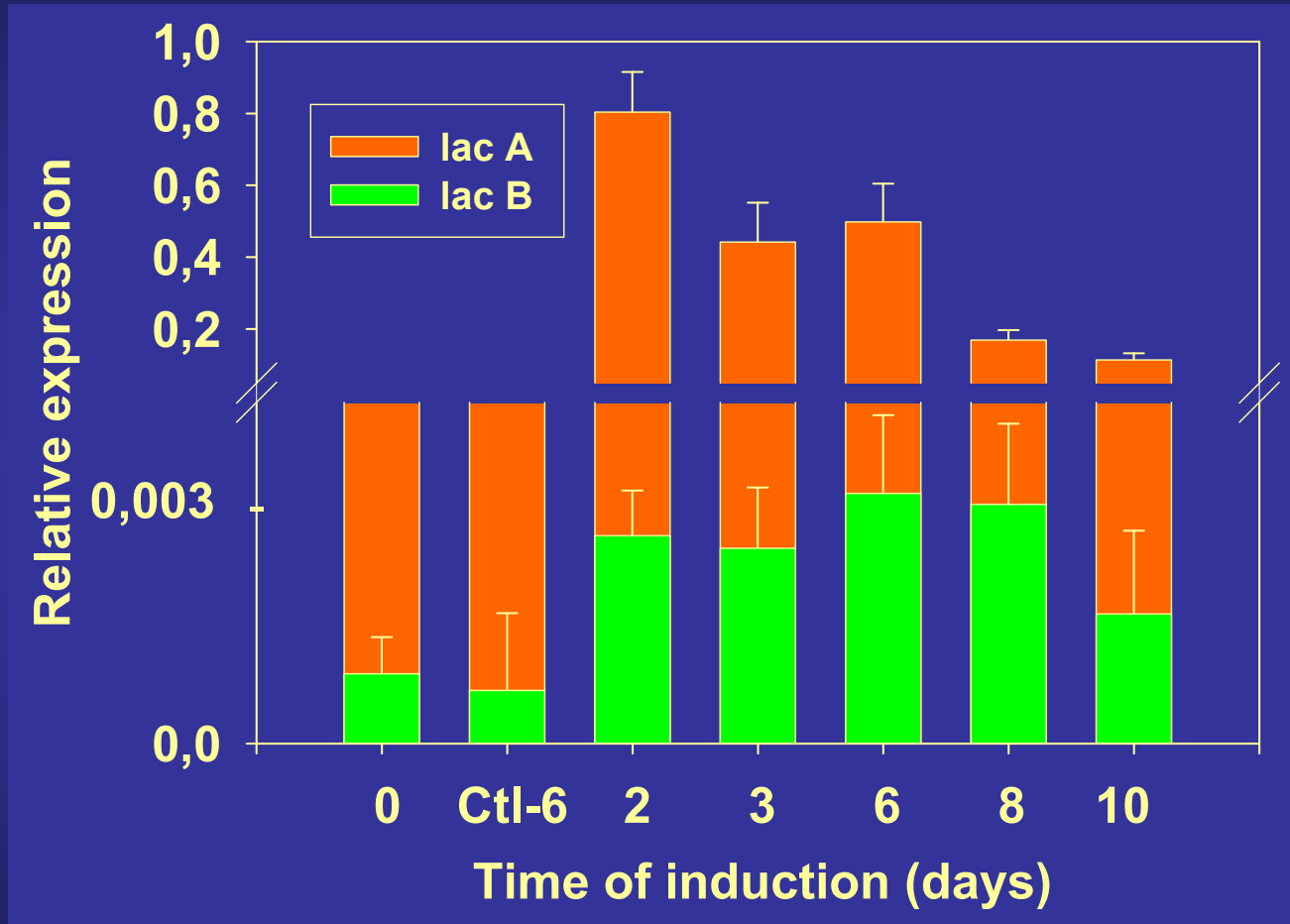




# Low level of activity: 4-n-nonylphenol, lac A



# Can lac B be induced by xenobiotics?



Chemical concentration: 100  $\mu$ M

# Effect of xenobiotics on the production of laccase proteins

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- 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

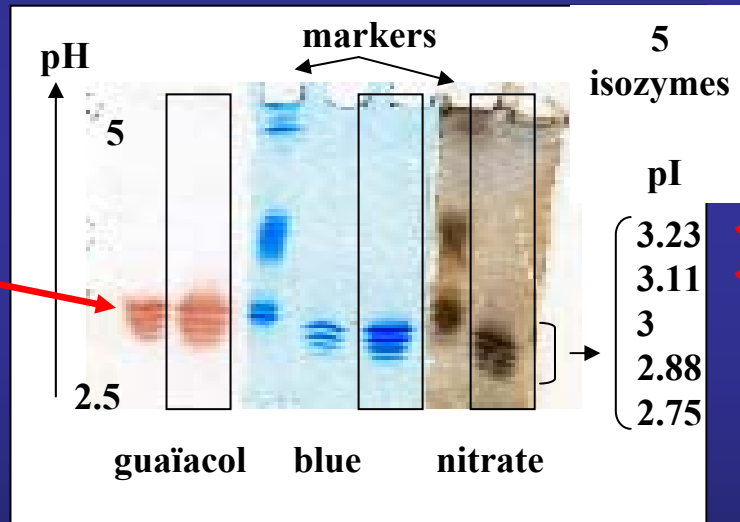
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# Separation and identification of lac A isoforms

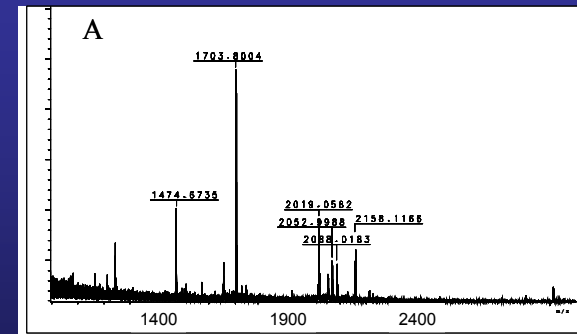
1<sup>st</sup> migration  
mass

2<sup>nd</sup> migration  
pI

Maldi-TOF



MW Da  
58463  
59776



# Conclusions

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- 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



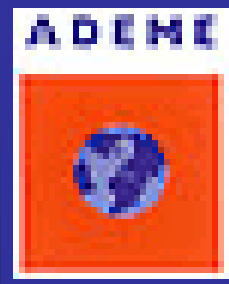
# Acknowledgements

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