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# Interference of soil contaminants with laccase activity during the transformation of complex mixtures of polycyclic aromatic hydrocarbons in liquid media

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# Introduction

Laccase-producing fungi are often presented as efficient tools for the bioremediation of soils and liquid wastes polluted by PAHs, but the efficiency of the enzymes has not been clearly demonstrated

## **Aims of the study:**

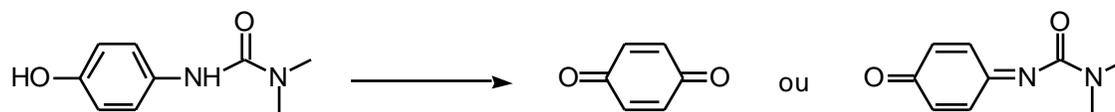
- to determine the efficiency of fungal laccases to transform synthetic mixtures of PAHs
- to assay their use for the transformation of PAHs extracted from polluted soils
- to evidence the interference of contaminants co-extracted from the polluted soils with PAH transformation

# Properties of the fungal laccases

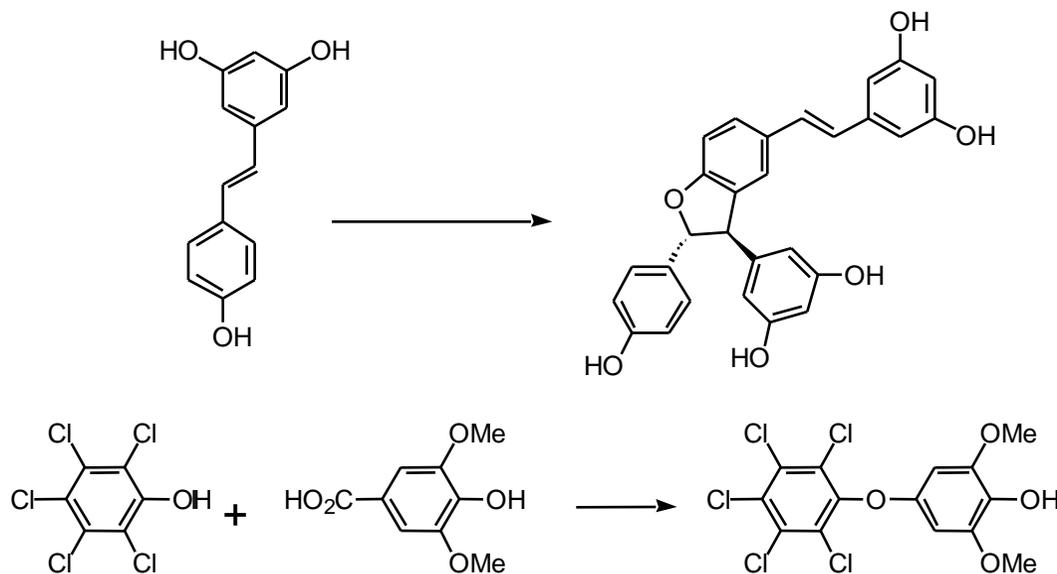
- Distribution: fungi, bacteria, higher plants, insects
- Function: lignin degradation, morphogenesis, pathogenesis  
transformation of xenobiotics
- Structure: dimeric or tetrameric glycoprotein, with 4 Cu,  
exocellular, 520 to 550 aa, 60 to 80 kDa  
secreting a N-terminal peptide
- Regulation:  
inhibitors: small anions, metals, fatty acids, chelating agents  
inducers: culture medium, lignin and related compounds,  
xenobiotics
- Equation:  $2RH + \frac{1}{2}O_2 \rightarrow 2R^\bullet + H_2O$

# Main catalyzed reactions

- RH = Phenolic compound  
oxidation, bond cleavage: C-C, C-N,...

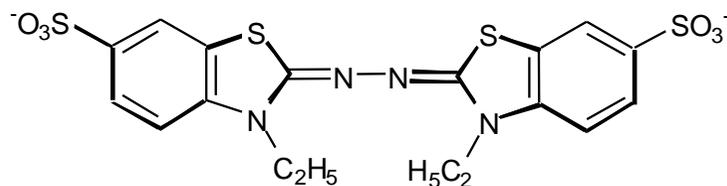
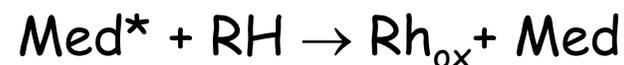


oxidative coupling

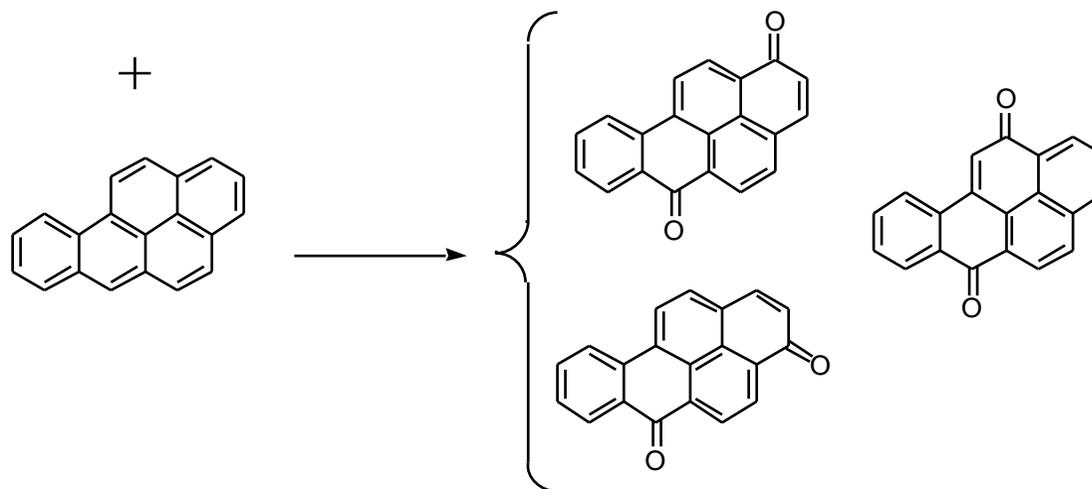


- RH = non phenolic compound

→ a redox mediator is needed:  $\text{Lac} + \text{Med} \rightarrow \text{Med}^*$



2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid): ABTS



## Experimental

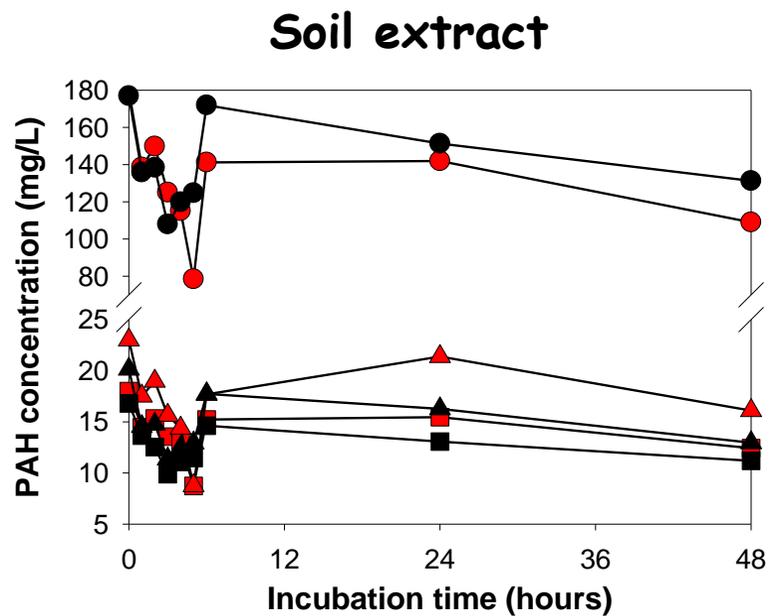
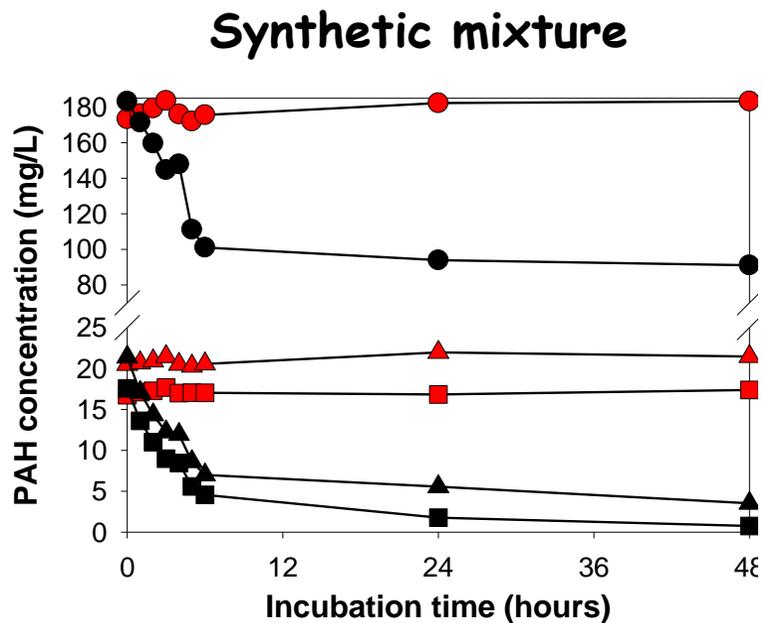


2-liter bioreactors containing:

- 1.5 L citrate/phosphate buffer pH 3, 0.1 M
- 20 mL Tween 20
- 360 mg PAHs dissolved in 500 mL acetonitrile
- 2 mM ABTS (redox mediator)
- 4000 U laccase of *Pycnoporus cinnabarinus*

incubated in the dark at 30°C for 48 hours

# Transformation of PAHs



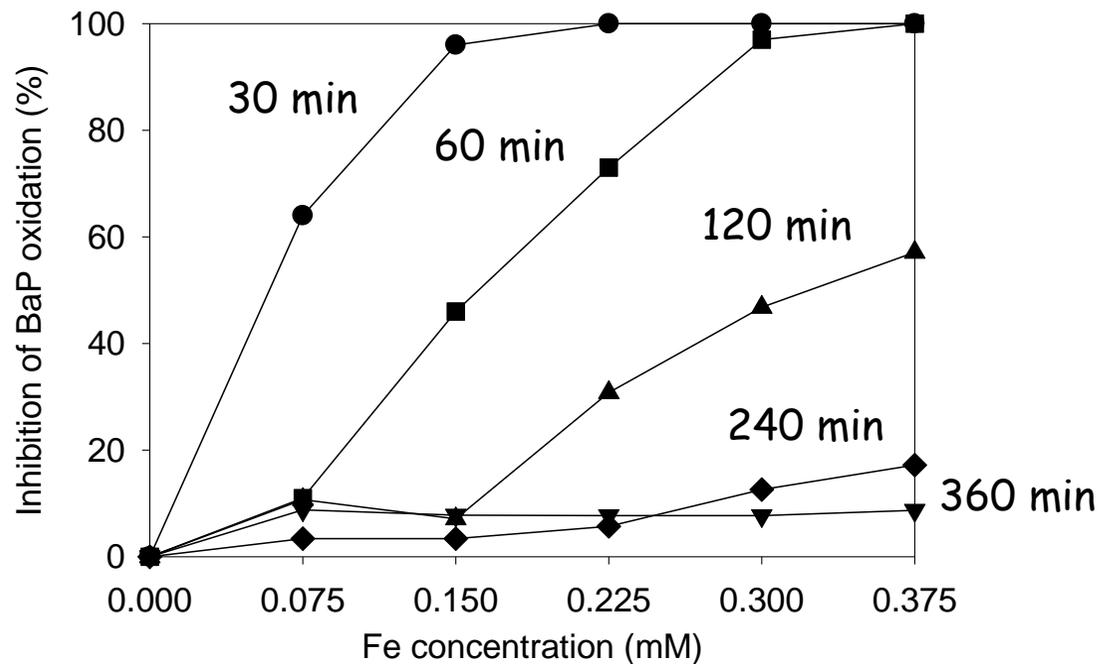
● mixture, ■ anthracene, ◆ benzo[a]pyrene, -lac, +lac

Common names	Amounts		Transformation products
	Initial (mg L <sup>-1</sup> )	After 48 h (%)	
<b>Naphtalene</b>	<b>10.0</b>	<b>1.2</b>	
Acenaphthylene	4.0	31.3	1,8-naphthalic acid anhydride
<b>Acenaphtene</b>	<b>3.0</b>	<b>17.4</b>	1,8-naphthalic acid anhydride
Fluorene	5.0	53.7	9-fluorenone
Phenanthrene	25.0	57.2	9,10-phenanthrene dione
<b>Anthracene</b>	<b>17.0</b>	<b>4.3</b>	9,10-anthracene dione
Fluoranthene	22.0	59.3	
Pyrene	20.0	57.3	1,6- 1,8-pyrene diones
Benzo[a]anthracene	9.0	68.6	7,12-benzo[a]anthracene dione
<b>Chrysene</b>	<b>5.0</b>	<b>95.6</b>	
Benzo[b]fluoranthene	17.0	63.5	
<b>Benzo[k]fluoranthene</b>	<b>15.0</b>	<b>99.3</b>	
<b>Benzo[a]pyrene</b>	<b>21.0</b>	<b>16.5</b>	1,6- 3,6- 6,12-benzo[a]pyrene diones
Dibenzo[ah]anthracene	1.0	73.7	
<b>Benzo[ghi]perylene</b>	<b>1.0</b>	<b>80.0</b>	
Indeno[123cd]pyrene	3.0	71.8	
16 PAHs	178.0	49.7	



# Effect of $K_4Fe(CN)_6$ on BaP oxidation by $ABTS^{++}$

2 mL 0.1 M citrate-phosphate buffer pH 3: 5 mM peroxodisulfate  
0.1 mM ABTS, 0.1 mM BaP, 0.075 to 0.375 mM  $K_4Fe(CN)_6$



## Conclusions

- fungal laccases are able to transform 10 PAHs in a synthetic mixture of 16 compounds. The reaction needs the presence of a redox mediator
  - metals, present in polluted soils, inhibit PAH transformation in withdrawing the electrons delivered by the mediator
  - as the inhibition by metals is limited in time, other unknown mechanisms may explain the lack of activity of the laccases
- Are laccases really efficient in polluted soils?  
How can we enhance their potential?

## Perspectives

- the results presented here have been obtained by using purified laccases from *P. cinnabarinus*. Similar data have been obtained later with enzymes from *T. versicolor*
  - we have now cloned and sequenced a gene encoding for a laccase of *T. versicolor*
  - we have expressed it in *E. coli*, soon in the yeast *Yarrowia lipolytica*
- the directed evolution of the enzyme should also be performed