

Supplementary information

Additional file 1

Effect of hydrogen peroxide and carbon-to-nitrogen ratio on growth and biochemical profile in oleaginous *Mucoromycota*

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Table S1. Range of H₂O₂ sublethal concentrations evaluated for each fungal strain.

Species	<i>Mucor circinelloides</i>	<i>Rhizopus stolonifer</i>	<i>Mucor circinelloides</i>	<i>Umbelopsis vinacea</i>	<i>Mortierella alpina</i>	<i>Mortierella hyalina</i>	<i>Absidia glauca</i>	<i>Cunninghamella blakesleeana</i>	<i>Lichtheimia corymbifera</i>
Collection no.	CCM F220	VKM F400	VI04473	CCM F539	ATCC 32222	VKM F1629	CCM F451	CCM F705	CCM F8077
Abbreviation	MC	RS	MCVI	UV	MA	MH	AG	CB	LC
H ₂ O ₂ (mM)	0	0	0	0	0	0	0	0	0
	0.438	0.5	0.25	0.0625	0.0625	0.0625	0.125	0.25	0.25
	0.563	0.563	0.5	0.125	0.125	0.125	0.25	0.5	0.5
	0.875	1	1	0.25	0.25	0.25	0.5	1	1
	1.125	1.125	2	0.5	0.5	0.5	1	2	2
	1.75	2	4	1	1	1	2	4	4
	2.25	2.25	8	2	2				
	3.5	4							
	4.5	4.5							
	7	8							
	9								
	14								

Table S2. Values of minimal fungicidal concentration (MFC) and minimal inhibitory concentration (MIC) for the set of Mucoromycota strains studied. n.a.: not available.

Strain	Collection number	MFC (H ₂ O ₂ mM)	MIC (H ₂ O ₂ mM)
<i>Mucor circinelloides</i>	CCM F220	25	18.7
<i>Mucor circinelloides</i>	VI04473	12.5	9.38
<i>Cunninghamella blakesleeana</i>	CCM F705	9.38	9.38
<i>Rhizopus stolonifer</i>	VKM F400	9.38	9.38
<i>Lichtheimia corymbifera</i>	CCM F8077	9.38	4.69
<i>Mortierella alpina</i>	ATCC 32222	n.a.	4
<i>Absidia glauca</i>	CCM F451	3.13	3.13
<i>Umbelopsis vinacea</i>	CCM F539	2.34	1.56
<i>Mortierella hyalina</i>	VKM F1629	1.17	1.17

* MFC calculation it is based in the concentration that kills 99.9% of the initial number of spores used while the MIC assay for *M. alpina* was based on mycelium. Therefore, MFC from *M. alpina* was technically impossible to define in such terms.

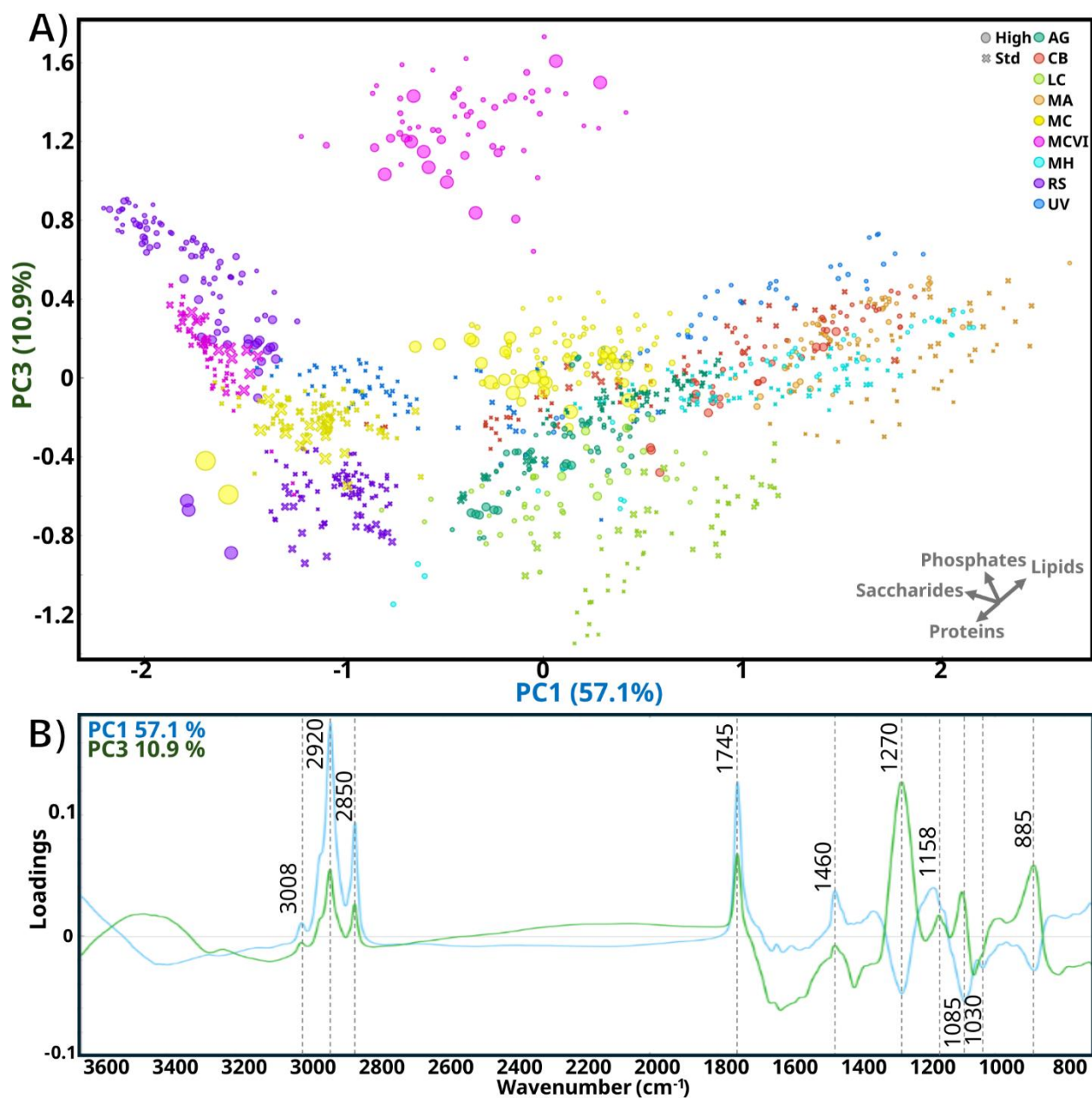


Figure S1. PCA score and loadings plots of FT-IR spectra of *A. glauca* (AG), *C. blakesleeana* (CB), *L. corymbifera* (LC), *M. alpina* (MA), *M. circinelloides* (MC and MCVI strains), *M. hyalina* (MH), *R. stolonifer* (RS), and *U. vinacea* (UV) grown at standard (×) and high (●) C/N and different concentrations of H₂O₂ represented by relative proportional symbol size (larger symbol size corresponds to higher concentration). A) Score plot using PC1 and PC3 with an explained variance of 57.1% and 10.9% respectively. A vector axes are provided as approximate indications of the directions of the relative increase of each metabolite. Color transparent dash-line arrows indicate the progression of each species from standard to high C/N conditions. B) Loadings plot of PC1 and PC3 with the most relevant peaks assigned. The explained variance for the first five PC was: 57.1%, 16.5%, 10.9%, 4.7%, and 3%.

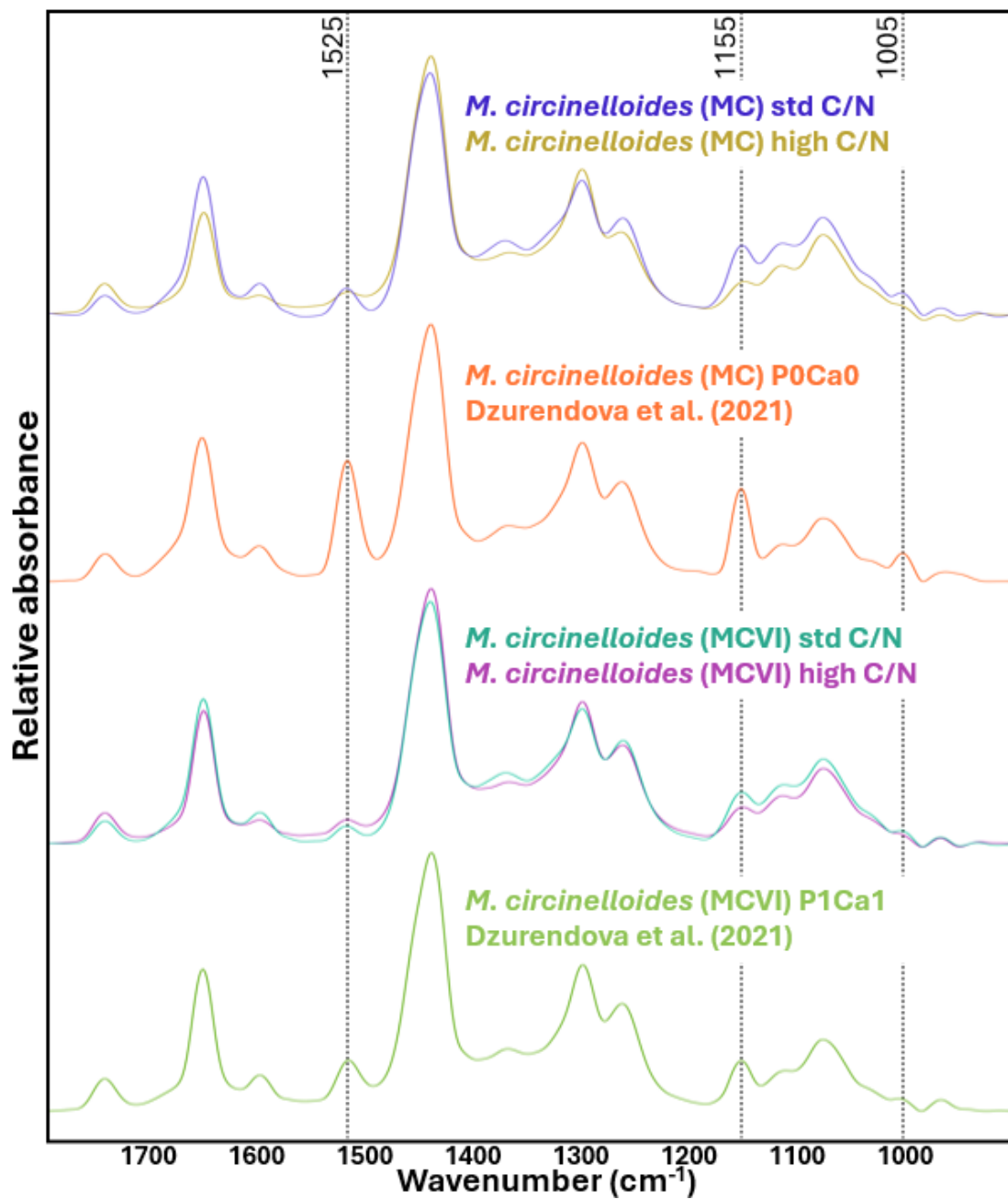


Figure S2. Comparison of FT-Raman spectra from *Mucor circinelloides* strains (MC and MCVI) grow at standard and high C/N medium and FT-Raman spectra of the same strains from Dzurendova et al. (2021) chosen at specific conditions (P: phosphate level, Ca: calcium level), which contained higher amounts of carotene than in the present study. Wavenumbers 1525, 1155 and 1005 cm⁻¹ are marked to indicate the presence of carotenes in the samples.

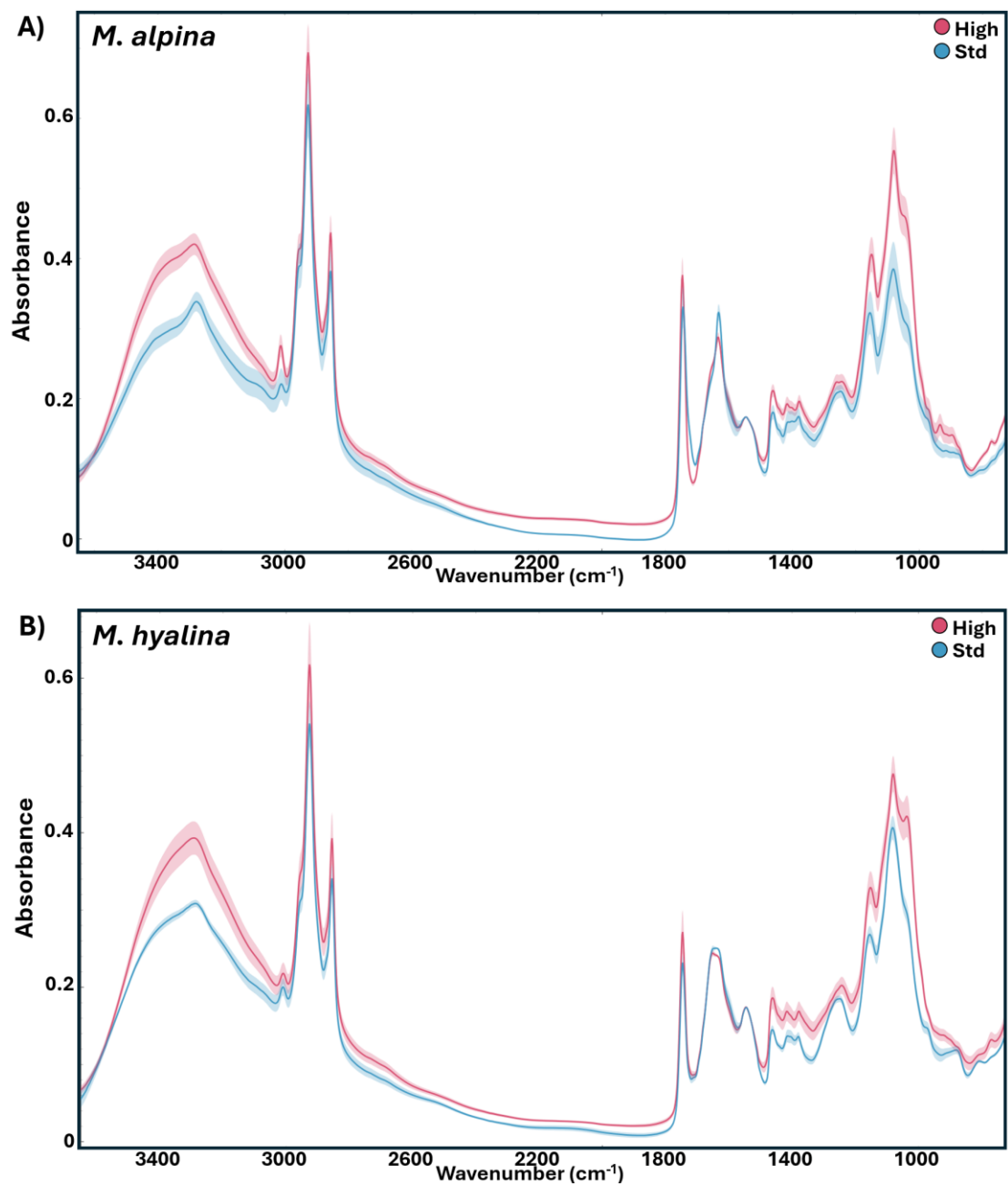


Figure S3. A) Spectra normalized by amide II from A) *Mortierella alpina* (MA) and B) *Mortierella hyalina* (MH) grown at standard and high C/N without H₂O₂.

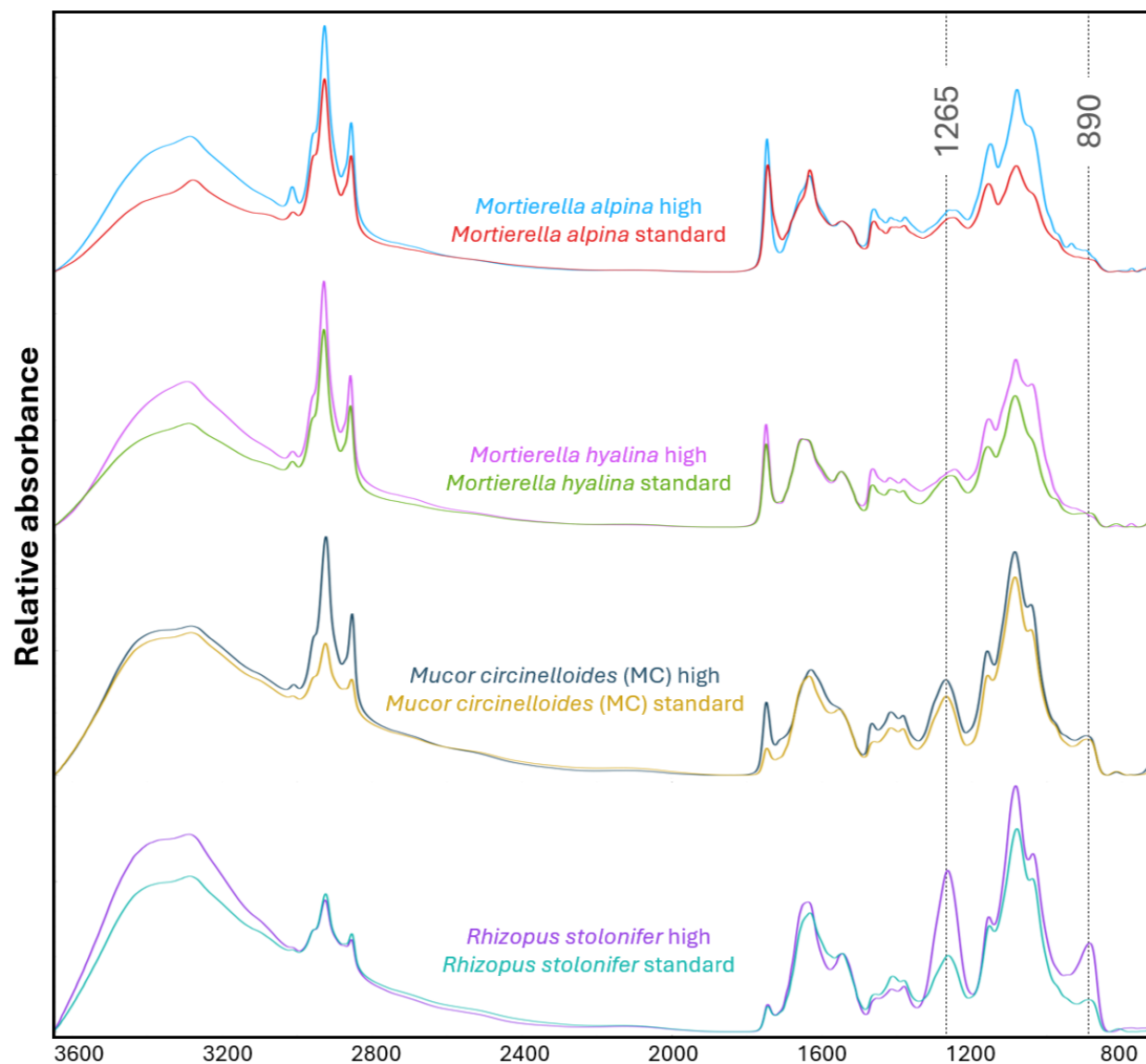


Figure S4. Comparison of phosphate-associated peaks (1265 cm^{-1} and 890 cm^{-1}) in FTIR-HTS spectra from *M. alpina*, *M. hyalina*, *M. circinelloides* (MC), and *R. stolonifer* grown at standard and high C/N media without H_2O_2 . Spectra were preprocessed by rubber band baseline correction and normalized by amide II.

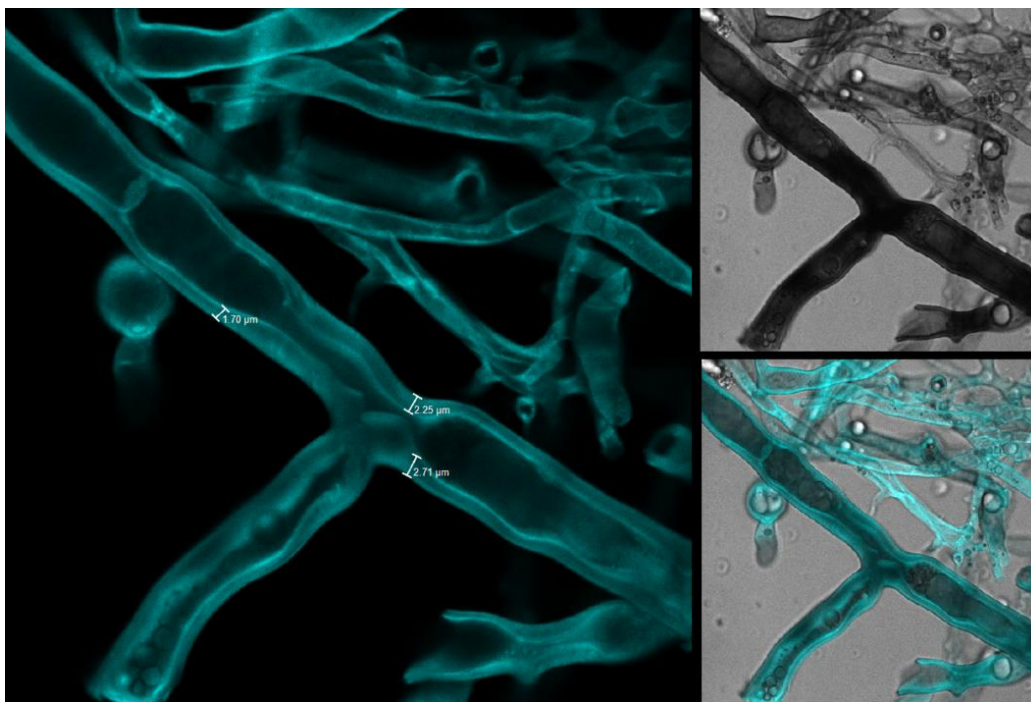


Figure S5. Pictograms of *Cunninghamella blakesleeana* stained with calcofluor white stain observed with confocal microscopy. View of fluorescence and brightfield channel separately and merged. The thickness of cell walls is marked with white bars and the corresponding value (1.7-2.7 μm).

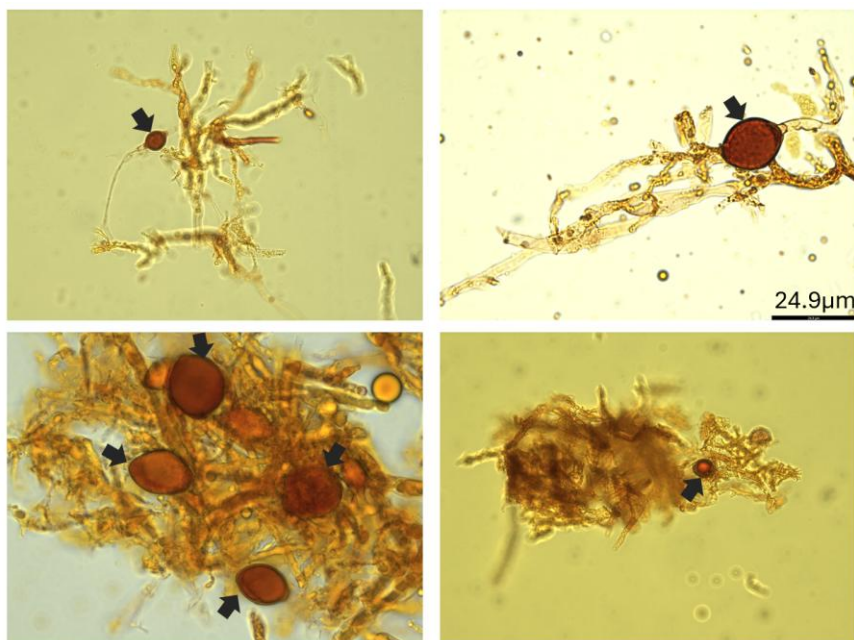


Figure S6. Pictograms of *Mortierella hyalina* grown in standard C/N medium stained with Lugol's reagent. Black arrows were included to indicate the presence of chlamydospores. The dark brown coloration produced by the staining indicates high content of glycogen and/or lipids.

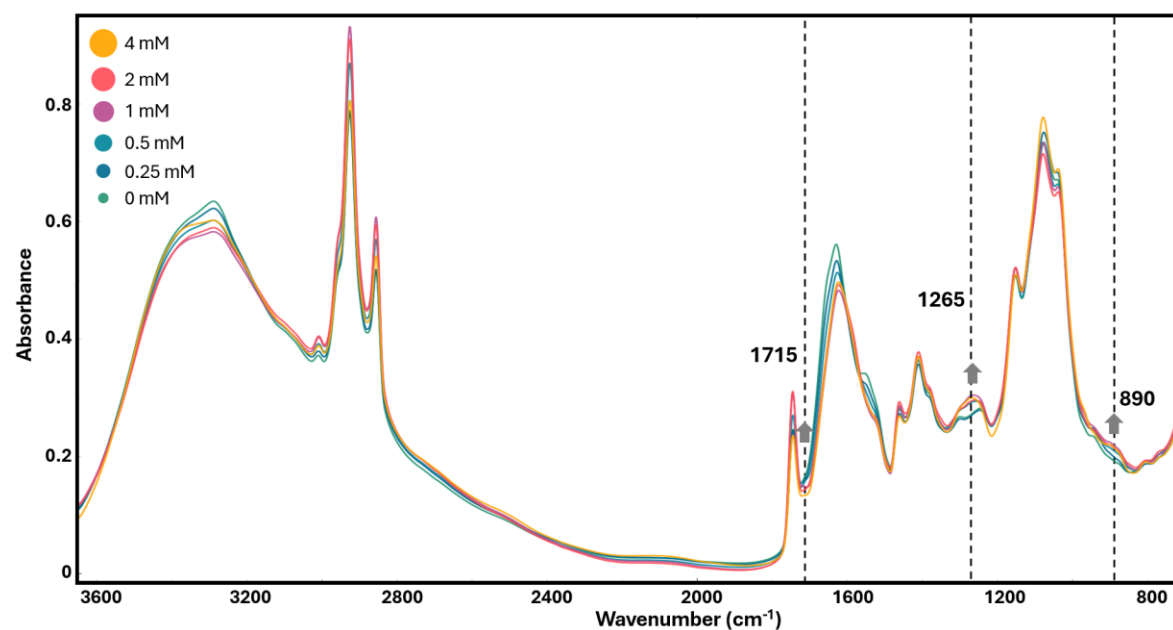


Figure S7. EMSC corrected spectra of *L. corymbifera* grown in standard C/N medium under different concentrations of H_2O_2 . Dash lines and grey arrows indicate small increases in specific wavenumbers that can be associated with protein phosphorylation and lipid peroxidation.