

Antagonistic potential of *Wickerhamomyces anomalus* against phytopathogens causing Olive Anthracnose and Chestnut Ink Disease



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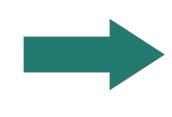
Background

Olive Anthracnose (OA) and Chestnut Ink Disease (CID)

Eukaryotic phytopathogens,
 Colletotrichum fungi and Phytophthora oomycetes.

Current disease management

- Mostly through chemical fungicides.
- Ineffective and harmful for the environment.
- Increasingly under strict legislation impeding utilization.



URGENT EFFECTIVE AND ECO-FRIENDLY ALTERNATIVES NEEDED

Yeasts as Fungal Antagonists

- Well known antagonistic activity against other yeasts, fungi and bacteria
- Often applied in food spoilage control and excellent candidates for in field biological control

Aims and Strategy

Explore the strong and recognized antagonistic potential of Wickerhamomyces anomalus towards more sustainable management

towards more sustainable management of OA and CID

Co-culturing fungus / yeast

Solid and liquid media

I. Viability
assays
Re-culturing

Re-culturing

MB Staining
Microsocopy

III. SEM

Microscopy

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Cellulase

β-glucanase

Chitinase

CAS-Agar Assay

DNS

assay

II. Enzymatic &

siderophore activity

Wickerhamomyces anomalus
Industrial strain LBCM 1105

VS.

Olive Anthracnose Chestnut Ink Disease



Colletotrichum gloeosporioides Colletotrichum nymphaeae Colletotrichum godetiae



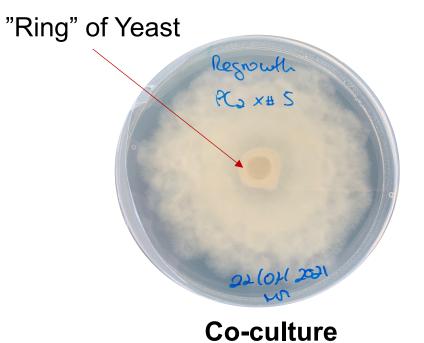
Phytophthora cinnamomi Phytophthora cambivora

Results

I. Viability assays



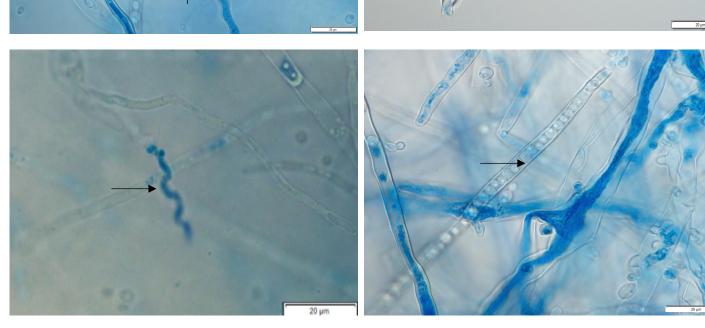
ControlPathogen single culture



W. anomalus vs. P. cinnamomi

Growth on all Viability is combinations retained

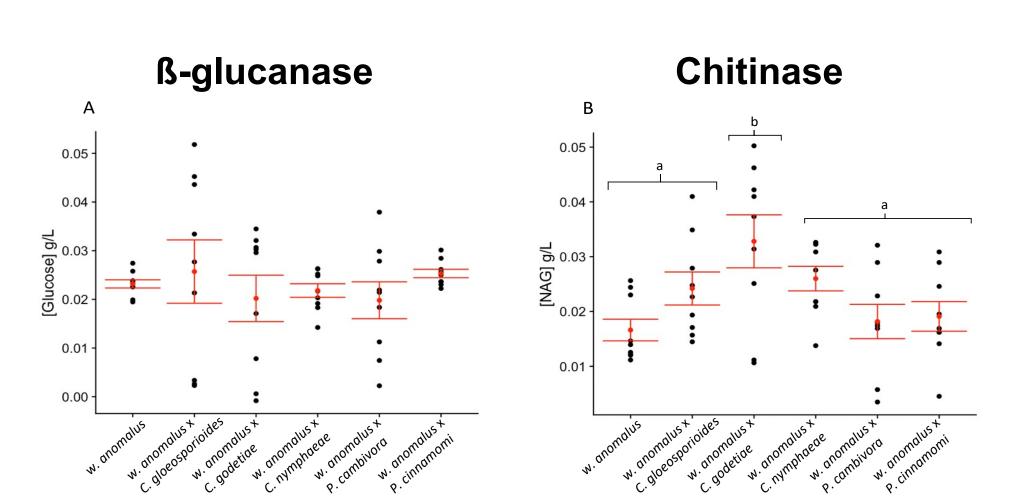




MB staining on all combinations

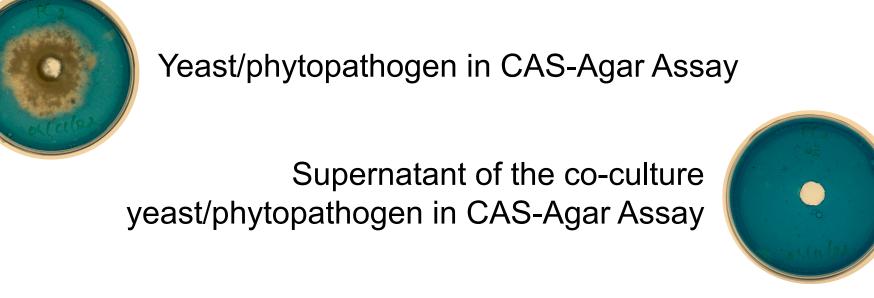
- Part of all fungal cultures are dead
- No dose-dependence → cell contact is needed
- Emptied hyphae and coiled hyphae
- Ruptured hyphae filled w/ yeast cells

II.Enzymatic and siderophore activity



ß-glucanase and Chitinase activity observed in all co-cultures **but** also on yeast single culture

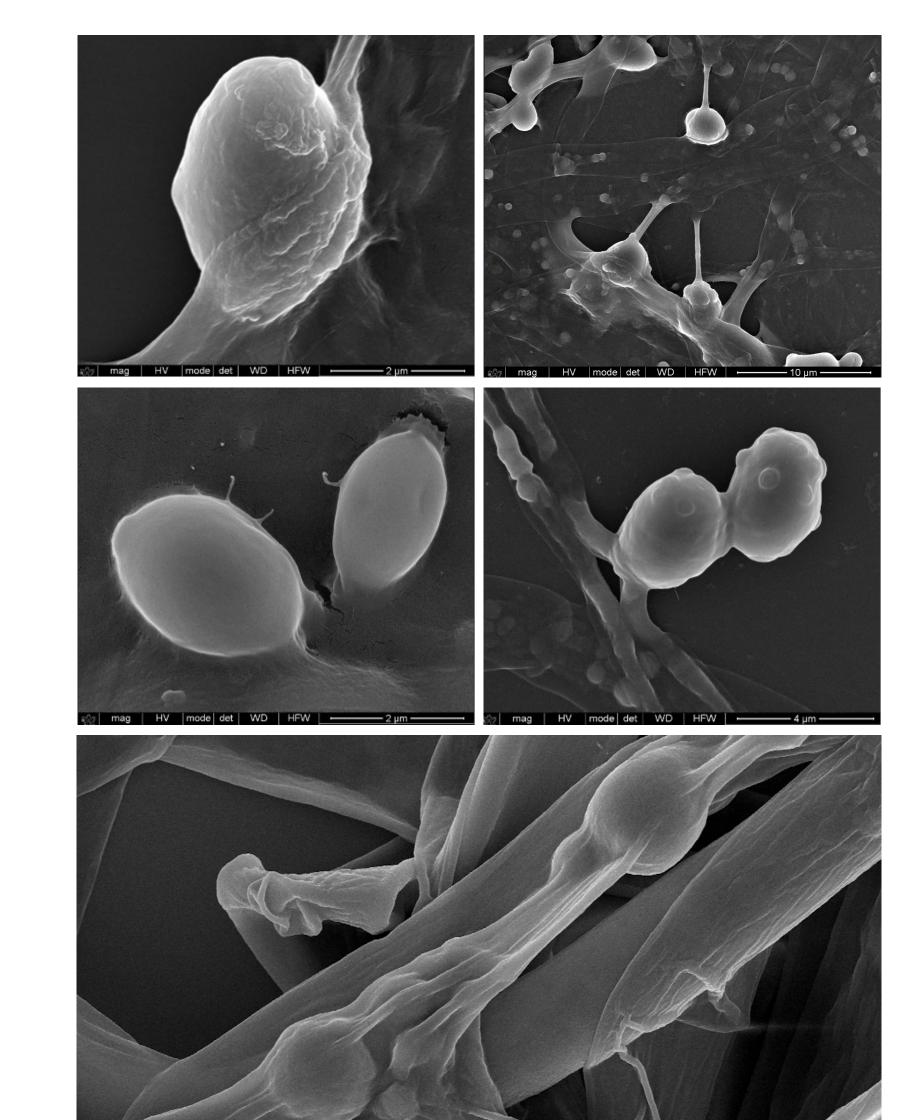
No **cellulase** activity



No **siderophore** activity

NO INFLUENCE ON ANTAGONISM

III.SEM Microscopy



- Veil-like structure ALL FUNGI covering yeasts in attachment points to hyphae
- **Tube-like** structures connecting yeasts to hyphae
- Fimbriae-like filaments around yeasts
- Cell wall fusion
 between yeasts and hyphae
- Spherical structures inside hyphae
- C. godetiae C. nymphaeae
- C. nymphaeae C. gloeosporioides
- C. gloeosporioides
- P. cinnamomi

YEAST PREDATORY BEHAVIOR

Take-home message

The results from the enzymatic assays clearly show that the production and release of enzymes to the extracellular space is not *per se* a condition for them being involved in antagonism. *W. anomalus* behaves as a **contact predator** of **filamentous fungi** and **comycetes**, and interacts differently with different opponents, which suggest species specific recognition mechanisms, and decreases the danger of indiscriminate impact in wild microbial populations. Results confirm this yeast as a serious candidate for *in field* assays on the biological control of OA and CID.

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