Yeast-Derived Biomolecule Mixtures from Wine Lees Display Antimicrobial Activity: an integrated bioprocess approach against grapevine pathogens

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Supplementary materials

Table S1 Table showing products' coding based on their inoculation modalities, fermentation medium, yeast's biomass conducted procedure, protein and FAN concentration of the final products

Table S2 Positive ionization mode (ESI+) and negative ionization mode (ESI-) intensities from each feature (Excel file ESM_2)

Table S3 Table of each marker with the corresponding *p-value* and FDR, FC and F-statistic (Excel file ESM_3)

Table S4 Table of the specific correlation and *p-value* of the biomarker with each pathogen (Excel file ESM_4)

Table S5 Biomarkers annotated at level 2. Putative identity of the biomarkers, including the respective intensity, ANOVA clustering (Tukey-Kramer HSD, with a=0.05) and *in silico* hydrophobicity, charge and pI (Excel file ESM_5)

Fig. S1 Yeast growth kinetics in preliminary laboratory fermentations in YPD broth

Fig. S2 Biomass production at the end of alcoholic fermentations all expressed in % (w/v)

Fig. S3 Fermentation kinetics expressed in released CO_2 (g/L) over time and Fermentation rate expressed in released CO_2 (g/L/h) over time

Fig. S4 Yeast growth kinetics (total viable population)

Fig. S5 Biomass production at the end of alcoholic fermentation all expressed in % (w/v)

Fig. S6 Lignin and phenolic compounds quantification on wood tissue

Fig. S7 Hierarchical Clustering Heatmap of features and growth capacity of *Phaeoacremonium minimum* (A) and *Phaeomoniella chlamydospora* (B) correlation matrix Heatmap

Table S1 Table showing products' coding based on their inoculation modalities, fermentation medium, yeast's biomass conducted procedure, protein and FAN concentration of the final products

Code	Inoculation modalities	Fermentation medium / Grape must	Yeast biomass procedure	Product's name	Protein Concentration (mg/L) for HMW-BM and FAN (mg/L) for LMW-BM
S1	Monoculture of indigenous *S. cerevisiae* strain (S1)	Laboratory grape juice medium	Autolysis	HMW-BM-S1	2440.7 ± 143.4
			Hydrolysis	LMW-BM-S1	382.44 ± 1.76
S3	Monoculture of commercial *S. cerevisiae* (S3)	Laboratory grape juice medium	Autolysis	HMW-BM-S3	2460.0 ± 311.8
			Hydrolysis	LMW-BM-S3	379.53 ± 1.92
S1H	Sequential inoculation of commercial *Hanseniaspora sp* and indigenous *S. cerevisiae* strain (S1)	Laboratory grape juice medium	Autolysis	HMW-BM-S1H	2224.0 ± 289.3
			Hydrolysis	LMW-BM-S1H	380.21 ± 1.15
S3H	Sequential inoculation of commercial *Hanseniaspora sp.* and commercial *S. cerevisiae* strain (S3)	Laboratory grape juice medium	Autolysis	HMW-BM-S3H	2186.0 ± 211.4
			Hydrolysis	LMW-BM-S3H	366.76 ± 1.64
M1S3	Monoculture of commercial *S. cerevisiae* (S3)	Grape must: Savatiano (M1)	Hydrolysis	M1S3	250.66 ± 0.50
M2S3	Monoculture of commercial *S. cerevisiae* (S3)	Grape must: Roditis (M2)	Hydrolysis	M2S3	228.84 ± 14.21
M3S3	Monoculture of commercial *S. cerevisiae* (S3)	Grape must: Moschofilero (M3)	Hydrolysis	M3S3	254.39 ± 25.34
M1HS3	Sequential inoculation of commercial *Hanseniaspora sp.* and commercial *S. cerevisiae* strain (S3)	Grape must: Savatiano (M1)	Hydrolysis	M1HS3	241.27 ± 2.66
M2HS3	Sequential inoculation of commercial *Hanseniaspora sp.* and commercial *S. cerevisiae* strain (S3)	Grape must: Roditis (M2)	Hydrolysis	M2HS3	290.43 ± 11.78
M3HS3	Sequential inoculation of commercial *Hanseniaspora sp.* and commercial *S. cerevisiae* strain (S3)	Grape must: Moschofilero (M3)	Hydrolysis	M3HS3	257.75 ± 22.67

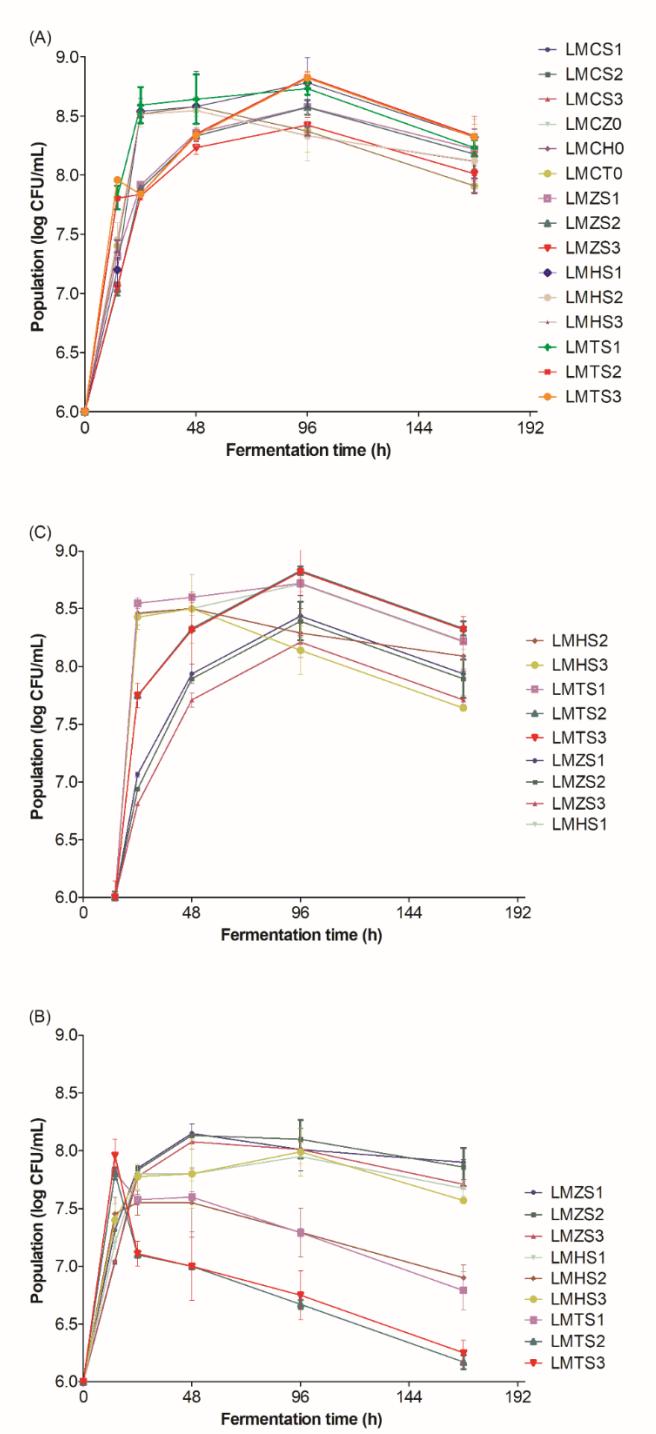

Fig. S1 Yeast growth kinetics in preliminary laboratory fermentations in YPD broth: (A) Total viable population (TVP) in all fermentations; (B) Non-*Saccharomyces* population in sequential modalities, (C) *S. cerevisiae* population in sequential modalities. Coding: LM= laboratory medium, C= monoculture, S1=*S. cerevisiae* S1, S2=*S. cerevisiae* S2, S3= *S. cerevisiae* S3, Z=*Z. bailii*, H= *Hanseniaspora sp.*, T=*T. delbrueckii.* Average value of three experiments ± standard deviation.

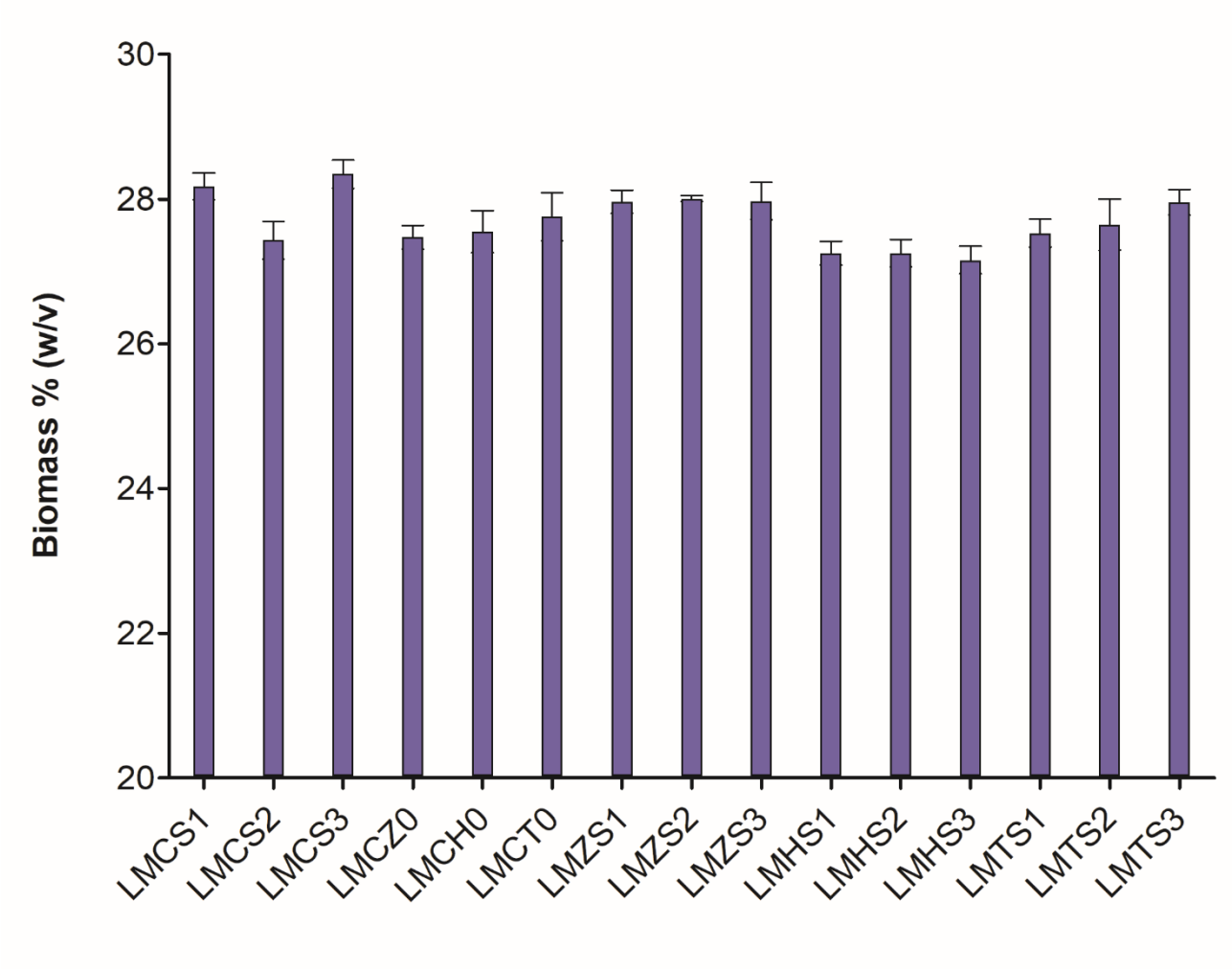

Fig. S2 Biomass production at the end of alcoholic fermentations all expressed in % (w/v). Average value of three experiments ± standard deviation. Coding: LM= laboratory medium, C= monoculture, S1=*S. cerevisiae* S1, S2=*S. cerevisiae* S2, S3= *S. cerevisiae* S3, Z=*Z. bailii*, H= *Hanseniaspora sp.*, T=*T. delbrueckii.* Average value of three experiments ± standard deviation.

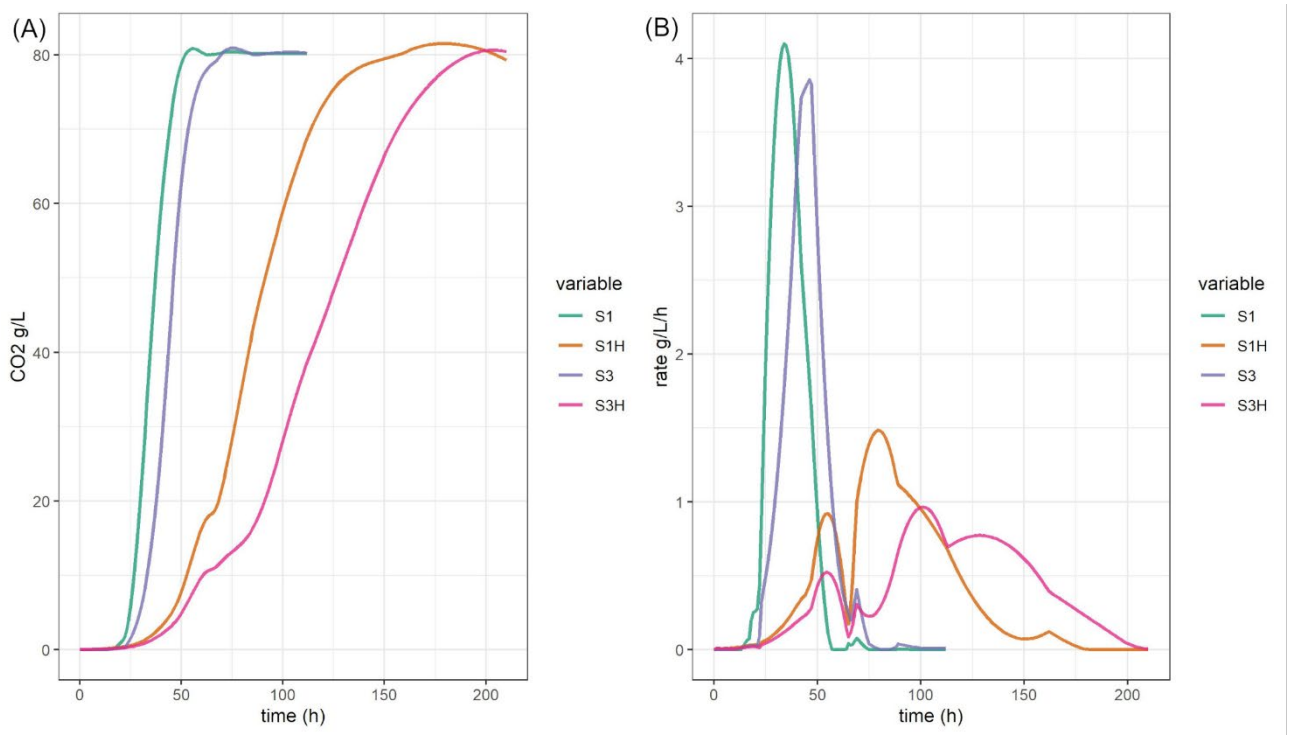

Fig. S3 (A) Fermentation kinetics expressed in released CO_2 (g/L) over time and (B) Fermentation rate expressed in released CO_2 (g/L/h) over time in pure (S1 (*S. cerevisiae*), S3 (*S. cerevisiae*) and mixed (S1H, S3H) cultures. Average values of three experiments, standard deviation <5%.

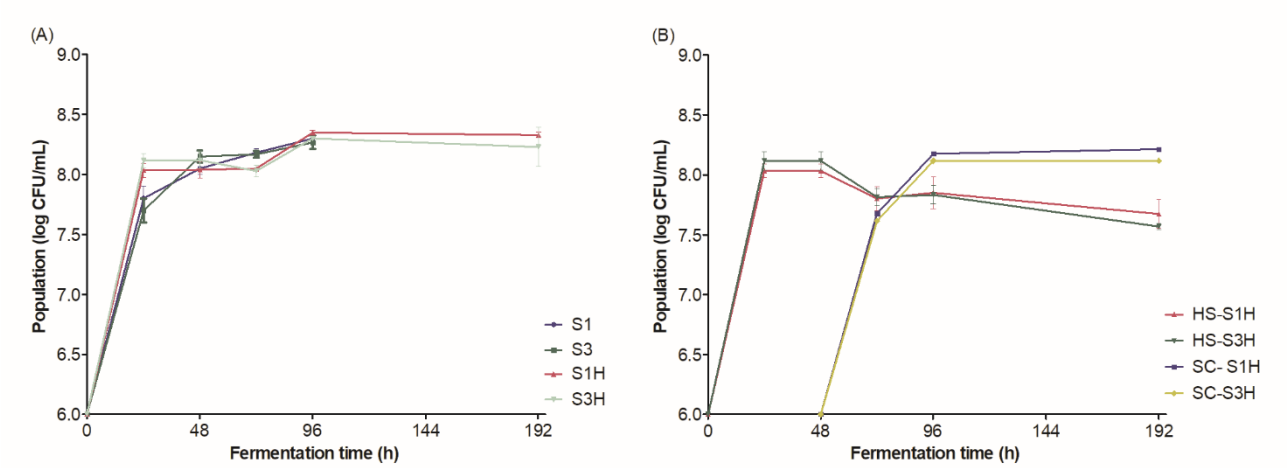

Fig. S4 Yeast growth kinetics: (A) Total viable population in pure cultures S1 (*S. cerevisiae*), S3 (*S. cerevisiae*) and in sequential inoculation schemes with *Hanseniaspora sp.* (S1H, S3H); (B) *S. cerevisiae* (SC) and *Hanseniaspora sp.* (HS) populations in sequential cultures (S1H and S3H). Average value of three experiments ± standard deviation.

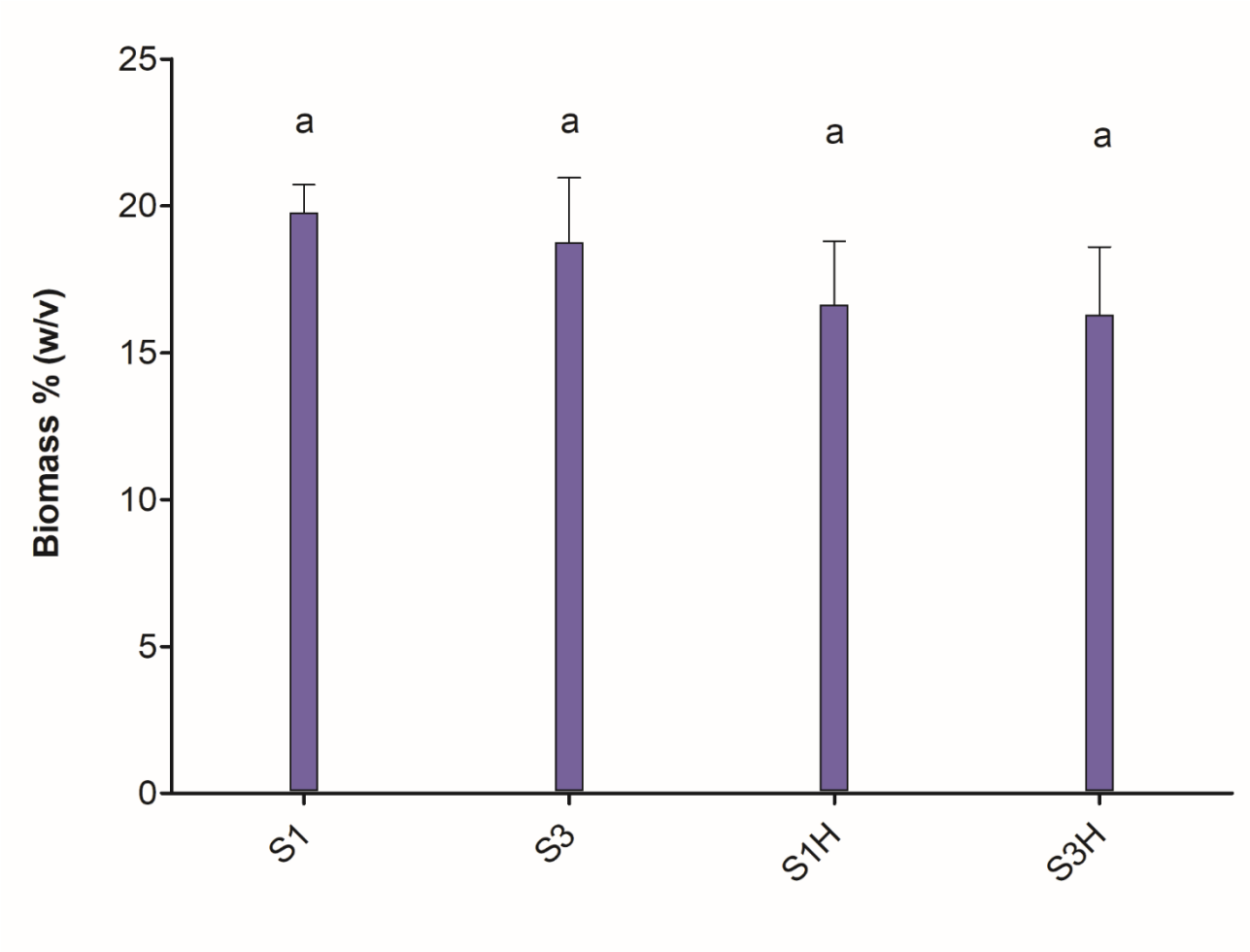

Fig. S5 Biomass production at the end of alcoholic fermentation all expressed in % (w/v). Average value of three experiments ± standard deviation. Different letters denote statistically significant differences ($p < 0.05$) based on ANOVA followed by Tukey's post hoc test.

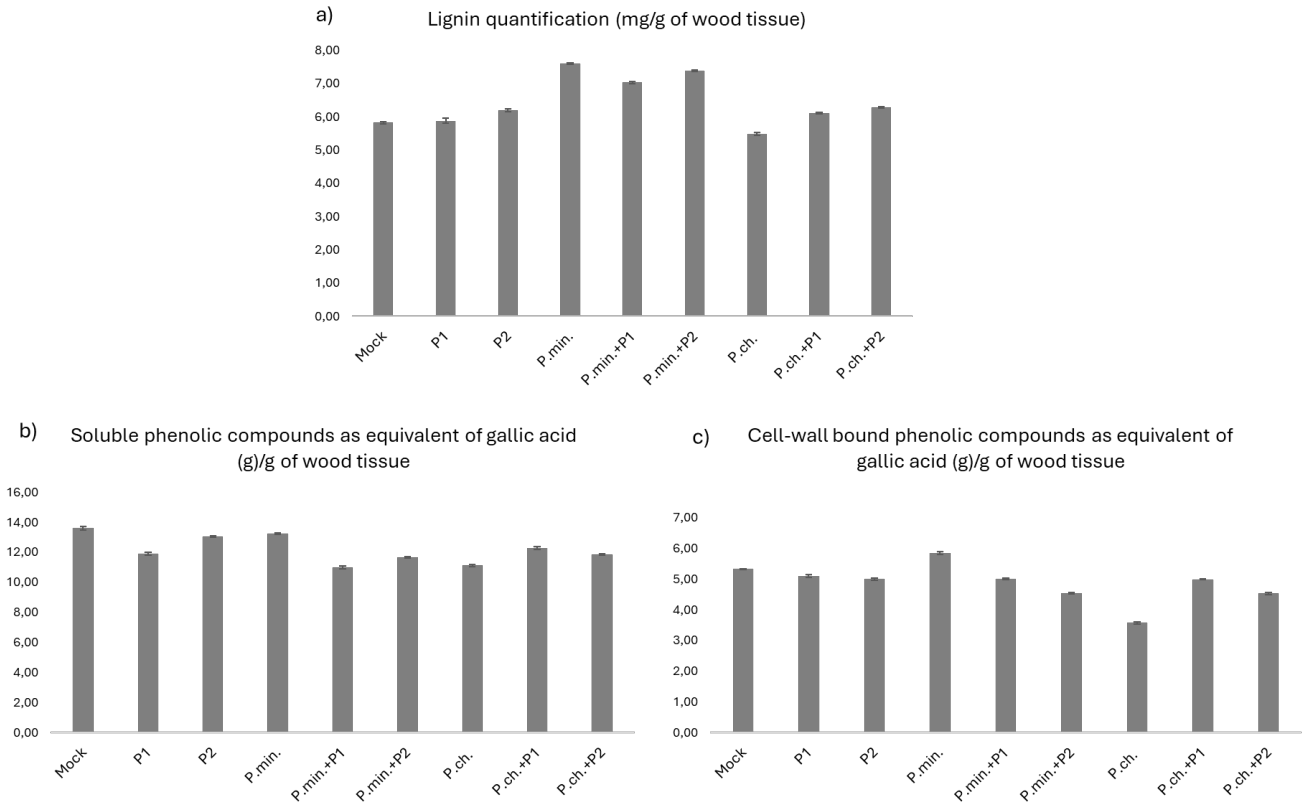

Fig. S6 Lignin (a), soluble phenolic compounds (b), and cell-wall phenolic compounds (c) quantification on 112 dpi wood tissue. All samples were tested in triplicates. Columns represent means of the three replications and the vertical bars indicate standard errors. Columns with an asterisk (*) are significantly different from the control, as determined by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons with the control (*p-value* < 0.05).

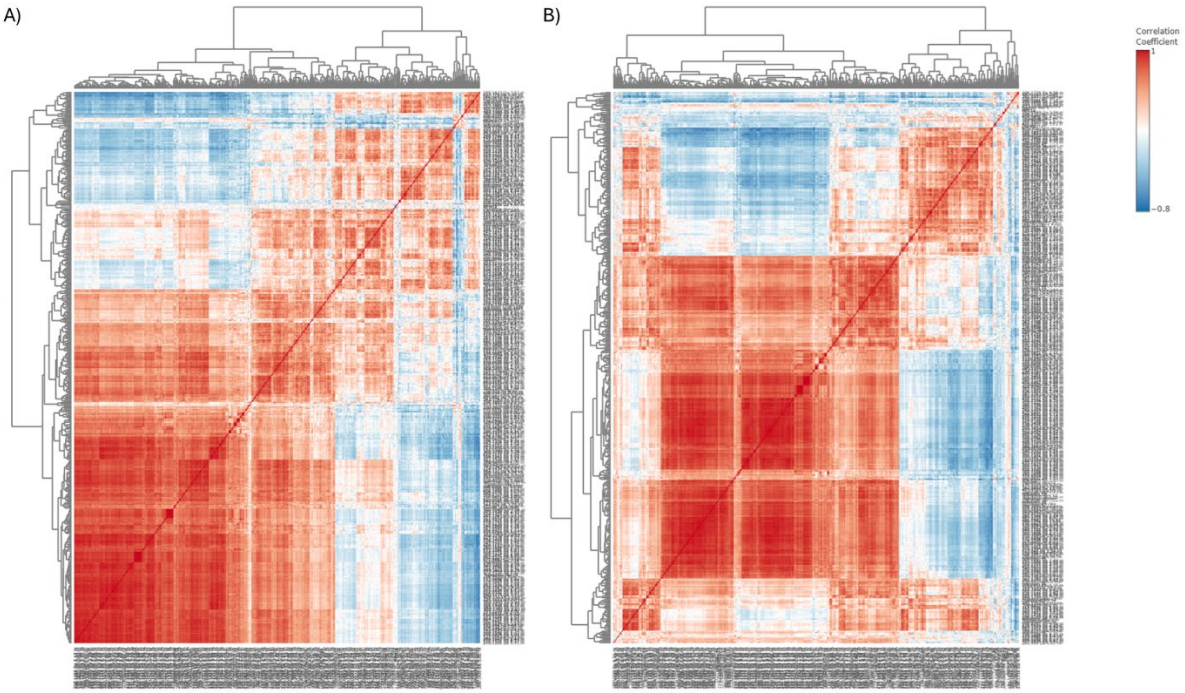

Fig. S7 Hierarchical Clustering Heatmap of features and growth capacity of *Phaeoacremonium minimum* (A) and *Phaeomoniella chlamydospora* (B) correlation matrix Heatmap. Red and blue intensity higher positive and negative statistical correlations between sample and marker. The acronyms represent different growth capacities of different pathogens (the blue color shows on the acronym represents growth inhibition).