# Universidad de La Frontera

What are the characteristics of white rot fungi that make them interesting candidates for solving some environmental problems?

WHITE-ROT FUNGI

Ligninolytic enzymes

Secondary metabolites

WRF play important roles in ecosystems

## WHITE-ROT FUNGI

## ENVIRONMENTAL APPLICATIONS

**Ligninolytic enzymes**

Bioremediation
- Pesticide degradation
- Detoxification of industrial dyes

**Fungal volatile organic compounds (FVOCs)**

Chemical markers of fungal presence
Biopesticides

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# Natural products of *Anthracophyllum discolor*: ligninolytic enzymes and antifungal volatile compounds


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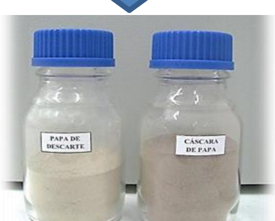
# Objective

To evaluate the production of ligninolytic enzymes and antifungal volatile organic compounds (VOCs) by *A. discolor* Sp4 using potato peels (PP) and discarded potato (DP) as low cost nutritional supports.

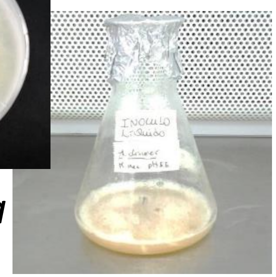
**Processing potato wastes**




**Inoculum**

*A. discolor*

*Agar media*



*Liquid media*

**1. Production of ligninolytic enzymes**

- Laccase
- Manganese peroridase
- Lignin peroxidase

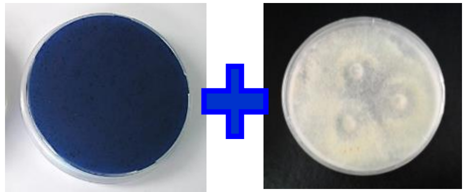
**2. Production of antifungal volatile compounds**

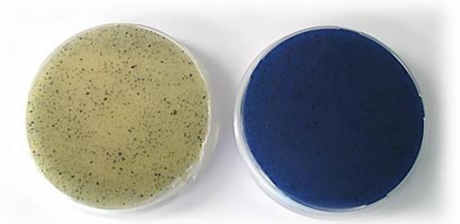
# 2. Methodology

# 1. Production of ligninolytic enzymes

## 1.1. Dye decolorization test

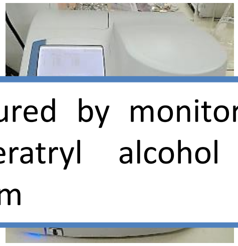
Remazol Brilliant Blue R (RBBR) (0.05% w/v)





1. **Kirk** + agar
2. Discarded potato agar (**DPA**)
3. Potato peel agar (**PPA**)

## 1.2. Ligninolytic enzyme activities

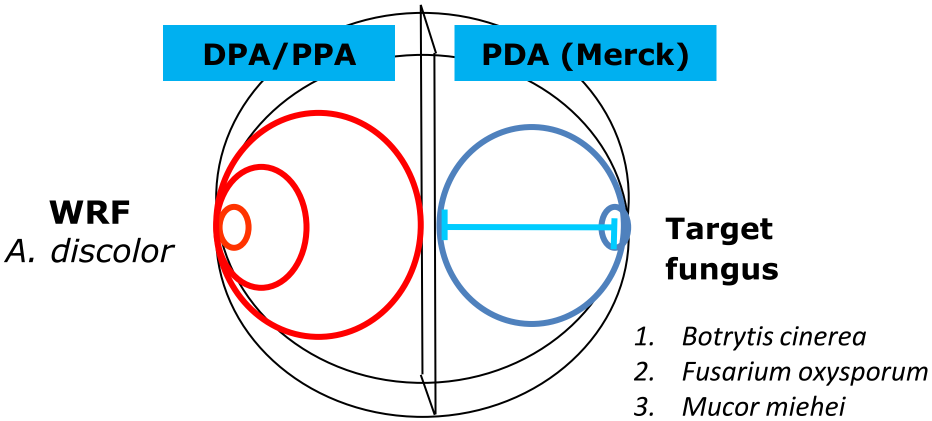


**Lac a** indepe (DMP)

**MnP a** oxidati adding

**LiP activity** was measured by monitoring the oxidation of veratryl alcohol to veratraldehyde at 310 nm

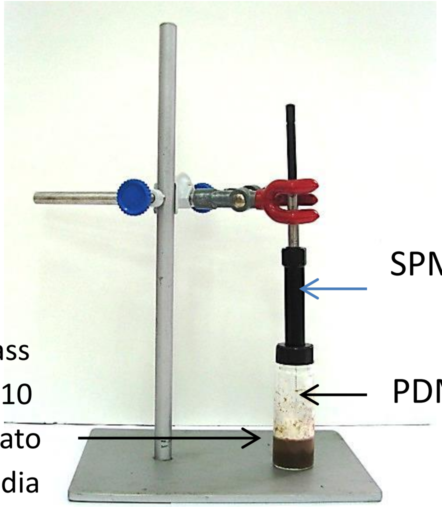
# 2. Production of antifungal volatile compounds




Bi-compartmented Petri dish assay

Analisys of VOCs effect by screening electron microscopy (SEM).

# 2. Production of volatile compounds




Solid phase micro-extraction (SPME)

Analisys of VOCs by gas chromatography coupled to mass spectrometry (GC-MS)

# 1. Production of ligninolytic enzymes

## 1.1. Dye decolorization test

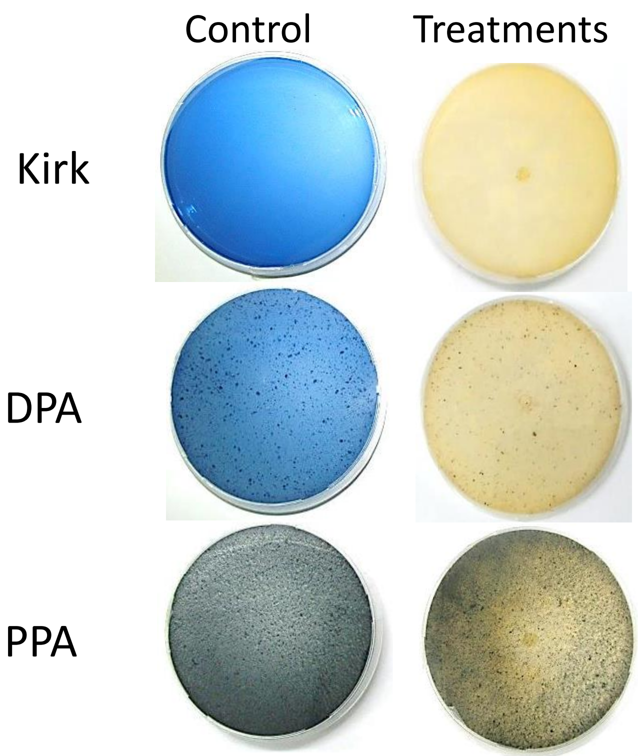



**Figure 1.** Decolorization RBBR (0.05% w/v) by *A. discolor* incubated during 21 days on discarded potato agar (DPA), potato peel agar (PPA) and modified Kirk medium.

## 1.2. Ligninolytic enzyme activities

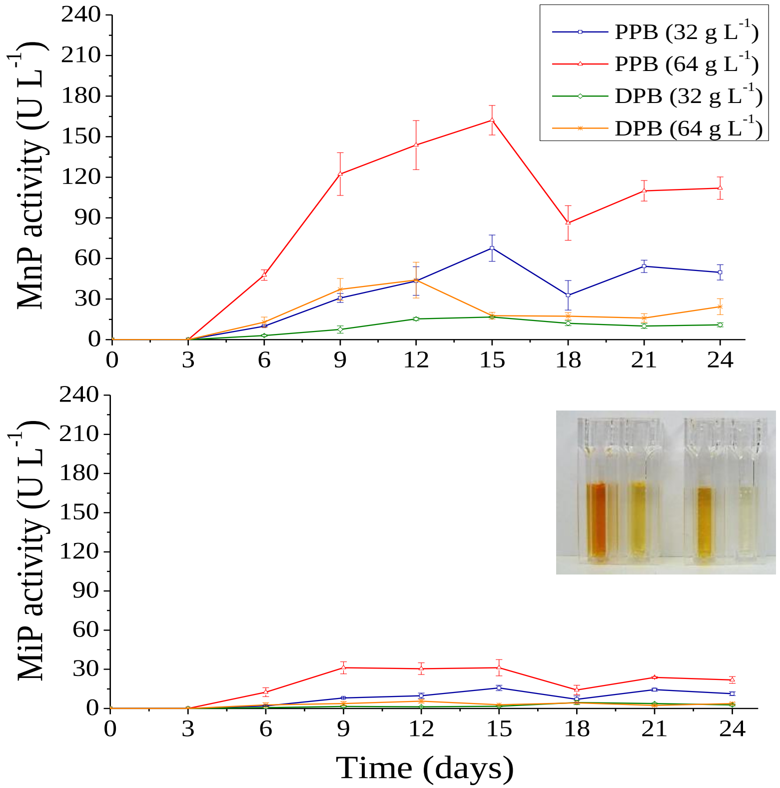



**Figure 2.** Manganese peroxidase (MnP) and manganese-independent peroxidase (MiP) activities by *A. discolor* Sp4 cultured in potato peel broth (PPB) and discarded potato broth (DPB) at two potato powder concentrations.

# 2. Production of volatile compounds

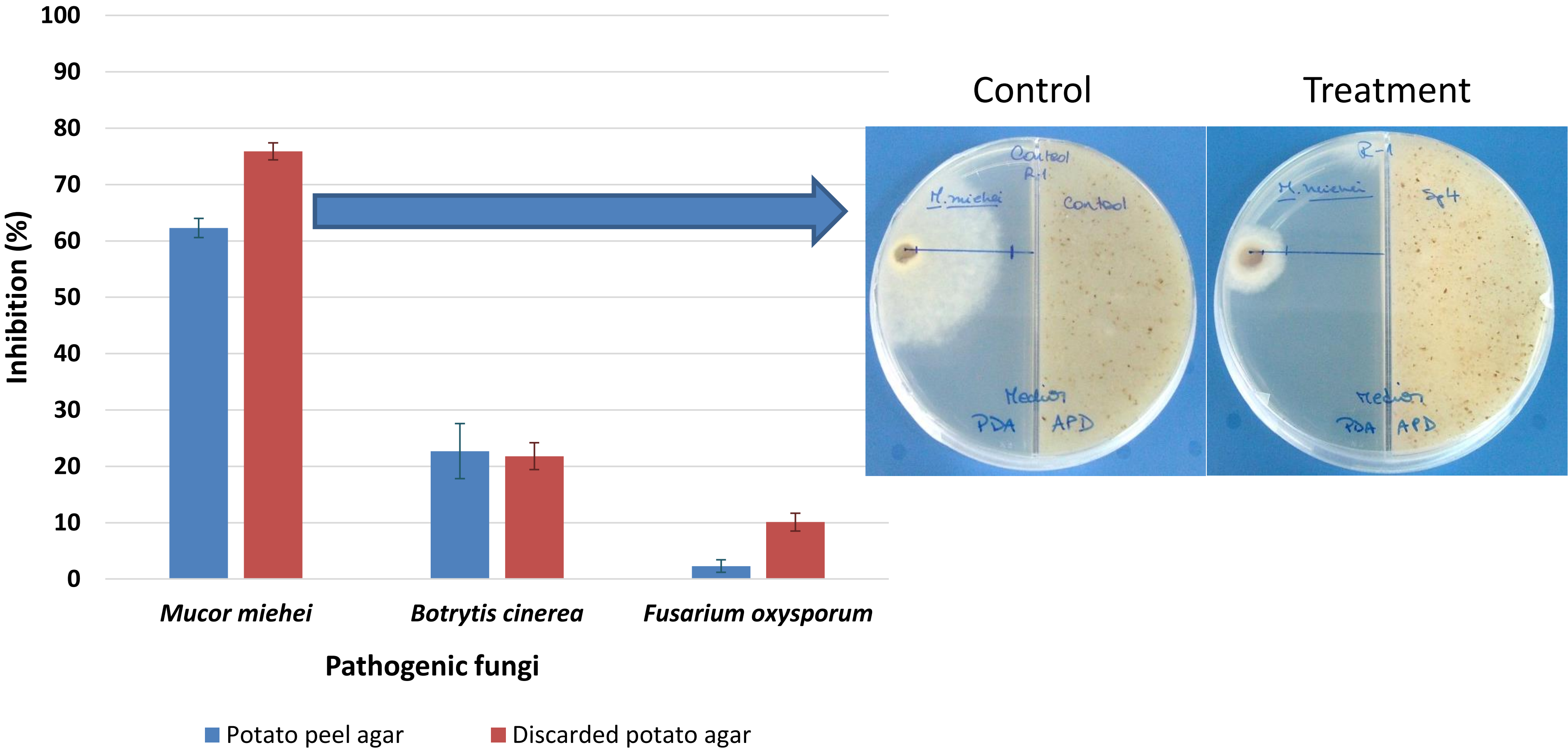



**Figure 3.** Antifungal activity of volatile compounds released from *A. dicolor* against *B. cinerea*, *F. oxysporum* and *M. miehei*.

## 2. Production of volatile compounds

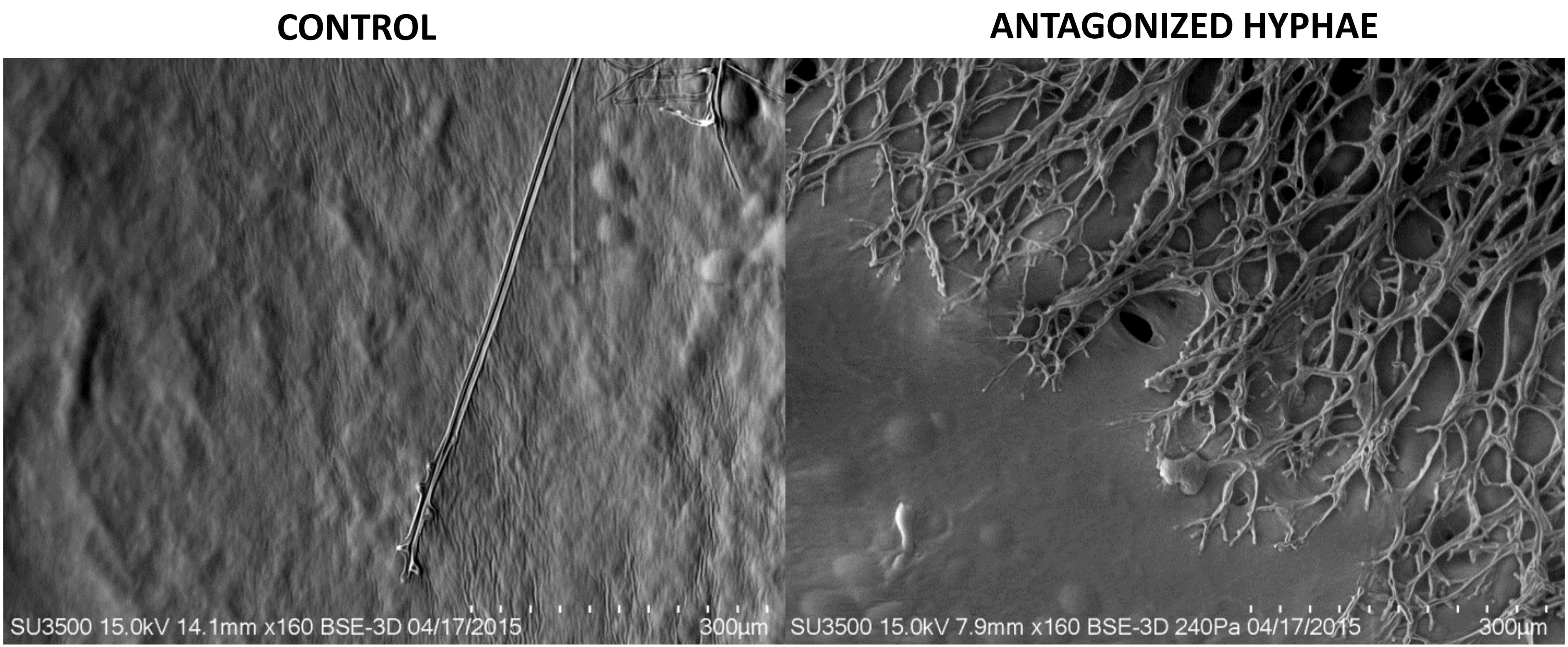
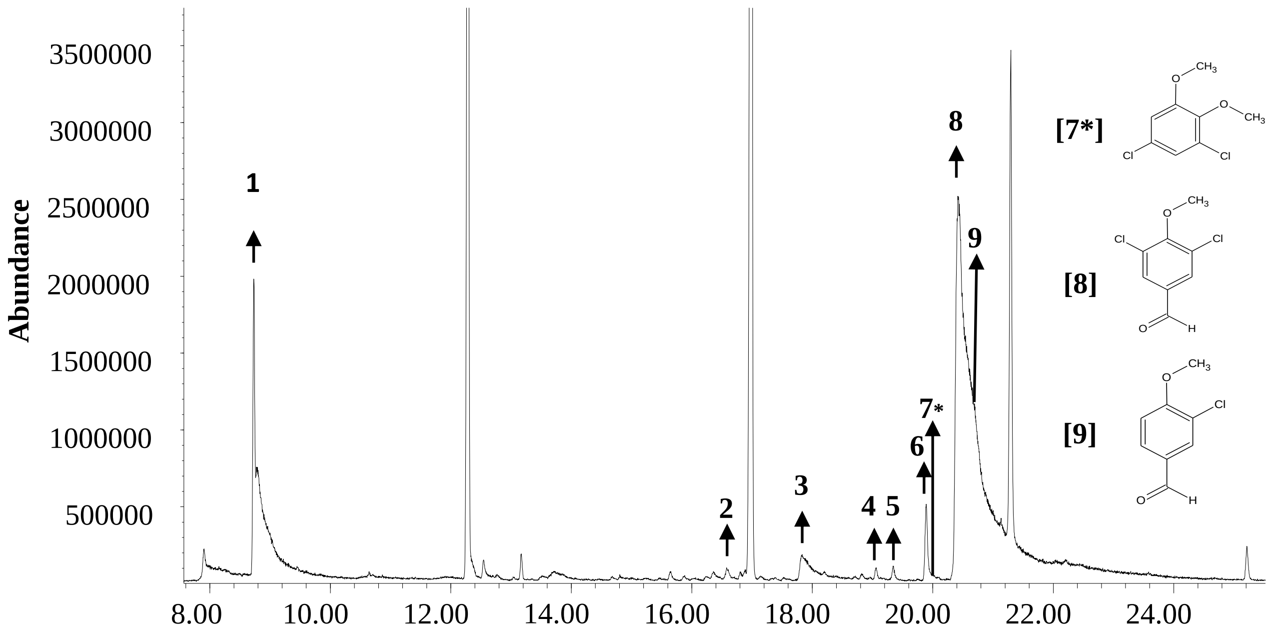



**Figure 4.** SEM images of untreated control hyphae of the target fungus *M. miehei* (left) and the antagonized hyphae of *M. miehei* exposed to the antifungal volatiles of *A. discolor* Sp4 (right).

# 2. Production of volatile compounds




The mass spectrums of peaks pointed to the structures of:

[1] 1-heptanol

[2] 2-butyl-1-octanol

[3] 3-ethyl-1,2-dihydroquinoxalin-2-one

[4] α-bisabolene

[5] 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene

[6] bulnesene

[7] 1,5-dichloro-2,3-dimethoxybenzene*

[8] 3,5-dichloro-4-methoxybenzaldehyde

[9] 3-chloro-4-methoxybenzaldehyde.

**Figure 5**. Gas chromatogram of VOCs released from *A. discolor* Sp4 cultured on DPA medium (discarded potato agar). Peaks appearing in the control vial were omitted from the list (N=3).

# Conclusions

The fungus *Anthracophyllum discolor* Sp4 are able to produce ligninolytic enzymes using potato wastes as nutritional support.

VOCs released from *A. discolor* Sp4 showed antifungal activity against *Mucor miehei* and *Botrytis cinerea*. The antifungal activity seems to be related to the production of sesquiterpenes and chlorinated aromatic compounds.

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