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Towards understanding of fungal biocontrol mechanisms of different yeasts antagonistic to *Botrytis cinerea* through exometabolomic analysis

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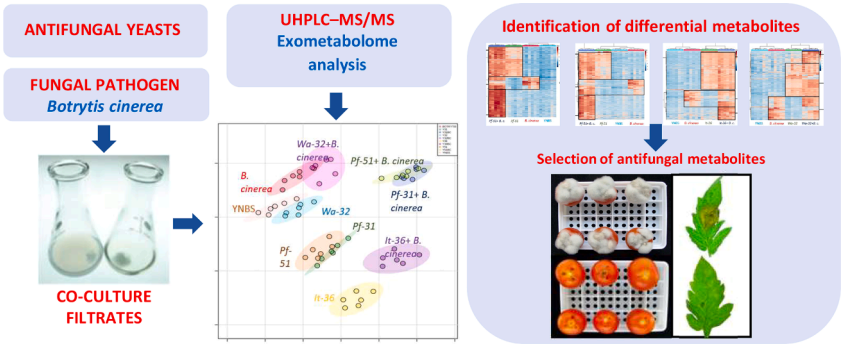
HIGHLIGHTS

- The yeast exometabolome changes after co-culture with a fungal pathogen.
- Both general and yeast strain-specific metabolites are differentially secreted.
- Several metabolites have antifungal properties *in vitro* but only two *in vivo*.
- Trans-cinnamic acid is the best antifungal metabolite secreted by yeasts.
- Indole-3-carboxaldehyde shows lower levels of protection.

GRAPHICAL ABSTRACT



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ABSTRACT

There is increased interest in research on yeasts as potential phytopathogen biocontrol agents due to increasing restrictions in the use of chemical pesticides. Yeast strains from a range of genera and species have been reported to inhibit postharvest decay in different fruits. However, the mechanisms behind these yeast biocontrol capacities have not been completely deciphered because they are complex and act synergistically. In this study, we performed a thorough untargeted analysis of the exometabolome generated in a co-culture of the fungal plant pathogen *Botrytis cinerea* with four antagonistic yeast strains: *Pichia fermentans* (two strains), *Issatchenkia terricola* and *Wickerhamomyces anomalus*. As a result, general and strain-specific antifungal mechanisms and molecules were identified. The *P. fermentans* strains secreted the highest number of differential metabolites to the extracellular medium when co-cultured with *B. cinerea*. *In vitro* antagonistic and *in vivo* pathogen protection assays were performed with the selected metabolites. Among a plethora of 46 differentially secreted metabolites related to yeast-fungus competitive interaction, the phenylpropanoid *trans*-cinnamic acid and the alkaloid indole-3-carboxaldehyde were identified as the best antagonistic metabolites against gray mold infection under *in vivo* protection assays. Both metabolites caused damage to the fungal membrane and increased ROS generation in spores of *B. cinerea*. In addition, enhanced yeast secretion to the extracellular medium of oxylipins, dipeptides, alkaloids or antibiotics deserve to be further investigated as signaling or antagonistic molecules. This study opens

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the door to future investigations of roles of these molecules in yeast metabolism and application of this knowledge for biotechnological purposes.

1. Introduction

Botrytis cinerea is one of the most prominent plant pathogenic fungi, known for its ability to infect more than 1400 plant species in different organs including leaves, flowers and fruits, both under field conditions and during the postharvest period. It has been estimated that gray mold infection causes losses of 10–100 billion US dollars worldwide (Hua et al., 2018). Nowadays, the use of synthetic chemicals against fungal pathogens in food chain production and commercialization is being restricted due to the development of fungal resistance and negative environmental impacts that have changed public perception and agronomic management. However, there is increasing interest in developing alternative biopesticides based on microorganisms with antifungal activity. In particular, several genera of bacteria (i.e. *Bacillus, Pantoea, Pseudomonas)* and filamentous fungi (i.e. *Trichoderma, Gliocladium*) that are already known to show antifungal activity have been proposed as treatments against *B. cinerea* infection (Elad and Stewart, 2004; Haidar et al., 2016; Roca-Couso et al., 2021). Regarding yeasts, several strains of different genera and species have been reported to be good antagonistic microorganisms with potential as phytopathogen biocontrol agents (Chen et al., 2018; Csutak et al., 2013; Hua et al., 2018; Pawlikowska et al., 2019; Santos et al., 2004). In fact, previous reports have shown that a variety of yeasts can inhibit postharvest decay by *B. cinerea* in a range of fruits (Fernandez-San Millan et al., 2021; Lutz et al., 2013; Oro et al., 2018; Parafati et al., 2015; Ruiz-Moyano et al., 2016; Saravanakumar et al., 2008; Spadaro and Droby, 2016).

Among the mechanisms contributing to the biocontrol capacities of yeasts, enzyme secretion, parasitism, production of volatile organic compounds, induction of systemic resistance and competition for space and nutrients have been described (Freimoser et al., 2019). In our previous studies (Fernandez-San Millan et al., 2021, 2020) several yeast strains of *Metschnikowia pulcherrima*, *Issatchenkia terricola*, *Pichia fermentans* and *Wickerhamomyces anomalus* showed excellent biocontrol abilities against *B. cinerea* on postharvest grapes and tomato fruits. In addition, *W. anomalus* protected tomato plants from infection by *Verticillium dahliae* and *Fusarium oxysporum*. We have also observed that the biocontrol effect seems to involve multiple mechanisms (Fernandez-San Millan et al., 2021), and this is supported by earlier studies (Liu et al., 2019). In particular, we showed that these yeast strains are able to synthesize cell wall-degrading enzymes, solubilize nutrients from the surrounding medium, promote plant growth, develop biofilms, or secrete iron chelating agents. However, although yeasts can secrete diffusible compounds, it has been reported that they do not produce as many secondary metabolites as filamentous fungi or bacteria (Freimoser et al., 2019; Roca-Couso et al., 2021), and little is known about metabolite-mediated antagonism in yeast against fungal plant pathogens.

Among yeast metabolites that have been described for their biocontrol activity (reviewed in Freimoser et al., 2019), most are protein-like killer toxins. Other kinds of secondary metabolites with antifungal activity have been rarely identified, but include 2-methylene-succinic acid, 2-propylacrylic acid, aureobasidins or liamocins, which are produced by the yeast-like fungus *Aureobasidium pullulans* (Freimoser et al., 2019; Price et al., 2017; Takesako et al., 1991; Zain et al., 2009). In fact, such studies have opened the door to a new field of research of secondary metabolites with potential use as biocontrol agents. However, the exometabolome of most yeast genera remains unexplored.

Two general approaches for the identification of bioactive compounds have been used to date (Siedler et al., 2019), mainly in bacteria and fungi. The first of these are bioassay-guided experiments, where different extracts are analyzed followed by identification of particular antagonistic compounds in the bioactive fractions, and secondly, high resolution mass spectrometry analysis to identify novel compounds with subsequent validation of their bioactivity (Ahsan et al., 2017; Arora et al., 2020; Awaad et al., 2012; Crowley et al., 2013; Fernandez-San Millan et al., 2022; Siedler et al., 2019). Microbial interactions activate secondary metabolite gene clusters that are not expressed or are only weakly expressed under monoculture conditions and give rise to the production of new chemical substances such as antimicrobials, pharmaceuticals, or improvers of flavor and aroma for beverages and foods (Arora et al., 2020; Bertrand et al., 2013a,b; Fernandez-San Millan et al., 2022; Jones and Wang, 2018; Kumar Verma et al., 2022; Meilin et al., 2019; Oppong-Danquah et al., 2018, 2020). In this context, a previous study performed in our laboratory optimized a co-culture protocol to analyze for the first time the exometabolome generated in a yeast-fungus antagonistic interaction (Fernandez-San Millan et al., 2022). Hence, two *M. pulcherrima* yeast strains were co-cultured with *B. cinerea* and the metabolomic analysis of the culture filtrates resulted in the identification of several new antifungal compounds among a profusion of metabolites related to such competitive interaction. Indeed, 3-amino-5-methylhexanoic acid, biphenyl-2,3-diol and sinapaldehyde are metabolites secreted by yeast cells that have shown high levels of protection in postharvest conditions against *B. cinerea* infection (Fernandez-San Millan et al., 2022). Nine additional metabolites showed only *in vitro* antifungal abilities. In general, the individual contribution of each identified metabolite is difficult to quantify and validate *in vivo* when assayed individually under laboratory conditions, and the data suggests instead that fungal inhibition occurs by a synergistic or additive effect among different compounds and strategies (Crowley et al., 2013; Le Lay et al., 2016).

In the last few years, an increasing number of studies have identified microorganisms with antifungal activity for crop or postharvest bioprotection (Crowley et al., 2013; de Souza et al., 2019; Fernandez-San Millan et al., 2021; Ferraz et al., 2016; Freimoser et al., 2019; Gross et al., 2018; Pawlikowska et al., 2019; Pertot et al., 2017; Raspor et al., 2010; Siedler et al., 2019). However, although the characterization of the microbial spectrum is important, there is an increasing interest in understanding the principles underlying the antagonistic activity. This requires interpreting yeast performance under the conditions of co-culture with the pathogen. Thus, metabolomic analysis will complement the global view of the antagonistic mechanisms essential to yeast biocontrol strategies, allowing us to answer crucial questions: Are common metabolic pathways among different yeast genera being enhanced during an antagonistic process? Are there any species-specific compounds that contribute significantly to the biocontrol abilities of a related species? Do any of the antifungal compounds identified have enough potential to become a new fungicide of agronomic interest?

The aim of this study was performing a broad exometabolomic analysis to address these questions. Our hypothesis was that secreted metabolites that accumulate after *B. cinerea* inoculation may be partly responsible for the antifungal activity of yeasts. Therefore, we have performed a *B. cinerea* co-culture assay with four yeast strains from three different genera and species that are highly antagonistic against gray mold. The exometabolome in culture filtrates has been characterized, and several antifungal compounds produced by different metabolic pathways have been identified and analyzed under *in vitro* and *in vivo* conditions. The comparison of these results with the already known antagonistic *M. pulcherrima* exometabolome has allowed us to find common and yeast-strain-specific antifungal compounds and broaden the general knowledge of yeast metabolic performance during interactions with phytopathogenic fungi.

2. Materials and methods

2.1. Yeast and fungal strains

Native yeast strains isolated from grapes (*Vitis vinifera* cv. Grenache) were used for this study: *Issatchenkia terricola* It-36, *Pichia fermentans* strains Pf-31 and Pf-51, and *Wickerhamomyces anomalus* Wa-32 (Fernandez-San Millan et al., 2021, 2020). Yeast cells were stored in cryovials (VWR, Spain) at −80 °C for long-term storage and plated onto YMA (Yeast Mannitol Agar) medium. For metabolomic analysis, yeast cells were grown in YNBS (Yeast Nitrogen Base) liquid culture, supplemented with 20 g/L glucose and 5.0 g/L ammonium sulfate at pH 6.8. For other experiments in liquid culture, yeast cells were grown in YPD (Yeast Peptone Dextrose) broth. Flasks were incubated on a rotary shaker at 150 rpm and 28 °C for 2 d. A TC20 cell counter (Bio-Rad, Hercules, USA) was used to determine the cell concentration.

Botrytis cinerea strain 20754 (CECT (Paterna, Spain) was used for co-culture confrontation for metabolomic analysis and screening of antagonistic metabolites. The fungus was grown on PDA (Potato Dextrose Agar) medium plates. PDB (Potato Dextrose Broth) or YNBS were used for liquid culture. The suspensions of fungal conidia were prepared as described previously (Fernandez-San Millan et al., 2021). All media were purchased from Condalab (Spain).

2.2. Co-culture assay and exometabolomic analysis

2.2.1. Analysis of B. cinerea growth inhibition by yeast culture filtrates

To analyze the antagonistic effect of yeast culture filtrates (CFs) at two concentrations on the mycelial growth of *B. cinerea*, a previously reported method was used (Fernandez-San Millan et al., 2022; Spadaro et al., 2002). Briefly, 12 h yeast cultures (10^6 cells mL^{-1}) in YNBS media were filtered (0.22 μm cellulose filters, Millipore) and diluted at 2:1 and 1:2 (filtrate:YNBS) ratios (high and low concentration respectively). Thirty μL of a 10^3 conidia mL^{-1} *B. cinerea* suspension were inoculated into 3 mL CFs dilutions in glass tubes (15 mm of diameter), which were incubated on a rotary shaker at 150 rpm and 28 °C for 3 d. Mycelium diameter was measured in triplicate after pouring the glass tube content in a petri dish. YNBS medium and non-diluted CFs were used as controls. Final concentrations of all medium components were adjusted to be equal in all treatments. The experiment was repeated twice.

2.2.2. B. cinerea co-culture with yeast cells

For analysis of the exometabolome, each yeast strain was co-cultured individually with *B. cinerea* as previously described (Fernandez-San Millan et al., 2022). Briefly, 100 μL of a 10^3 conidia mL^{-1} *B. cinerea* suspension were inoculated into flasks with 10 mL of YNBS medium. After 48 h incubation at 150 rpm and 28 °C, 100 μL (10^6 cell mL^{-1}) of the yeast pre-culture were added to each flask of *B. cinerea* and co-cultured for 12 additional hours. Monocultures of each yeast strain and *B. cinerea* were prepared as controls. Cells were subsequently centrifuged at 2000 *g* and filtered (0.22 μm cellulose filters) for metabolomic analysis. Three replicates were made for each condition.

2.2.3. Metabolomic analysis

The method used for the metabolomic analysis was previously described (Fernandez-San Millan et al., 2022). Briefly, 5 mL of freeze-dried CFs were resuspended in 1 mL of extraction solution (H_2O: MeOH:HCOOH (90:10:0.01)) and 5 μL of each biological replicate were randomly injected twice into the instrument for a total of six samples per condition. An ACQUITY UPLC I-Class System coupled to a SYNAPT G2-S high-definition mass Spectrometer MS/MS detector (Waters®) was used for the analysis. The chromatography was performed with a Kinetex 2.6 μm EVO C18 UPLC column (Phenomenex Inc.). The conditions for chromatography and accurate mass detection are described in Gamir et al. (2020). The MassLynx 4.2 and DataBridge packages provided by Waters enabled data acquisition and transformation. An XCMS library for peak-picking in R, and MetaboAnalyst 5.0 and MarVis-suite 2.0 software were used for data analysis and interpretation (Kaever et al., 2015; Pang et al., 2021). Three confidence levels for metabolite identification were assigned: matching with a reference standard from an internal library with more than 200 metabolites (Level 1); tentative identification based on MS/MS online spectrum matching (Level 2); and putative *m/z* identification based on online database matching (Level 3) (Schrimpe-Rutledge et al., 2016). Online matching for accurate mass was performed with the Kyoto Encyclopedia of Genes and Genomes (Kegg) (https://www.genome.jp/kegg/) with an error mass of 1 mDA. The MS/MS spectrum search was conducted against the Massbank (https://massbank.eu/MassBank/Search) and Metlin (https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage) databases.

2.3. In vitro screening of yeast metabolites against B. cinerea conidia germination

A range of commercially available metabolites was purchased and a 100 mM stock solution was prepared following the manufacturer's protocols. To avoid pH interference with *B. cinerea* growth, the pH of each stock solution was adjusted between 5.5 and 7.

For *in vitro* antifungal screening of metabolites against conidia germination of *B. cinerea*, 100 μL of 10^4 conidia mL^{-1} were spread out on PDA plates and a 5 mm-diameter hole was made with a cork borer as described (Fernandez-San Millan et al., 2022). Metabolites (100 μL of 100 mM stock solution) were deposited inside each hole and corresponding solvents were included as controls. After 3 to 4 d of incubation at 28 °C, a halo zone was visible on plates that included metabolites with antifungal effects. Four replicates were made for each metabolite.

2.4. In vivo screening of yeast metabolites against B. cinerea postharvest fruit spoilage

All of the commercially available metabolites were assayed for postharvest antifungal properties against *B. cinerea* infection on cherry tomatoes (*Solanum lycopersicum* var. cerasiforme) as described previously (Fernandez-San Millan et al., 2021; Magoye et al., 2020). Briefly, three wounds were made per tomato and each wound was inoculated with 5 μL (1, 10 or 100 mM) of the metabolite or 5 μL of the corresponding solvent (MES 10 mM, ethanol, methanol or chloroform) in negative control tomatoes. After complete air drying of the drop, 5 μL of a 10^4 conidia mL^{-1} *B. cinerea* suspension were deposited on each wound. Six tomatoes were set in a plastic support inside a plastic tray with water-soaked paper for 14 d at 25 °C in the dark. The disease incidence (DI) was measured after 7 d as described (Parafati et al., 2015): DI % (percentage of infected wounds) = (number of infected wounds / number of total wounds) × 100. The disease severity (DS) was measured after 14 d using a 0-to-4 scale: 0 = no symptoms; 1 = 1–25 % of the surface with fungal damage; 2 = 26–50 %; 3 = 51–75 %; 4 = 76–100 %. Subsequently the DS percentage was calculated (Parafati et al., 2015): DS %= [∑ (number of infected fruits in each scale × disease scale)/ (total fruits analyzed × highest disease scale)] × 100. This experiment was repeated three times.

2.5. Antifungal assay of yeast metabolites against B. cinerea infection in leaves

Five μL of metabolites (100 mM) and the commercial fungicide Cabrio® (500 μg mL^{-1}; 550 mg g^{-1} metiram and 50 mg g^{-1} pyraclostrobin; Basf) were applied to the abaxial surface of the leaves of adult tomato plants grown in the greenhouse. Cabrio® was included as a control that shows good protection against gray mold in leaves. After air drying, the leaves were wounded and inoculated with fungal conidia as described before for tomato fruits. The inoculated leaves were maintained at room temperature in high relative humidity conditions. After 4 d, single leaflets were cut off and the lesion diameter was measured in

two perpendicular directions and averaged. Five leaflets per leaf and three leaves per treatment were infected. The experiment was performed three times.

2.6. Analysis of the effects of metabolites on B. cinerea

2.6.1. Effective concentration value (EC_{50}) of metabolites

The *B. cinerea* strain used in this study was tested for its sensitivity to the most bioactive metabolites. To calculate the 50 % effective concentration (EC_{50}, i.e. the metabolite concentration (μg mL^{-1}) causing a 50 % reduction in the mycelial growth rate compared to an unamended control) for both selected metabolites, PDA plates containing 0, 25, 50 or 100 μg mL^{-1} of *trans*-cinnamic acid (CA) or indole-3-carboxaldehyde (ICAld) were seeded with 5 μL of a 10^4 *B. cinerea* spore solution in the center of the plate. Cabrio® was included as a commercial fungicide control at concentrations of 0, 5, 25 or 75 μg mL^{-1}. Mycelial radial growth was measured after 5 d at 20 °C and the EC_{50} value for each metabolite and the fungicide was calculated by regression of the percentage inhibition of radial growth (EC_{50} calculator Bioquest; https://www.aatbio.com/tools/ec50-calculator). There were four replicates per treatment. The experiment was performed twice.

2.6.2. Effect of metabolites on B. cinerea cell membrane permeability and intracellular level of reactive oxygen species (ROS)

Measurements of cell membrane permeability were undertaken according to Fernandez-San Millan et al., (2022) and Wang et al., (2021), to assess the leaching of cell contents. Briefly, metabolites at EC_{50} were added to 3 d mycelial cultures in PDB media. As controls, mycelia amended with the corresponding amounts of solvents were used. After a 2 h incubation in a rotary shaker at 28 °C and 150 rpm, mycelia were collected from each flask, rinsed twice with distilled water and suspended again in distilled water (0.2 g of mycelium per mL). Conductivity data were taken at 30 min intervals with a SensION portable conductivity-meter (Hach, Loveland, CO, USA). After 4 h, samples were boiled for 10 min and final conductivity was quantified. The relative conductivity (%) was calculated as (conductivity at each time / final conductivity) × 100. Three repeats were performed for each treatment. The entire experiment was performed twice.

The method described by Cheng et al., (2022) was used to determine the protein leakage with slight modifications. *B. cinerea* (10^3 spores mL^{-1}) was cultured in PDB at 25 °C for 72 h. Samples (1 g) of the resulting mycelium were then incubated with 300 mg L^{-1} of metabolites for 2 h. Cultures were centrifuged at 4 °C at 3000 *g* for 5 min and the pellet was washed three times. The final pellet was resuspended with 1 mL of sterilized distilled water, and then cell breakage was induced with an ultrasonic disintegrator (Sonifier 250; Branson-Emerson, Leicester, UK) with two intervals of 30 s at maximum power. After that, the supernatant was separated by centrifugation at 4 °C at 20 000 *g* for 10 min and the remaining intracellular soluble protein was determined with the Bradford protein assay. Three repeats were performed for each treatment. The entire experiment was performed twice.

For measurement of intracellular ROS production and cell membrane integrity of *B. cinerea* spores, a suspension of 10^5 spores mL^{-1} was cultured in PDB medium containing the selected metabolites (at EC_{50} concentration) for 2 h at 22 °C. Then, spores were stained for 30 min at room temperature with the different dyes: 2, 7-dichlorodihydrofluorescein diacetate (5 μM DCHF-DA; Sigma-Aldrich) and propidium iodide (10 μg mL^{-1} PI; Sigma-Aldrich), respectively (Fernandez-San Millan et al., 2022; Zhang et al., 2015). After washing twice with phosphate-buffered saline (PBS), the fluorescence of spores was observed under a microscope (Leica MC170 HD, excitation 488 nm and emission 525 nm). The percentage of fluorescent spores with respect to the total counted under brightfield was calculated by observing 4–6 samples per treatment. The experiment was performed twice.

2.6.3. Oxalic acid secretion by B. cinerea in the presence of metabolites

Measurements of oxalic acid secretion by *B. cinerea* into the extracellular media in liquid culture conditions were calculated in the presence or absence of metabolites as previously described (Fernandez-San Millan et al., 2022). Three replicates were conducted per treatment and the experiment was repeated twice.

2.7. Data analysis

One-way ANOVA with a Tukeýs pairwise comparison ($p < 0.05$) was used for evaluating differences among treatments. DI and DS data were transformed into arcsine square-root values as described (Fernandez-San Millan et al., 2021) to normalize the distribution before performing the statistical analysis. Different letters have been used to indicate means that differ significantly. Experiments were analyzed with SPSS.24 software (SPSS, Chicago).

3. Results

3.1. Yeast strains produce extracellular compounds with antagonistic activity against B. cinerea growth

Four yeast strains were selected among a collection of 69 strains isolated from grapes (Fernandez-San Millan et al., 2020) to undertake the exometabolomic analysis: *Issatchenkia terricola* It-36, *Pichia fermentans* Pf-31 and Pf-51 and *Wickerhamomyces anomalus* Wa-32. Two criteria were established for the selection: (i) high antagonistic activity against *B. cinerea* under postharvest conditions and (ii) production of extracellular antifungal compounds. For the first criterion, the selection was made according to results observed by Fernandez-San Millan et al. (2021). In this previous work, among 20 strains that showed *in vitro* antagonistic effects, only six showed effective postharvest protection against gray mold infection in tomato and grape. It was recently demonstrated (Fernandez-San Millan et al., 2022) that two of them, *M. pulcherrima* Mp-22 and Mp-30, were able to produce extracellular antifungal metabolites. This fact prompted us to investigate whether the remaining four strains also secreted antifungal compounds into the extracellular medium, therefore fulfilling the second criterion for exometabolomic analysis. Hence, cell-free aseptic culture filtrates (CFs) of the four strains were included at two different concentrations as part of the culture medium for *B. cinerea* growth. The results showed a concentration-dependent inhibitory effect of CFs on mycelium development (Fig. 1). The highest inhibition rate at low concentrations of CFs was observed with the It-36 strain (three times reduction in mycelial growth relative to the control). For all strains tested, fungal growth was totally suppressed with high concentrations of the yeast CFs.

3.2. Global exometabolome profiling by UPLC-MS/MS

B. cinerea and the four yeast strains selected were co-cultured to conduct an untargeted metabolomic assay to discover the metabolites that were differentially secreted into the extracellular medium in the presence of fungus. In order to compare the results with those observed in *M. pulcherrima* strains (Fernandez-San Millan et al., 2022), an identical co-culture procedure was performed, and we ensured that yeast growth was in the exponential phase 12 h after inoculation. A principal component analysis (PCA) revealed that the loading plots of Wa-32-secreted metabolites were closer to the control medium (YNBS) and *B. cinerea* loading plots than the other yeast plots (Fig. 2). On the other hand, we observed similar behavior between the two *P. fermentans* strains, Pf-31 and Pf-51. When comparing monoculture (Pf-31 and Pf-51) and co-culture filtrates (Pf-31 + *B. cinerea* and Pf-51 + *B. cinerea*), the loading plots showed a different secretion profile (Fig. 2). Finally, It-36 displayed the most impactful result among all yeast strains. The loading plots of either It-36 or It-36 co-cultured with *B. cinerea* (It-36 + *B. cinerea*) displayed the greatest difference compared to the control

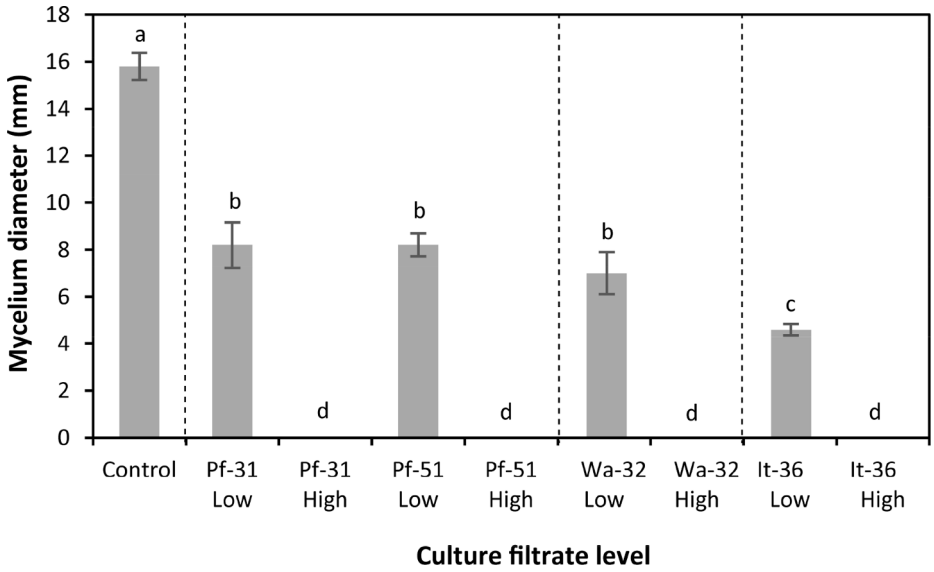

Fig. 1. Antagonistic activity of *P. fermentans* (Pf-31 and Pf-51), *I. terricola* It-36 and *W. anomalus* Wa-32 culture filtrates on mycelium growth of *B. cinerea* in liquid medium. Control: without yeast culture filtrate. Low: culture filtrate/YNBS medium at a 1:2 ratio. High: culture filtrate/YNBS medium at a 2:1 ratio. Data are presented as the means ± SE (n = 6). Values with the same letter do not differ significantly ($p < 0.05$) according to the Tukey's multiple range test.

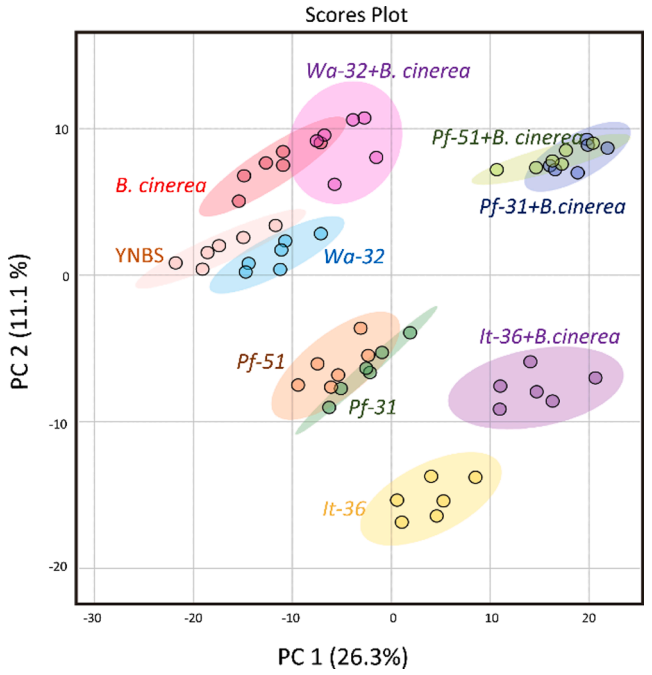

Fig. 2. Principal component analysis (PCA) showing UPLC-QTOF-MS/MS data of culture filtrates. PCA was elaborated with 9746 signals (7084 ESI + and 2662 ESI-) of It-36, Pf-31, Pf-51, and Wa-32 incubated with or without *B. cinerea*. YNBS: culture medium. *B. cinerea*: fungal culture. Wa-32, Pf-31, Pf-51 and It-36: yeast strains. Wa-32 + *B. cinerea*, Pf-31 + *B. cinerea*, Pf-51 + *B. cinerea* and It-36 + *B. cinerea*: yeast-fungus co-culture.

medium in the first principal component (PC1), indicating the maximum percentage of variability in the analysis (Fig. 2).

A heatmap analysis was carried out to identify those signals with enhanced intensity in Pf-31, Pf-51, It-36 and Wa-32 CFs in the presence of the fungal pathogen (Fig. 3). To perform the analysis, signals with $p > 0.05$ (Kruskal-Wallis) were extracted to guarantee variability among the samples. The heatmap analysis of ESI + and ESI- signals from the four yeast strains displayed a cluster of features with greater accumulation in CFs samples from the co-culture with *B. cinerea* (cluster 1), a cluster of signals constitutively secreted by the yeasts (cluster 2) and, finally, a cluster of signals secreted by *B. cinerea* alone (cluster 3). From the signals in cluster 1, a tentative identification of 46 secreted metabolites was established (Table 1) based on our internal library and online accurate-mass and fragmentation spectra matching. Among the most representative metabolites, we found several oxocarboxylic acid and amino acid-related compounds, dipeptides, oxylipins, and purine or phenylpropanoid derivatives (Table 1).

3.3. *In vitro antifungal activity of metabolites*

Identified metabolites that were commercially available were tested in an *in vitro* assay to evaluate their biocontrol potential against *B. cinerea*. Six out of the 14 tested metabolites showed a significant inhibitory effect on *B. cinerea* spore germination and mycelial growth in plate assays (Fig. 4 and Fig. S1). It was observed that *trans*-cinnamic acid (CA) was the most potent inhibitor in these conditions, followed by kynurenic acid and indole-3-carboxaldehyde (ICAld).

3.4. *In vivo antifungal activity of metabolites: Control of B. cinerea postharvest tomato spoilage*

The 14 commercially available metabolites were also analyzed for *in vivo* protection against *B. cinerea* infection in cherry tomatoes. This assay of experimental infection was previously demonstrated to be adequate to select protective metabolites against gray mold (Fernandez-San Millan et al., 2022). As a result, only two metabolites (CA and ICAld) were able to significantly control fruit spoilage. CA was able to reduce the disease incidence four times compared to the control without protection in the most concentrated application (Fig. 5A and C). The disease severity was also reduced below 10 %, demonstrating a high protection effect in tomato. The incidence and severity of damage was not reduced with respect to the control at the lower concentration (1 mM). The other protective metabolite was ICAld, which even though it did not reduce disease incidence, managed to lower disease severity to 50 %, but only at the higher concentration (100 mM) (Fig. 5B and C).

3.5. *Antagonistic efficacy of bioactive metabolites in leaves*

As *B. cinerea* is also a preharvest pathogen that infects other parts of the plant besides harvested fruits, experiments on tomato potted plants

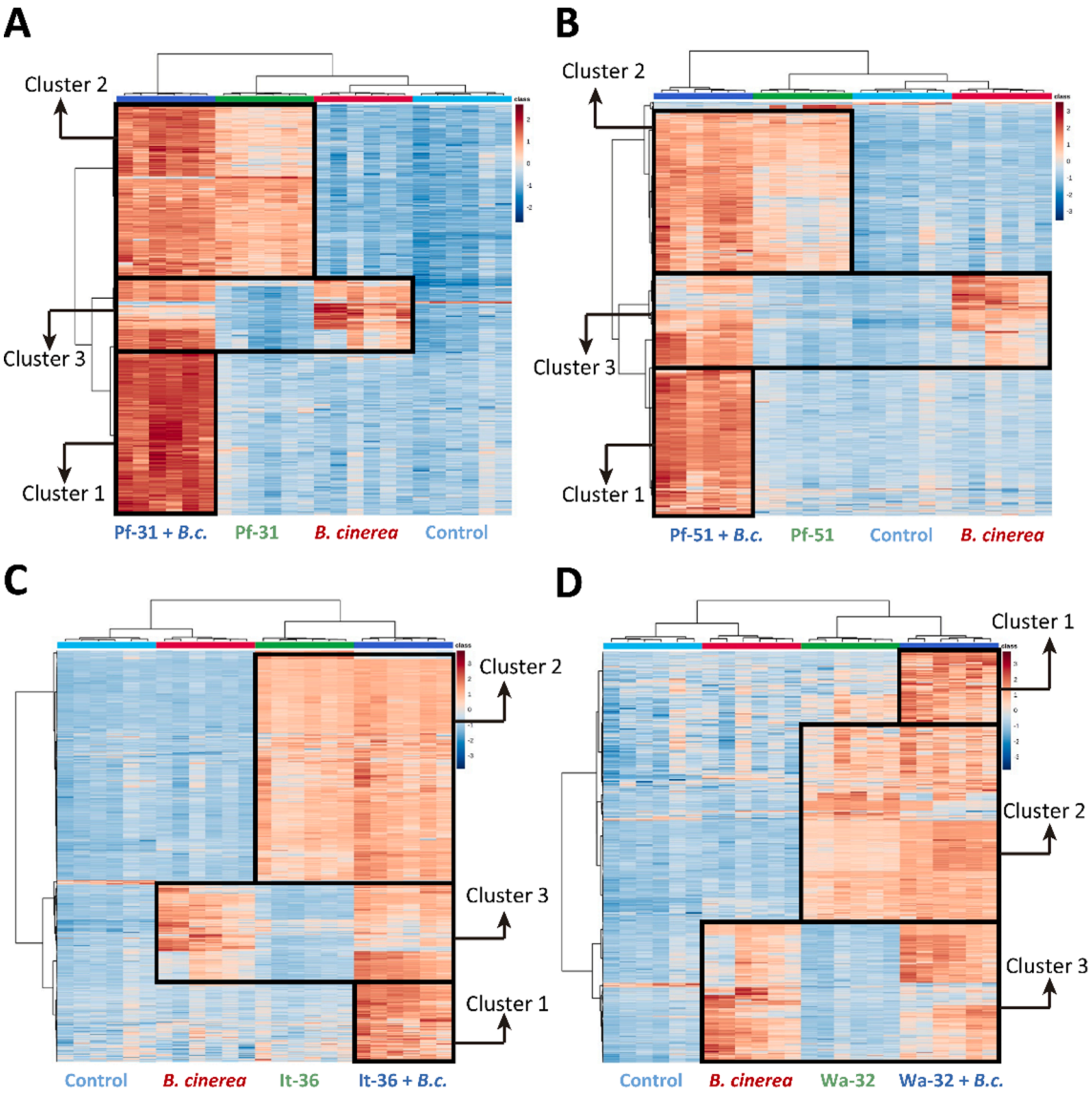

Fig. 3. Heatmap analysis of culture filtrates. (A) Pf-31, (B) Pf-51, (C) It-36 and (D) Wa-32 signals from ESI + and ESI- with p-value < 0.05 were used in the heatmap analysis. The relative abundance of the signals was calculated by normalizing the chromatographic pick area with the dry weight of the culture. Control: culture medium. *B. cinerea*: fungal culture. Pf-31, Pf-51, It-36 and Wa-32: yeast strains. Pf-31 + *B.c.*, Pf-51 + *B.c.*, It-36 + *B.c.* and Wa-32 + *B.c.*: yeast-fungus co-culture. The heatmap analysis shows a graphical representation of differentially accumulated compounds within the samples. Cluster 1 contains signals with greater accumulation in yeast-fungus co-culture. Cluster 2 contains signals with greater accumulation in yeast cultures and yeast-fungus co-culture. Custer 3 contains signals with greater accumulation in fungal cultures.

were performed to assay the protection of metabolites on leaves (Fig. 6). The chemical fungicide Cabrio® reduced by 76 % the progress of the infection at 500 μg mL^{-1}. The development of infection compared to the control was reduced by 42 % with ICAld at 14 μg mL^{-1} (100 mM) when the leaves were inoculated with the fungal spores. In contrast, when treated with CA at the same concentration, a 70 % decrease of de infection was obtained, which was nearly equal to the efficacy obtained by Cabrio®.

3.6. *Characterization of the antifungal mechanisms of bioactive metabolites*

3.6.1. *Fungicidal strength of metabolites against B. cinerea*

The fungicidal strength of CA and ICAld was measured *in vitro* by determining the concentration that inhibited fungal growth by 50 % (also called EC_{50}) of both the metabolites and the commercial fungicide Cabrio®. Their inhibitory effects on mycelium growth were positively correlated with the concentration increase in metabolites and the fungicide (Fig. 7). The inhibitory EC_{50} was 52 and 50 μg mL^{-1} for CA and ICAld, respectively (352 and 345 μM). The EC_{50} of the commercial fungicide was also calculated as a reference value, being 23 μg mL^{-1}, which is in the range referenced in the literature (Pokorny et al., 2016).

3.6.2. *Effect of metabolites on the intracellular level of ROS, cell membrane integrity and permeability of B. cinerea*

To investigate possible underlying mechanisms of metabolites against gray mold infection, ROS production and loss of membrane integrity in *B. cinerea* spores were measured by fluorescence microscopy. It was observed that the metabolites CA and ICAld caused an increase in ROS production, as can be observed and quantified by a visible green fluorescence (Fig. 8A and Fig. S2A). In addition, both metabolites also damaged the integrity of the spore membranes compared to the control, and this effect was significantly higher in the presence of CA (Fig. 8A and Fig. S2B). Measurements of the membrane permeability of *Botrytis*

Table 1
List of secreted metabolites identified by MS/MS data.

Compound	*m/z*	ESI	Yeast Strain	Functional category	Identification level	Antifungal effects *in vitro / in vivo*
Feruloylputrescine	255.085	+	Pf-31	Phenylpropanoid and alkaloid biosynthesis	3	–
Hydroxyphenyllactic acid	181.050	–	Pf-51	Phenylpropanoid biosynthesis	2	*In vitro*
Trans-cinnamic acid	147.044	–	It-36, Pf-31, Pf-51	Phenylpropanoid and alkaloid biosynthesis	2	*In vitro / In vivo*
Indole-3-carboxaldehyde	146.060	+	It-36, Wa-32	Indole alkaloid biosynthesis	2	*In vitro / In vivo*
2-Succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate	329.090	+	It-36	Ubiquinone and terpenoid-quinone biosynthesis	3	–
Mevalonolactone	131.070	+	It-36	Terpenes and steroids biosynthesis	2	*In vitro*
Piperidine	86.097	+	Wa-32	Tropane, piperidine and pyridine alkaloid biosynthesis	3	No
Quinolinate	168.031	+	Pf-31, Pf-51	Biosynthesis of alkaloids / Trp metabolism-kynurenine pathway	3	No
Kynurenic acid	190.050	–	Pf-31, Pf-51	Trp metabolism-Kynurenine pathway	2	*In vitro*
p-Hydroxyphenylacetylglycine	210.075	+	Pf-31, Pf-51	Tyr metabolism	3	–
Succinic acid	117.018	–	Pf-51	Citrate cycle	2	*In vitro*
Ketoglutaric acid	145.013	–		Citrate cycle / 2-Oxocarboxylic acid metabolism	1	No
2-Isopropylmaleate 2-Isopropylmalic acid	157.049	–	It-36, Pf-31, Pf-51, Wa-32	2-Oxocarboxylic acid metabolism Val, Leu and Ile biosynthesis	3	–
2-Isopropylmalic acid	175.060	–	It-36, Pf-31, Pf-51	2-Oxocarboxylic acid metabolism Val, Leu and Ile biosynthesis	2	No
3-Methyl-2-oxopentanoic acid	129.054	–	Pf-31, Pf-51, Wa-32	2-Oxocarboxylic acid metabolism Val, Leu and Ile biosynthesis	2	–
But-1-ene-1,2–4-tricarboxylate/but-1-ene-1,2–3-tricarboxylate	187.023	–	Wa-32	2-Oxocarboxylic acid metabolism Lys synthesis	3	–
Ketovaline	115.038	–	It-36, Pf-51	2-Oxocarboxylic acid metabolism Val, Leu and Ile biosynthesis	3	–
n-Hydroxy-l-isoleucine	148.097	+	Pf-31, Wa-32	2-Oxocarboxylic acid metabolism Val, Leu and Ile biosynthesis	3	–
Homocitrate	205.034	–	Wa-32	2-Oxocarboxylic acid metabolism Lys biosynthesis	3	–
Glutamine	147.077	+	Pf-31, Pf-51	Amino acid	1	–
Phenylalanyne	166.087	+	It-36, Pf-31	Amino acid	1	–
Tryptophan	203.082	–	It-36	Amino acid	2	–
Ile-Tyr	295.165	+	Pf-31, Pf-51	Dipeptide	2	–
Val-Leu / Val-Ile	231.170	+	It-36, Pf-31, Pf-51, Wa-32	Dipeptide	2	No
Glutathionylspermidine	435.242	+	Pf-31	Glutathione metabolism	3	–
12,13 DiHOME-dihydroxyoctadec-9-enoic acid	313.237	–	Pf-51	Linoleic acid metabolism	3	–
9,12,13 TriHOME- trihydroxy-9-octadecenoic acid	329.232	–	It-36, Pf-31, Pf-51	Linoleic acid metabolism	3	–
Lauroyl-CoA	203.082	–	It-36	Fatty acid elongation	3	–
Phosphodimethylethanolamine	170.060	+	It-36	Glycerophospholipid metabolism	3	–
2-Hydroxycaproic acid	131.070	–	It-36, Pf-31, Pf-51	Aromatic compounds degradation	3	–
2-Hydroxy-6-oxo-(2′-aminophenyl)-*hexa*-2,4-dienoate	234.077	+	Pf-51	Aromatic compounds degradation	3	–
2-hydroxy-6-oxo-6-(2′-hydroxyphenoxy)-*hexa*-2,4-dienoate	251.059	+	Pf-51	Aromatic compounds degradation	3	–
Benzene 1,2,4 triol	125.023	–	Wa-32	Aromatic compounds degradation	3	No
3-Methylpyrrole-2,4-dicarboxylic acid	168.029	–	Pf-31, Pf-51	Coumermycin and novobiocin antibiotics biosynthesis	2	–
Tetracenomycin A2	423.102	+	Wa-32	Biosynthesis of type II polyketide antibiotic products	3	–
Cellohexaose	989.321	–	Wa-32	Starch and sucrose metabolism	3	–
Cellotetraose	665.213	–	It-36, Pf-31, Pf-51	Starch and sucrose metabolism	3	–
5′-Inosine monophosphate	347.036	–	It-36, Pf-31, Pf-51, Wa-32	Purine metabolism	2	No
Adenine	136.062	+	It-36, Pf-31, Pf-51	Purine metabolism	2	–
cAMP	330.061	+	Pf-51	Purine metabolism / Signalling and metabolic pathways	2	–
Thymidine	241.083	–	Pf-51	Pyrimidine metabolism	3	No
Pantetheine	277.121	–	Pf-31	Pantothenate and CoA biosynthesis	3	–

(*continued on next page*)

Table 1 (*continued*)

Compound	*m/z*	ESI	Yeast Strain	Functional category	Identification level	Antifungal effects *in vitro / in vivo*
Pantoic acid	147.065	–	It-36, Pf-31, Pf-51, Wa-32	Pantothenate and CoA biosynthesis	3	–
Heteropyrithiamine	202.120	+	It-36	Thiamine metabolism	3	–
2,5-Diamino-6-(5-phospho-d-ribosylamino) pyrimidin-4(3 h)-one	354.080	+	Pf-31, Pf-51, Wa-32	Riboflavin metabolism	3	–
2-Hydroxy-6-oxo-6-(2′-hydroxyphenyl)-*hexa*-2,4-dienoate	233.043	–	Pf-51	Riboflavin metabolism	3	–

Identification levels: level 1, validated with a reference standard; level 2, tentative identification by exact mass and online fragmentation spectrum; level 3, tentative identification by online database *m/z* match. -: metabolite not tested. No: no *in vitro / in vivo* antifungal effects.

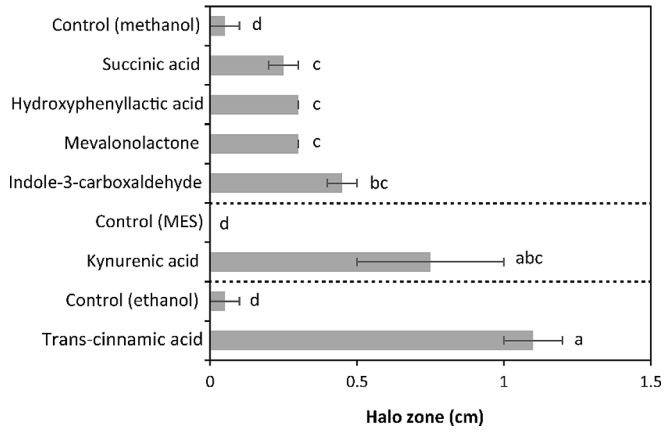

Fig. 4. *In vitro* plate assay of the antagonistic activity of bioactive metabolites against *B. cinerea* spore germination. Data (halo zone) are presented as the means ± SE (n = 4). Groups of metabolites dissolved with the same solvent (methanol, MES or ethanol) are separated by dashed lines. Controls: solvents without metabolite.

mycelia by quantification of electrolyte leakage corroborated the negative effect on membrane integrity of the CA or ICAld treatment (Fig. 8B). The electric conductivity of mycelia treated with metabolites increased gradually over time at higher ratio than the controls. The highest leakage was observed when mycelia were treated with CA at EC_{50} from 90 to 240 min, indicating that CA induces more severe damage than ICAld.

Results obtained from protein leakage measurements of *B. cinerea* mycelia exposed to both metabolites were also consistent with the results of membrane damage. After the 6-h treatment, there was a significant decrease in the intracellular protein content of the fungal cells treated with CA or ICAld at EC_{50} (Fig. 8C), and these values were 20 and 13 % lower than the control, respectively.

3.6.3. *Effect of metabolites on oxalic acid content in the extracellular media*

Oxalic acid secretion into the extracellular medium by *B. cinerea* was calculated in the presence or absence of CA and ICAld. Higher content of this acid has been related to higher levels of pathogenicity and sclerotia development (Wang et al., 2021). It was observed that CA significantly reduced oxalic acid secretion after 48 h of mycelial growth, but unexpectedly, the presence of ICAld increased the secretion of this acid (Fig. 8D).

4. Discussion

Yeast metabolites have been mainly studied with analytical techniques that are inadequate for a more detailed analysis of their role (Sailwal et al., 2020). Metabolomics has emerged as a powerful tool that may help such exploration and provide a comprehensive overview of yeast metabolism in terms of signaling and carbon mapping, as well as production of secondary metabolites of economic relevance or associated with pathogenicity or antagonism (Mülleder et al., 2016; Roullier-Gall et al., 2020; Sailwal et al., 2020). In particular, many yeast strains have been isolated and reported to be efficient agents of biocontrol against several plant and postharvest pathogens (Cabañas et al., 2020; Díaz et al., 2020; Fernandez-San Millan et al., 2021; Hilber-Bodmer et al., 2017; Oztekin and Karbancioglu-Guler, 2021; Ruiz-Moyano et al., 2016; Sipiczki et al., 2020), although their commercialization still continues to be difficult, and there are few yeast-based products on the market (Díaz et al., 2020; Droby et al., 2016; Dukare et al., 2019; Freimoser et al., 2019; Zhang et al., 2018). Metabolomics may help to understand the antagonistic mechanisms of biocontrol yeasts and to identify new antifungal substances that could reach the market more easily than a living organism-based product. Indeed, application of synthetic chemical fungicides can generate pathogen resistance and public concern over environmental contamination and food safety. Therefore, new effective strategies need to be urgently developed for controlling fungal infections in crops (El-Baky and Amara, 2021). In a previous study (Fernandez-San Millan et al., 2022), it was shown that metabolomic analysis of CFs produced during the co-culture of a plant pathogen with its biocontrol yeast was a valid system for finding metabolites involved in such antagonistic mechanisms. Through this methodology, three new compounds produced by *M. pulcherrima* strains with *in vitro* and *in vivo* fungicidal effects were described. By assaying four additional strains of three different genera, the present study reveals species-specific and general metabolic mechanisms related to fungal antagonism. Indeed, the current untargeted metabolomic study has shown relevant differences in the exometabolome between yeast monocultures and the co-cultures with *B. cinerea*. Enhanced levels or the new appearance of several yeast metabolites in the presence of *B. cinerea* clearly indicates that such compounds must have an essential function in the yeast-fungus interaction. Furthermore, Granucci et al. (2015) already speculated that the extracellular medium may be a storage place for some metabolites with roles other than central metabolism of the cell (e.g. regulation of metabolism, cell-to-cell communication or detoxification).

The extracellular fraction of the four yeast strains showed an increase in aromatic metabolites, such as *trans*-cinnamic acid (CA) and its related compound feruloylputrescine, as well as indole-3 carboxaldehyde (ICAld), quinolinate, piperidine, benzene 1,2,4 triol, and hydroxyphenyllactic acid, to name a few. Some metabolites related to the tricarboxylic acid cycle including succinic or ketoglutaric acid, as well as oxylipins and other central carbon metabolites such as sugars and amino acids, also increased in the presence of *B. cinerea*. Among a total of 46 identified metabolites, 12 were found to be common for at least three of the four strains analyzed, suggesting their implication in general mechanisms. The 12 compounds included several metabolites related to the 2-oxocarboxylic acid metabolism, and purine and vitamin's biosynthesis (Table 1), suggesting an enhancement of the central metabolism of yeast in the presence of the fungus. In addition, other metabolites such as CA, the dipeptide Val-Leu or three oxylipins were secreted by at least three yeast strains. It was also observed that the *I. terricola* It-36 strain secreted more metabolites than the other yeast species studied, as shown in the PCA analysis. However, the

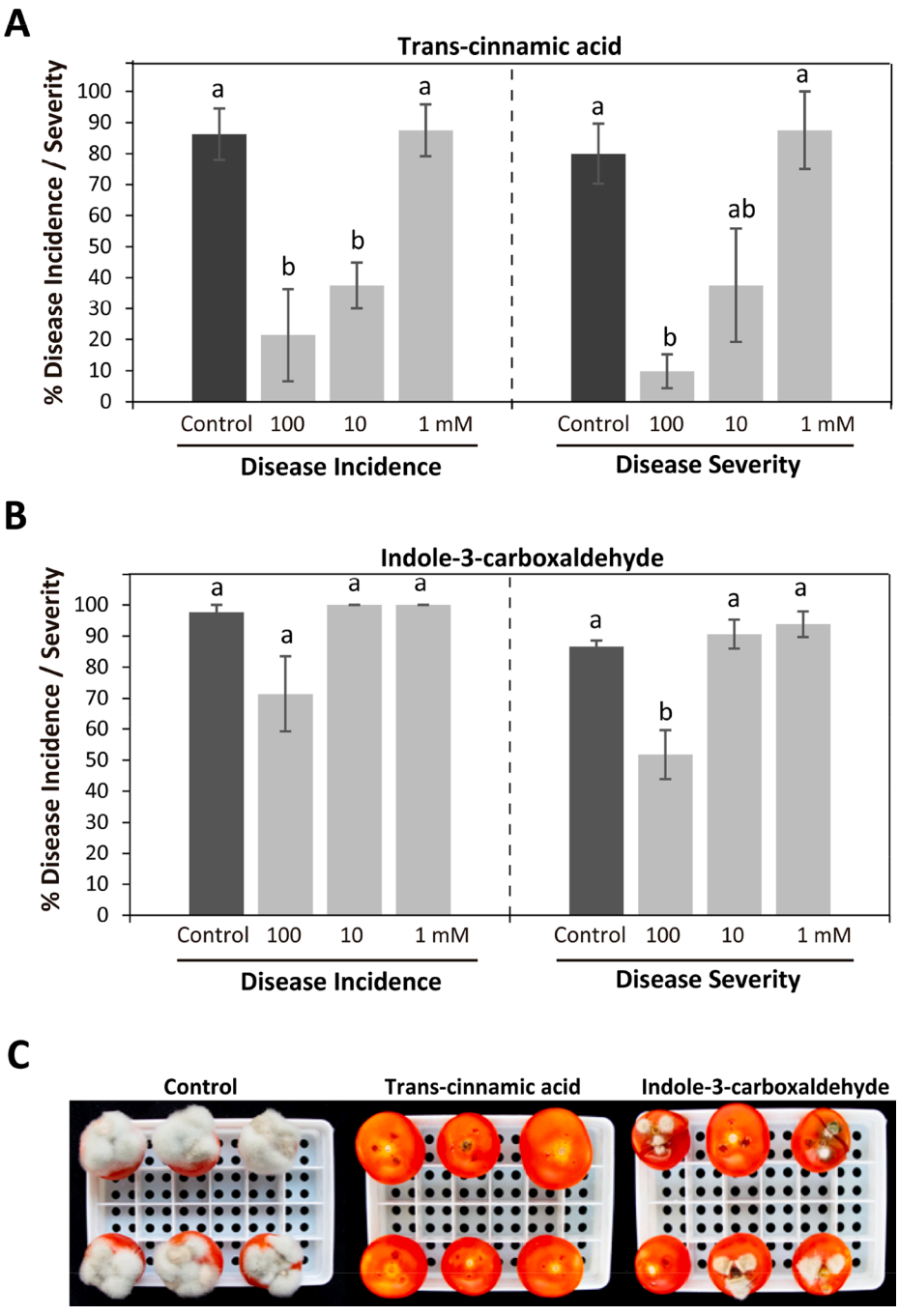

Fig. 5. Biocontrol of postharvest gray mold in tomato. Disease incidence and disease severity of cherry tomatoes inoculated with *B. cinerea* and treated with *trans*-cinnamic acid (A) or indole-3-carboxaldehyde (B) at different concentrations (100 – 10 – 1 mM). (C) Example of biocontrol of gray mold in tomatoes by both metabolites (100 mM). Data are presented as the means ± SE (n = 36 for tomato). Values with the same letter do not differ significantly ($p < 0.05$) according to the Tukey's multiple range test. Control: infected tomatoes without any treatment.

metabolomic analysis showed that the *P. fermentans* Pf-31 and Pf-51 strains secreted the highest number of differential metabolites into the extracellular medium when co-cultured with *B. cinerea*. Both strains showed quite similar general behavior, indicating conserved and specific metabolic mechanisms at the species level, as previously observed with *M. pulcherrima* strains (Fernandez-San Millan et al., 2022). On the other hand, Wa-32 had the simplest exometabolome, although it was the only strain that secreted higher levels of an antibiotic in the presence of *B. cinerea*. The antibiotic was tetracenomycin, whose secretion has only been reported before from actinomycetes of the genus *Streptomyces* and *Saccharotrix* (Liu et al., 2018). In addition, Wa-32 also produced piperidine, a natural alkaloid with a wide range of biological functions, and that serves as a structural element of many alkaloids with pharmaceutical applications (Wang et al., 2020).

4.1. Two yeast metabolites displayed antifungal activity in vivo

Only the 14 commercially available metabolites could be tested as pure reference compounds for growth inhibition of *B. cinerea*. Among them, two had antifungal activity *in vivo* and *in vitro*, and four showed only some antagonistic capacity in the *in vitro* assays.

The most bioactive metabolite was CA, found in the exometabolome of the It-36, Pf-31 and Pf-51 strains. Cinnamic acid is an aromatic carboxylic acid that is usually found in the *trans* configuration (Rodrigues et al., 2019). CA synthesis is the first step of the phenylpropanoid pathway and the source of most hydroxycinnamic acid derivatives like coumaric, caffeic, sinapic and ferulic acids (Desjardins, 2008). *M. pulcherrima* strains also were reported to produce metabolites from the phenylpropanoid pathway, including p-coumaroyl quinic acid and 2,3-dihydroxybenzoate (Fernandez-San Millan et al., 2022), thereby

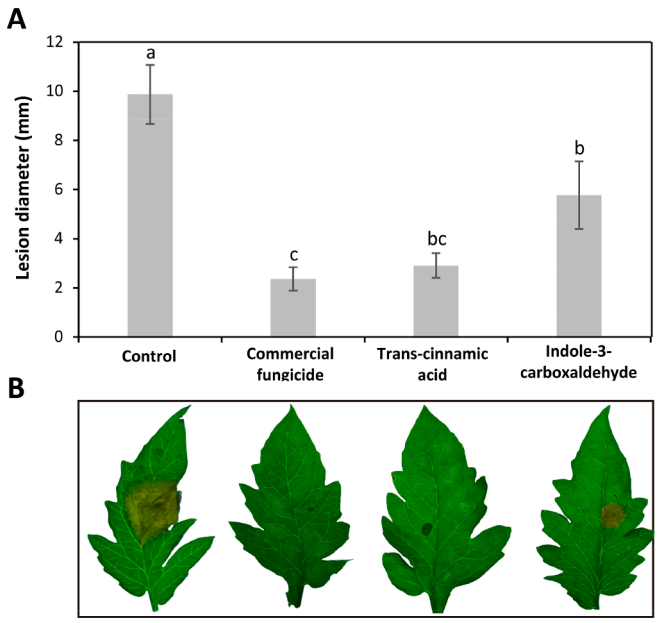

Fig. 6. Biocontrol of gray mold in tomato leaves. (A) Lesion diameter in leaflets inoculated with *B. cinerea* and treated with *trans*-cinnamic acid or indole-3-carboxaldehyde (100 mM). Data are presented as the means ± SE (n = 15). Values with the same letter do not differ significantly ($p < 0.05$) according to the Tukey's multiple range test. Control: infected leaflets only treated with the corresponding solvent. Fungicide: infected leaflets only treated with the fungicide Cabrio®. (B) Examples of leaflets treated with the corresponding compound 4 days after infection with *B. cinerea*.

confirming the importance that this pathway might have in yeast-fungal interactions. CA and its derivatives are secondary metabolites that have been reported to induce pathogen resistance in plants (Métraux, 2002; Zhang et al., 2015). In addition, CA has antioxidant, antibacterial and antifungal properties (Alberto and Dorian, 2013; Beyki et al., 2014; Bock et al., 2014; Korošec et al., 2014; Laverty et al., 2015; Sova, 2012; Wang et al., 2021). In fact, such an antifungal effect of CA has already been described against postharvest decay caused by *B. cinerea* on table grapes (Zhang et al., 2015) and *Penicillium italicum* on mandarins (Li et al., 2019). CA also showed antifungal activity against *Aspergillus flavus* (Ribes et al., 2017), *Geotrichum citri-aurantii* (Cheng et al., 2022), *Fusicladium*, *Colletotrichum* (Bock et al., 2014) and *Sclerotinia* (Wang et al., 2019). Although it was originally isolated from cinnamon bark and other plants (Guzman, 2014), several organisms are also able to secrete CA into the extracellular fraction. For example, Datta et al., (2017) demonstrated that cultures of the probiotic *S. cerevisiae* var. boulardii were rich in polyphenolic metabolites such as CA or vanillic acid. Bock et al. (2014) observed that the nematode *Photorhabdus luminescens* also secreted CA, and these authors demonstrated its *in vitro* antifungal activity against *Colletotrichum* spp. and *Fusicladium effusum*. In this case, the EC_{50} was between 10 and 200 μg $mL^{-}1$ of CA, which is in the range of the EC_{50} calculated in our study for *B. cinerea* inhibition *in vitro* and in other studies (Wang et al., 2021, 2019; Zhang et al., 2015).

In our experimental conditions, CA was a powerful antifungal molecule that also protected tomato leaves from gray mold as it was described for oilseed rape infected by *Sclerotinia sclerotiorum* (Wang et al., 2019).

CA bioactivity has been exploited in agriculture as a raw material for plant-derived pesticides or fungicides, growth promoters, and vegetable and fruit preservatives (Letsididi et al., 2018). In addition to the antifungal properties of CA, its innocuity has encouraged the use of this compound in the formulation of pharmaceuticals, cosmetics and food products (Chalabaev et al., 2008; Cheng et al., 2022). It can be obtained by extraction from plants or by chemical synthesis, but both manufacturing processes have shortcomings, such as the low concentration in plant material or the generation of toxic byproducts (Vargas-Tah and Gosset, 2015). Alternatively, eco-friendly methods for CA production are being developed to enable its biotechnological production by genetically engineering microbial hosts, including *S. cerevisiae* and bacteria like *Escherichia coli*, among others (Vargas-Tah and Gosset, 2015). Although many microbial species do not constitutively synthesize CA, we have demonstrated that under particular conditions, such as co-culture with other organisms, CA synthesis may be induced in yeast strains of *P. fermentans* and *I. terricola* species. In the future, this finding might help to produce CA for industry in a cost-effective way. The detection of CA or other bioactive compounds in the exometabolome of yeasts suggests the possibility of using particular strains for industrial scale production of interesting compounds. As previously demonstrated (Fernandez-San Millan et al., 2022), other yeast species such as *M. pulcherrima* are able to secrete metabolites with interesting biotechnological applications including antibiotics, alkaloids, long chain fatty acids or bile acids.

The second metabolite selected for postharvest tomato protection against gray mold was the alkaloid ICAld, a tryptophan-derived compound related to indole-3-acetic acid metabolism. Its antimicrobial activity has been related to camalexin biosynthesis in plants, an antimicrobial phytoalexin that accumulates in tissues exposed to infection (Devys and Barbier, 1991; Zook and Hammerschmidt, 1997). Contreras-Cornejo et al. (2011) found that *Trichoderma virens* grown under axenic conditions produces ICAld, and that exogenous applications of this metabolite increased camalexin accumulation in *Arabidopsis thaliana* seedlings. Powell et al. (2020) identified indole and its derived metabolites, including ICAld, as molecules derived from cruciferous vegetal species or from commensal bacteria (Swimm et al., 2018) that promote protection in response to damage by acute stressors. Additionally, ICAld is the precursor of indole-3-carboxylic acid, a well-known secondary metabolite that induces defense priming in *A. thaliana* and *Plectosphaerella cucumerina* (Gamir et al., 2014). In a recent study, Duan et al. (2019) also showed that the endophytic fungus *Irpex lacteus* displayed antimicrobial activity and was able to synthesize ICAld.

In our study, the secretion of ICAld to the extracellular medium was enhanced in the presence of *B. cinerea* by strains of two different genera, It-36 and Wa-32. ICAld reduced the disease severity of gray mold in tomatoes by 40 % at the highest concentration tested of 100 mM, although the disease incidence was similar to the control. Lower concentrations of the metabolite were not effective at preventing damage from infection. A reduction in the infection in tomato leaves was also observed following ICAld treatment. However, although ICAld was less efficient than CA, the EC_{50} calculated *in vitro* was similar among the two metabolites: 52 and 50 μg mL^{-1} for CA and ICAld respectively.

We have observed that EC_{50} values calculated under *in vitro* conditions for both bioactive metabolites were lower than the effective concentrations observed *in vivo*. In fact, the EC_{50} calculated for CA in plates was 0.352 mM, but treatments below 1 mM did not protect tomatoes against *B. cinerea* infection and the same shift was observed for ICAld. Cheng et al. (2022) observed the same for CA application against *G. citri-aurantii*: a complete inhibition of growth was caused by 400 μg mL^{-1} CA *in vitro*, but a reduction in disease incidence below 50 % in citrus fruits was obtained after the application of 4000 μg mL^{-1}. Wang et al. (2021) also had to increase the concentrations of other antagonistic metabolites against *B. cinerea* under *in vivo* conditions. Reis et al. (2015) demonstrated that concentrations used in the field are many times higher than those determined in the laboratory, inferring that there is no direct relationship between the concentration recommended for field applications and the EC_{50} determined under controlled laboratory conditions due to different factors such as dilution in leaf tissues, low absorption rates or degradation.

After demonstrating their biocontrol effects, the fungicidal mechanisms of CA and ICAld were investigated. We found that upon treatment with both metabolites, *B. cinerea* was induced to form intracellular

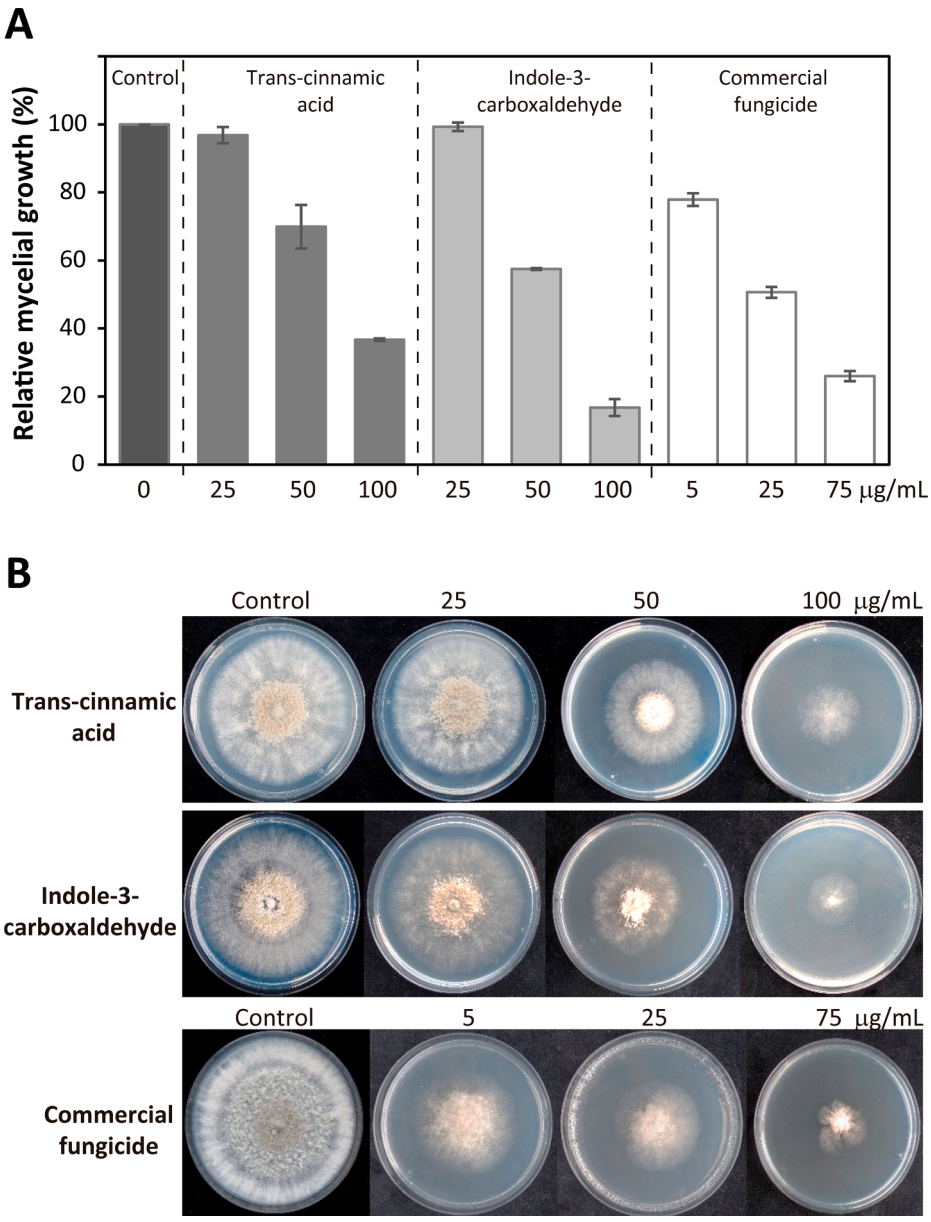

Fig. 7. Effect of *trans*-cinnamic acid, indole-3-carboxaldehyde and commercial fungicide Cabrio® on *in vitro* mycelial growth of *B. cinerea* (A). The mycelial growth relative to the control is shown in B. Each plate contains the same amount of the corresponding solvent of the metabolite. Data are presented as the means ± SE (n = 4).

reactive oxygen species (ROS), lost its membrane integrity and released cytoplasmic contents from the cells. Concomitantly, it has been reported that biotic or abiotic stresses may also induce intracellular ROS generation in fungal pathogens (Zhao et al., 2014; Zou et al., 2010) and similar loss of membrane integrity may release cytoplasmic contents (Cheng et al., 2022; Qin et al., 2010). These effects were more pronounced in spores and mycelia treated with CA than treatments with ICAld. Similar results have been obtained before with CA amendments against *B. cinerea* (Zhang et al., 2015) and other fungal pathogens such as *P. italicum* (Li et al., 2019), *Sclerotinia* (Wang et al., 2019) and *G. citri-aurantii* (Cheng et al., 2022). It is not surprising that both metabolites affect the membrane integrity of the fungus because its role in maintaining intracellular substances is vital to cell survival. In fact, the cell membrane is a target site for several antifungal substances (Avis, 2007; Hasim and Coleman, 2019).

Low secretion of oxalic acid from *B. cinerea* mycelia to the extracellular media elicited by the presence of CA was also observed, as had been previously reported for *Sclerotinia* (Wang et al., 2019). Oxalic acid secretion has been related to virulence and pathogenicity (Schoonbeek et al., 2007). Unexpectedly, ICAld generated an opposite result and oxalic acid increased significantly in the liquid medium. Further studies would be needed to explain this response. Other metabolites from *M. pulcherrima* strains also reduced the oxalic acid content or kept the levels unchanged (Fernandez-San Millan et al., 2022).

4.2. Several yeast metabolites only display in vitro antifungal activity

Among the 14 metabolites tested in this study, four had antifungal activity *in vitro* but not under *in vivo* conditions. These discrepancies were also found in the co-culture study with *M. pulcherrima* strains (Fernandez-San Millan et al., 2022): all the strains with *in vivo* bioactivity were also antagonistic *in vitro* but not the other way round. To explain this fact, we hypothesize that some metabolites may act synergistically against fungal development, as has been described before

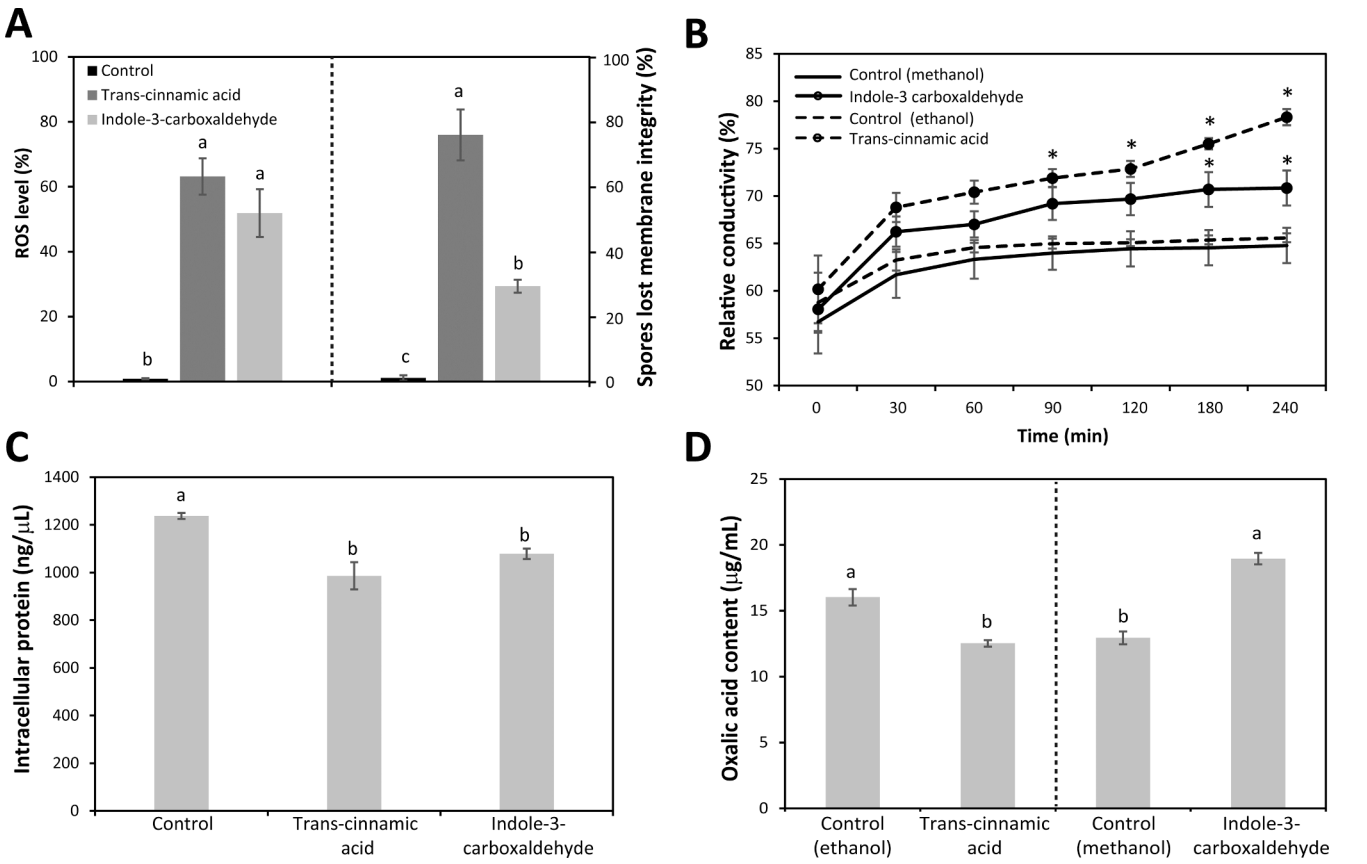

Fig. 8. Effect of the selected metabolites (100 mM) on ROS production and spore membrane integrity (A), mycelial cell membrane permeability (B), intracellular protein level (C) and oxalic acid content (D) of *B. cinerea* cultures. Data are presented as the means ± SE (n = 6). Values with the same letter do not differ significantly ($p < 0.05$) according to the Tukey's multiple range test. Control: fungal culture without any metabolite. Asterisks in B indicate statistical significance compared with the control, as determined by Student's *t*-test ($p < 0.05$).

(Crowley et al., 2013; Le Lay et al., 2016).

One such *in vitro* bioactive metabolites is succinic acid (SA), which was only overproduced by the Pf-51 strain. This is an organic acid localized at the crossroads of several metabolic pathways and it is involved in a wide range of cellular and metabolic activities, including those linked to the tricarboxylic acid cycle, such as branched-chain amino and fatty acid metabolism, carbohydrate pathways, ROS generation, and extra- and intra-cellular signaling (Nowicki and Gottlieb, 2015; Tretter et al., 2016). SA excretion is widespread among fungi, being a byproduct of fermentation by yeasts (Jayaram et al., 2014). Oren-Young et al. (2021) observed that the external addition of SA reduced virulence and prevented sclerotia formation by *B. cinerea*. Wu et al. (2011) showed that SA decreased conidia germination and the growth of *F. oxysporum*. In addition, pathogenic enzyme activity and mycotoxin production by the fungus were inhibited at higher concentrations of SA. In fact, boscalid is a succinate dehydrogenase inhibitor registered as an active ingredient for the treatment of gray mold on strawberries in Spain (Fernández-Ortuño et al., 2017) or on grapevine in France (Walker and Leroux, 2015), for example. The increasing intracellular levels of SA, due to the inhibition of succinate hydrogenase, evidences the importance of the concentration levels of this metabolite for the general survival of *B. cinerea*.

Kynurenic acid is another *in vitro* bioactive metabolite that was differentially secreted by both *P. fermentans* strains. It is a metabolite derived from tryptophan degradation along the kynurenine pathway, which is crucial in eukaryotic and prokaryotic cells for the catabolism of tryptophan and synthesis of the essential cofactor NAD^+ (Ohashi et al., 2017). Some species of the yeast genus *Saccharomyces* and *Yarrowia lipolitica* may also produce kynurenic acid during fermentation (Turska et al., 2019; Wróbel-Kwiatkowska et al., 2020; Yılmaz and Gökmen, 2019). In fact, its production at large scale is being investigated because it has important medical applications (Wróbel-Kwiatkowska et al., 2020). Although the role of extracellular secretion of kynurenic acid remains to be elucidated, it has been suggested that the kynurenine pathway can be essential for the detoxification of excess tryptophan (Ohashi et al., 2017). Ohashi et al. (2013) showed that *S. cerevisiae* secretes quinolinic acid into the medium, which is another metabolite of the kynurenine pathway that was also enhanced in both *P. fermentans* strains. These authors proposed that such metabolite secretions may be a strategy for reutilization and storage in order to maintain a suitable intracellular NAD^+ concentration, although the possibility of its excretion as a toxic molecule was not excluded.

Hydroxyphenyllactic acid (OH-PLA) had some antagonistic effect *in vitro* and also had higher levels of accumulation in the Pf-51 strain's CFs. Its antifungal activity has been previously described against *Penicillium nordicum* as a compound found in the CFs of lactic acid bacteria (Guimarães et al., 2018). In fact, phenyllactic acid and the related compound OH-PLA can display a broad range of inhibitory antifungal activity against several species, including some that produce mycotoxins, such as *Aspergillus ochraceus* or *Penicillium* spp. (Lavermicocca et al., 2000; Yoo et al., 2016).

Mevalonolactone is the last metabolite described with *in vitro* fungal inhibitory capacity and it was only found in It-36 CFs. It is the δ-lactone form of mevalonic acid, a precursor in the mevalonate pathway. Although rare as a metabolite, Niku-Paavola et al. (1999) identified mevalonolactone in the antimicrobial fraction of the bacterium *Lactobacillum plantarum*, which inhibited the growth of *Pantoea agglomerans* in synergy with lactic acid.

4.3. Other relevant secreted metabolites: Oxylipins and dipeptides

Of particular interest is the enhanced presence of oxylipins (hydroxy Di- and TriHOME fatty acids and 2-hydroxycaproic acid) produced by It-36, Pf-31 and Pf-51. They belong to a family of secondary metabolites derived from oxygenase-catalyzed reactions of fatty acids present in organisms from all kingdoms (Gessler et al., 2017; Qiu et al., 2021; Tsitsigiannis and Keller, 2007). Several studies have demonstrated the importance of oxylipins in regulating secondary metabolism and growth. In fungi, they have a strong impact on reproduction, development, adaptative responses and synthesis of secondary metabolites (including mycotoxins) (Gessler et al., 2017). For example, *Candida albicans* produces the oxylipin farnesol, an intermediate in the ergosterol biosynthesis pathway that regulates biofilm formation and quorum sensing (Langford et al., 2009). Deboever et al. (2020) showed the broad antimicrobial activity of several oxylipins against bacteria, fungi and oomycetes. An explanation for their antifungal activity was suggested by Sjögren et al. (2003), who proposed that oxylipins readily partition into the lipid bilayers of fungal membrane, increasing permeability and releasing intracellular electrolytes and proteins. Hydroxy-fatty acids show a broad range of antifungal activity (Pohl et al., 2011), although it depends on the position of the hydroxyl group (Liang et al., 2020). Indeed, a study found that oxylipins with hydroxylation at positions 9, 10, 12, or 13 (as those shown in our study) exhibited greater antifungal activity against *Penicillium* or *Aspergillus* than species with hydroxylation at position 2 or 18 (Liang et al., 2020). In fact, Cowley and Walters (2005) observed a reduction in the infection of barley leaves by the powdery mildew fungus *Blumeria graminis* when the same TriHOME oxylipin was applied that we have described from the CFs. In addition, current studies suggest an additional role for oxylipins in cell signaling (Beccaccioli et al., 2019). It is becoming increasingly clear that fungi, animals and plants utilize oxylipins as a common communication currency to elicit biological responses through an oxylipin 'language' that mostly operates via mechanisms that resemble quorum sensing (Affeldt et al., 2012; Deboever et al., 2020; Fischer and Keller, 2016). Our findings of oxylipins in CFs of three of the yeast strains might support their important role in microbial interaction.

Another interesting finding is the presence of the Val-Leu dipeptide in CFs of all strains analyzed under co-culture conditions in this study and in the one performed previously with the two strains of *M. pulcherrima* (Fernandez-San Millan et al., 2022). Further, both *P. fermentans* strains also secreted the Ile-Tyr dipeptide. Although we did not observe any antifungal effect when *B. cinerea* was challenged by the dipeptide, the enhanced accumulation of dipeptides in CFs must have a significant role in the microbial interaction, so this deserves further investigation. Currently, little is known concerning the function of proteogenic dipeptides (Moreno et al., 2021). In a recent publication about protein-metabolite interactions (PMIs) in *S. cerevisiae*, Luzarowski et al. (2021) showed that several molecules co-elute with proteins, with half of them being proteogenic dipeptides. Indeed, Val-Leu was one of the dipeptides found associated with these yeast proteins. However, Luzarowski et al. (2021) focused their study on the dipeptide Ser-Leu, revealing its numerous protein interactors comprising metabolic enzymes, proteasomal subunits and chaperones. They also observed that Ser-Leu supplementation of the yeast culture medium led to acute metabolic changes and concluded that the role of the dipeptide is related to central metabolism and protein degradation. Examples of PMIs can be found in all protein functional classes, ranging from metabolic enzymes to structural proteins or signaling components, such as kinases or transcription factors (Grove, 2017; Hahn and Young, 2011; Kudryashov and Reisler, 2012; Lemmon and Schlessinger, 2010). Luzarowski et al. (2021) highlighted that regulation by PMIs can be especially important for single-cell organisms such as yeasts, which face constant changes in their environment and nutrient supply. In fact, Moreno et al. (2021) suggested that proteogenic dipeptides act as evolutionarily conserved small-molecule regulators at the nexus of metabolism, stress and protein degradation. In the present work, we were able to demonstrate that accumulation of dipeptides in response to the presence of *B. cinerea* is a general mechanism inherent to different genera and species of yeasts.

5. Conclusions

The validation of the yeast-fungal co-culture model with several genera has increased our knowledge about the microbial interaction and molecular mechanisms by which yeast cells antagonize other fungi. Deciphering the compounds and processes that mediate these interactions represents a continuous challenge. Our results highlight the yeast position as a resource of structurally diverse metabolites, which may affect interactions with other organisms and provide new compounds of interest for agriculture and industry. Clear differences among species and genera were found in the exometabolome profiles, with the *P. fermentans* strains secreting the largest number of differential metabolites into the extracellular medium when co-cultured with *B. cinerea*. In contrast, *W. anomalus* is a poor secretor of metabolites, which probably means that other antagonistic mechanisms are more relevant for this species.

Overall, in this study we have identified 46 differentially secreted metabolites and investigated the antifungal activity of 14 of them against gray mold. Our results have demonstrated that yeasts are able to produce a battery of antifungal substances that probably act in a synergistic way. In particular, CA and ICAld exhibited antifungal activity both *in vitro* and *in vivo*, and they could prevent the infection of *B. cinerea* by generating ROS and damaging the fungal membrane. We have also described the presence of other metabolites, such as oxylipins, an antibiotic or alkaloids, that despite not being assayed for their antifungal capacity, are of significant interest for their antagonistic interactions. Importantly, we have also described the presence of dipeptides in the exometabolome of all the strains analyzed, which opens the door to future investigations of their roles as key regulators of yeast metabolism.

CRediT authorship contribution statement

Alicia Fernandez-San Millan: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Jordi Gamir:** Methodology, Formal analysis, Investigation, Writing – original draft. **Luis Larraya:** Formal analysis, Methodology. **Inmaculada Farran:** Methodology, Funding acquisition. **Jon Veramendi:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2022.105033.

References

Affeldt, K.J., Brodhagen, M., Keller, N.P., 2012. *Aspergillus* oxylipin signaling and quorum sensing pathways depend on G protein-coupled receptors. Toxins (Basel). 4, 695–717. https://doi.org/10.3390/toxins4090695.

Ahsan, T., Chen, J., Zhao, X., Irfan, M., Wu, Y., 2017. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express 7, 54. https://doi.org/10.1186/s13568-017-0351-z.

Alberto, A.M., Dorian, P.C., 2013. Synthesis, characterization, thermal behavior, and antifungal activity of La(III) complexes with cinnamates and 4-methoxyphenylacetate. J. Rare Earths 31, 1106–1113. https://doi.org/10.1016/S1002-0721(12)60412-8.

Arora, D., Gupta, P., Jaglan, S., Roullier, C., Grovel, O., Bertrand, S., 2020. Expanding the chemical diversity through microorganisms co-culture: Current status and outlook. Biotechnol. Adv. 40, 107521 https://doi.org/10.1016/j.biotechadv.2020.107521.

Avis, T.J., 2007. Antifungal compounds that target fungal membranes: Applications in plant disease control. Can. J. Plant Pathol. 29, 323–329. https://doi.org/10.1080/07060660709507478.

Awaad, A.S., Nabilah, A.J.A., Zain, M.E., 2012. New antifungal compounds from *Aspergillus terreus* isolated from desert soil. Phyther. Res. 26, 1872–1877. https://doi.org/10.1002/ptr.4668.

Beccaccioli, M., Reverberi, M., Scala, V., 2019. Fungal lipids: Biosynthesis and signalling during plant-pathogen interaction. Front. Biosci. (Landmark Ed) 24(1), 168–181. https://doi.org/10.2741/4712.

Bertrand, S., Schumpp, O., Bohni, N., Bujard, A., Azzollini, A., Monod, M., Gindro, K., Wolfender, J.L., 2013a. Detection of metabolite induction in fungal co-cultures on solid media by high-throughput differential ultra-high pressure liquid chromatography–time-of-flight mass spectrometry fingerprinting. J. Chromatogr. A 1292, 219–228.

Bertrand, S., Schumpp, O., Bohni, N., Monod, M., Gindro, K., Wolfender, J.L., 2013b. De novo production of metabolites by fungal co-culture of *Trichophyton rubrum* and *Bionectria ochroleuca*. J. Nat. Prod 76, 1157–1165. https://doi.org/10.1021/np400258f.

Beyki, M., Zhaveh, S., Khalili, S.T., Rahmani-Cherati, T., Abollahi, A., Bayat, M., Tabatabaei, M., Mohsenifar, A., 2014. Encapsulation of *Mentha piperita* essential oils in chitosan-cinnamic acid nanogel with enhanced antimicrobial activity against *Aspergillus flavus*. Ind. Crops Prod. 54, 310–319. https://doi.org/10.1016/j.indcrop.2014.01.033.

Bock, C.H., Shapiro-Ilan, D.I., Wedge, D.E., Cantrell, C.L., 2014. Identification of the antifungal compound, trans-cinnamic acid, produced by *Photorhabdus luminescens*, a potential biopesticide against pecan scab. J. Pest Sci. 87, 155–162. https://doi.org/10.1007/s10340-013-0519-5.

Cabañas, C.M., Hernández, A., Martínez, A., Tejero, P., Vázquez-Hernández, M., Martín, A., Ruiz-Moyano, S., 2020. Control of *Penicillium glabrum* by indigenous antagonistic yeast from vineyards. Foods 9, 1864. https://doi.org/10.3390/foods9121864.

Chalabaev, S., Turlin, E., Bay, S., Ganneau, C., Brito-Fravallo, E., Charles, J.F., Danchin, A., Biville, F., 2008. Cinnamic acid, an autoinducer of its own biosynthesis, is processed via Hca enzymes in *Photorhabdus luminescens*. Appl. Environ. Microbiol. 74, 1717–1725. https://doi.org/10.1128/AEM.02589-07.

Chen, P.H., Chen, R.Y., Chou, J.Y., 2018. Screening and evaluation of yeast antagonists for biological control of *Botrytis cinerea* on strawberry fruits. Mycobiology 46, 33–46. https://doi.org/10.1080/12298093.2018.1454013.

Cheng, X., Yang, Y., Zhu, X., Yuan, P., Gong, B., Ding, S., Shan, Y., 2022. Inhibitory mechanisms of cinnamic acid on the growth of *Geotrichum citri-aurantii*. Food Control 131, 108459. https://doi.org/10.1016/j.foodcont.2021.108459.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Beltrán-Peña, E., Herrera-Estrella, A., López-Bucio, J., 2011. *Trichoderma*-induced plant immunity likely involves both hormonal- and camalexin dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungus *Botrytis cinerea*. Plant Signal. Behav. 6, 1554–1563. https://doi.org/10.4161/psb.6.10.17443.

Cowley, T., Walters, D., 2005. Local and systemic effects of oxylipins on powdery mildew infection in barley. Pest Manag. Sci. 61, 572–576. https://doi.org/10.1002/ps.1026.

Crowley, S., Mahony, J., Van Sinderen, D., 2013. Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. Trends Food Sci. Technol. 33, 93–109. https://doi.org/10.1016/j.tifs.2013.07.004.

Csutak, O., Vassu, T., Sarbu, I., Stoica, I., Cornea, P., 2013. Antagonistic activity of three newly isolated yeast strains from the surface of fruits. Food Technol. Biotechnol. 51, 70–77.

Datta, S., Timson, D.J., Annapure, U.S., 2017. Antioxidant properties and global metabolite screening of the probiotic yeast *Saccharomyces cerevisiae* var. boulardii. J. Sci. Food Agric. 97, 3039–3049. https://doi.org/10.1002/jsfa.8147.

de Souza, C., Christofoleti-Furlan, R.M., de Souza Miranda Muynarsk, E., Vinicius de Melo Pereira, G., Lucas, D.L., Basso, L.C., 2019. Biotechnological applications of nonconventional yeasts. In: Yeasts in biotechnology. IntechOpen, pp. 1–27. https://doi.org/https://doi.org/10.5772/57353.

Deboever, E., Deleu, M., Mongrand, S., Lins, L., Fauconnier, M.L., 2020. Plant–pathogen interactions: Underestimated roles of phyto-oxylipins. Trends Plant Sci. 25, 22–34. https://doi.org/10.1016/j.tplants.2019.09.009.

Desjardins, Y., 2008. Physiological and ecological functions and biosynthesis of health-promoting compounds in fruit and vegetables. Improv. Heal. Prop. Fruit Veg. Prod. 201–247 https://doi.org/10.1533/9781845694289.3.201.

Devys, M., Barbier, M., 1991. Indole-3-carboxaldehyde in the cabbage *Brassica oleracea*: A systematic determination. Phytochemistry 30, 389–391. https://doi.org/10.1016/0031-9422(91)83690-M.

Díaz, M.A., Pereyra, M.M., Picón-Montenegro, E., Meinhardt, F., Dib, J.R., 2020. Killer yeasts for the biological control of postharvest fungal crop diseases. Microorganisms 8, 1–14. https://doi.org/10.3390/microorganisms8111680.

Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D., Jijakli, M.H., 2016. The science, development, and commercialization of postharvest biocontrol products. Postharvest Biol. Technol. 122, 22–29. https://doi.org/10.1016/j.postharvbio.2016.04.006.

Duan, X., Xu, F., Qin, D., Gao, T., Shen, W., Zuo, S., Yu, B., Xu, J., Peng, Y., Dong, J., 2019. Diversity and bioactivities of fungal endophytes from *Distylium chinense*, a rare waterlogging tolerant plant endemic to the Three Gorges Reservoir. BMC Microbiol. 19, 1–14. https://doi.org/10.1186/s12866-019-1634-0.

Dukare, A.S., Paul, S., Nambi, V.E., Gupta, R.K., Singh, R., Sharma, K., Vishwakarma, R. K., 2019. Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. Crit. Rev. Food Sci. Nutr. 59, 1498–1513. https://doi.org/10.1080/10408398.2017.1417235.

Elad, Yigal, Stewart, A., 2004. *Botrytis*: Biology, pathology and control. Kluwer Academic Publishers, Netherlands, pp. 223–241. https://doi.org/10.1007/978-1-4020-2626-3.

El-Baky, N.A., Amara, A.A.A.F., 2021. Recent approaches towards control of fungal diseases in plants: An updated review. J. Fungi 7 (11), 900. https://doi.org/10.3390/jof7110900.

Fernández-Ortuño, D., Pérez-García, A., Tores, J.A., de Vicente, A., 2017. Resistencia a múltiples fungicidas en *Botrytis cinerea* en fresa. Agric. Vergel 327–330.

Fernandez-San Millan, A., Farran, I., Larraya, L., Ancin, M., Arregui, L.M., Veramendi, J., 2020. Plant growth-promoting traits of yeasts isolated from Spanish vineyards: Benefits for seedling development. Microbiol. Res. 237, 126480 https://doi.org/10.1016/j.micres.2020.126480.

Fernandez-San Millan, A., Larraya, L., Farran, I., Ancin, M., Veramendi, J., 2021. Successful biocontrol of major postharvest and soil-borne plant pathogenic fungi by antagonistic yeasts. Biol. Control 160, 104683. https://doi.org/10.1016/j.biocontrol.2021.104683.

Fernandez-San Millan, A., Gamir, J., Farran, I., Larraya, L., Veramendi, J., 2022. Identification of new antifungal metabolites produced by the yeast *Metschnikowia pulcherrima* involved in the biocontrol of postharvest plant pathogenic fungi. Postharvest Biol. Technol. 192, 111995 https://doi.org/10.1016/j.postharvbio.2022.111995.

Ferraz, L., Cunha, T., Da Silva, A., Kupper, K., 2016. Biocontrol ability and putative mode of action of yeasts against *Geotrichum citri-aurantii* in citrus fruit. Microbiol. Res. 188–189, 72–79. https://doi.org/10.1016/j.micres.2016.04.012.

Fischer, G.J., Keller, N.P., 2016. Production of cross-kingdom oxylipins by pathogenic fungi: An update on their role in development and pathogenicity. J. Microbiol. 54, 254–264. https://doi.org/10.1007/s12275-016-5620-z.

Freimoser, F., Rueda-Mejia, M., Tilocca, B., Migheli, Q., 2019. Biocontrol yeasts: Mechanisms and applications. World J. Microbiol. Biotechnol. 35, 1–19. https://doi.org/10.1007/s11274-019-2728-4.

Gamir, J., Pastor, V., Kaever, A., Cerezo, M., Flors, V., 2014. Targeting novel chemical and constitutive primed metabolites against *Plectosphaerella cucumerina*. Plant J. 78, 227–240. https://doi.org/10.1111/tpj.12465.

Gamir, J., Minchev, Z., Berrio, E., García, J.M., De Lorenzo, G., Pozo, M.J., 2020. Roots drive oligogalacturonide-induced systemic immunity in tomato. Plant, Cell & Environ. 44, 275–289. https://doi.org/10.1111/pce.13917.

Gessler, N.N., Filippovich, S.Y., Bachurina, G.P., Kharchenko, E.A., Groza, N.V., Belozerskaya, T.A., 2017. Oxylipins and oxylipin synthesis pathways in fungi. Appl. Biochem. Microbiol. 53, 568–579. https://doi.org/10.1134/S0003683817060060.

Granucci, N., Pinu, F.R., Han, T.L., Villas-Boas, S.G., 2015. Can we predict the intracellular metabolic state of a cell based on extracellular metabolite data? Mol. Biosyst. 11, 3297–3304. https://doi.org/10.1039/c5mb00292c.

Gross, S., Kunz, L., Müller, D.C., Santos Kron, A., Freimoser, F.M., 2018. Characterization of antagonistic yeasts for biocontrol applications on apples or in soil by quantitative analyses of synthetic yeast communities. Yeast 35, 559–566. https://doi.org/10.1002/yea.3321.

Grove, A., 2017. Regulation of metabolic pathways by MarR family transcription factors. Comput. Struct. Biotechnol. J. 15, 366–371. https://doi.org/10.1016/j.csbj.2017.06.001.

Guimarães, A., Venancio, A., Abrunhosa, L., 2018. Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*. In: Food additives and contaminants - Part A. Taylor & Francis, pp. 1803–1818. https://doi.org/10.1080/19440049.2018.1500718.

Guzman, J.D., 2014. Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. Molecules 19, 19292–19349. https://doi.org/10.3390/molecules191219292.

Hahn, S., Young, E.T., 2011. Transcriptional regulation in *Saccharomyces cerevisiae*: Transcription factor regulation and function, mechanisms of initiation, and roles of activators and coactivators. Genetics 189, 705–736. https://doi.org/10.1534/genetics.111.127019.

Haidar, R., Fermaud, M., Calvo-Garrido, C., Roudet, J., Deschamps, A., 2016. Modes of action for biological control of *Botrytis cinerea* by antagonistic bacteria. Phytopathol. Mediterr. 55 (3), 13–34. https://doi.org/10.14601/Phytopathol_Mediterr-18079.

Hasim, S., Coleman, J.J., 2019. Targeting the fungal cell wall: Current therapies and implications for development of alternative antifungal agents. Future Med. Chem. 11, 869–883. https://doi.org/10.4155/fmc-2018-0465.

Hilber-Bodmer, M., Schmid, M., Ahrens, C.H., Freimoser, F.M., 2017. Competition assays and physiological experiments of soil and phyllosphere yeasts identify *Candida subhashii* as a novel antagonist of filamentous fungi. BMC Microbiol. 17, 1–15. https://doi.org/10.1186/s12866-016-0908-z.

Hua, L., Yong, C., Zhanquan, Z., Boqiang, L., Guozheng, Q., Shiping, T., 2018. Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest decay in fruits and vegetables. Food Qual. Saf. 2, 111–119. https://doi.org/10.1093/fqsafe/fyy016.

Jayaram, V.B., Cuyvers, S., Verstrepen, K.J., Delcour, J.A., Courtin, C.M., 2014. Succinic acid in levels produced by yeast (*Saccharomyces cerevisiae*) during fermentation strongly impacts wheat bread dough properties. Food Chem. 151, 421–428. https://doi.org/10.1016/j.foodchem.2013.11.025.

Jones, J.A., Wang, X., 2018. Use of bacterial co-cultures for the efficient production of chemicals. Curr. Opin. Biotechnol. 53, 33–38. https://doi.org/10.1016/J.COPBIO.2017.11.012.

Kaever, A., Landesfeind, M., Feussner, K., Mosblech, A., Heilmann, I., Morgenstern, B., Feussner, I., Meinicke, P., 2015. MarVis-Pathway: Integrative and exploratory pathway analysis of non-targeted metabolomics data. Metabolomics 11, 764–777. https://doi.org/10.1007/s11306-014-0734-y.

Korošec, B., Sova, M., Turk, S., Kraševec, N., Novak, M., Lah, L., Stojan, J., Podobnik, B., Berne, S., Zupanec, N., Bunc, M., Gobec, S., Komel, R., 2014. Antifungal activity of cinnamic acid derivatives involves inhibition of benzoate 4-hydroxylase (CYP53). J. Appl. Microbiol. 116, 955–966. https://doi.org/10.1111/jam.12417.

Kudryashov, D.S., Reisler, E., 2012. Invited review: ATP and ADP actin states. Biopolymers 99. https://doi.org/10.1002/bip.22155.

Kumar Verma, D., Thyab Gddoa Al-Sahlany, S., Kareem Niamah, A., Thakur, M., Shah, N., Singh, S., Baranwal, D., Patel, A.R., Lara Utama, G., Noe Aguilar, C., 2022. Recent trends in microbial flavour compounds: A review on chemistry, synthesis mechanism and their application. Food. Saudi J. Biol. Sci. 29, 1565–1576. https://doi.org/10.1016/j.sjbs.2021.11.010.

Langford, M.L., Atkin, A.L., Nickerson, K.W., 2009. Cellular interactions of farnesol, a quorum-sensing molecule produced by *Candida albicans*. Future Microbiol. 4, 1353–1362. https://doi.org/10.2217/fmb.09.98.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., Gobbetti, M., 2000. Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl. Environ. Microbiol. 66, 4084–4090. https://doi.org/10.1128/AEM.66.9.4084-4090.2000.

Laverty, G., Mccloskey, A.P., Gorman, S.P., Gilmore, B.F., 2015. Anti-biofilm activity of ultrashort cinnamic acid peptide derivatives against medical device-related pathogens. J. Pept. Sci. 21, 770–778. https://doi.org/10.1002/psc.2805.

Le Lay, C., Coton, E., Le Blay, G., Chobert, J.M., Haertlé, T., Choiset, Y., Van Long, N.N., Meslet-Cladière, L., Mounier, J., 2016. Identification and quantification of antifungal compounds produced by lactic acid bacteria and propionibacteria. Int. J. Food Microbiol. 239, 79–85. https://doi.org/10.1016/j.ijfoodmicro.2016.06.020.

Lemmon, M.A., Schlessinger, J., 2010. Cell signaling by receptor tyrosine kinases. Cell 141, 1117–1134. https://doi.org/10.1016/j.cell.2010.06.011.

Letsididi, K.S., Lou, Z.X., Letsididi, R., Mohammed, K., Maguy, B.L., 2018. Antimicrobial and antibiofilm effects of trans-cinnamic acid nanoemulsion and its potential application on lettuce. LWT-FOOD Sci. Technol. 94, 25–32. https://doi.org/10.1016/j.lwt.2018.04.018.

Li, J., Li, H., Ji, S., Chen, T., Tian, S., Qin, G., 2019. Enhancement of biocontrol efficacy of *Cryptococcus laurentii* by cinnamic acid against *Penicillium italicum* in citrus fruit. Postharvest Biol. Technol. 149, 42–49. https://doi.org/10.1016/j.postharvbio.2018.11.018.

Liang, N., Dacko, A., Tan, A.K., Xiang, S., Curtis, J.M., Gänzle, M.G., 2020. Structure-function relationships of antifungal monohydroxy unsaturated fatty acids (HUFA) of plant and bacterial origin. Food Res. Int. 134 https://doi.org/10.1016/j.foodres.2020.109237.

Liu, B., Li, J., Chen, M., Hao, X., Cao, F., Tan, Y., Ping, Y., Wang, Y., Xiao, C., Gan, M., 2018. Seco-tetracenomycins from the marine-derived actinomycete *Saccharothrix* sp. 10-10. Mar. Drugs 16. https://doi.org/10.3390/md16100345.

Liu, Y., Yao, S., Deng, L., Ming, J., Zeng, K., 2019. Different mechanisms of action of isolated epiphytic yeasts against *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Postharvest Biol. Technol. 152, 100–110. https://doi.org/10.1016/j.postharvbio.2019.03.002.

Lutz, M.C., Lopes, C.A., Rodriguez, M.E., Sosa, M.C., Sangorrín, M.P., 2013. Efficacy and putative mode of action of native and commercial antagonistic yeasts against postharvest pathogens of pear. Int. J. Food Microbiol. 164, 166–172. https://doi.org/10.1016/j.ijfoodmicro.2013.04.005.

Luzarowski, M., Vicente, R., Kiselev, A., Wagner, M., Schlossarek, D., Erban, A., de Souza, L.P., Childs, D., Wojciechowska, I., Luzarowska, U., Górka, M., Sokołowska, E.M., Kosmacz, M., Moreno, J.C., Brzezińska, A., Vegesna, B., Kopka, J., Fernie, A.R., Willmitzer, L., Ewald, J.C., Skirycz, A., 2021. Global mapping of protein–metabolite interactions in *Saccharomyces cerevisiae* reveals that Ser-Leu dipeptide regulates phosphoglycerate kinase activity. Commun. Biol. 4, 1–15. https://doi.org/10.1038/s42003-021-01684-3.

Magoye, E., Pfister, M., Hilber-Bodmer, M., Freimoser, F., 2020. Competition assays to quantify the effect of biocontrol yeasts against plant pathogenic fungi on fruits. Bio-Protocol. 10, 1–11. https://doi.org/10.21769/bioprotoc.3518.

Meilin, Y., Yingxin, L., Shivakumar, P.B., Lu, L., Changlun, S., Zhiyong, L., Changyun, W., 2019. New metabolites from the co-culture of marine-derived actinomycete *Streptomyces rochei* MB037 and fungus *Rhinocladiella similis* 35. Front. Genet. 10 https://doi.org/10.3389/fmicb.2019.00915.

Métraux, J.P., 2002. Recent breakthroughs in the study of salicylic acid biosynthesis. Trends Plant Sci. 7 (8), 332–334. https://doi.org/10.1016/s1360-1385(02)02313-0.

Moreno, J.C., Rojas, B.E., Vicente, R., Gorka, M., Matz, T., Chodasiewicz, M., Peralta-Ariza, J.S., Zhang, Y., Alseekh, S., Childs, D., Luzarowski, M., Nikoloski, Z., Zarivach, R., Walther, D., Hartman, M.D., Figueroa, C.M., Iglesias, A.A., Fernie, A.R., Skirycz, A., 2021. Tyr-Asp inhibition of glyceraldehyde 3-phosphate dehydrogenase affects plant redox metabolism. EMBO J. 40, 1–16. https://doi.org/10.15252/embj.2020106800.

Mülleder, M., Calvani, E., Alam, M.T., Wang, R.K., Eckerstorfer, F., Zelezniak, A., Ralser, M., 2016. Functional metabolomics describes the yeast biosynthetic regulome. Cell 167, 553–565.e12. https://doi.org/10.1016/j.cell.2016.09.007.

Niku-Paavola, M.-L., Laitila, A., Mattila-Sandholm, T., Haikara, A., 1999. New types of antimicrobial compounds produced by *Lactobacillus plantarum*. J. Appl. Microbiol. 86, 29–35. https://doi.org/10.1046/j.1365-2672.1999.00632.x.

Nowicki, S., Gottlieb, E., 2015. Oncometabolites: Tailoring our genes. FEBS J. 282, 2796–2805. https://doi.org/10.1111/febs.13295.

Ohashi, K., Kawai, S., Murata, K., 2013. Secretion of quinolinic acid, an intermediate in the kynurenine pathway, for utilization in NAD+ biosynthesis in the yeast *Saccharomyces cerevisiae*. Eukaryot. Cell 12, 648–653. https://doi.org/10.1128/EC.00339-12.

Ohashi, K., Chaleckis, R., Takaine, M., Wheelock, C.E., Yoshida, S., 2017. Kynurenine aminotransferase activity of Aro8/Aro9 engage tryptophan degradation by producing kynurenic acid in *Saccharomyces cerevisiae*. Sci. Rep. 7, 6–13. https://doi.org/10.1038/s41598-017-12392-6.

Oppong-Danquah, E., Parrot, D., Blümel, M., Labes, A., Tasdemir, D., 2018. Molecular networking-based metabolome and bioactivity analyses of marine-adapted fungi co-cultivated with phytopathogens. Front. Microbiol. 9, 1–20. https://doi.org/10.3389/fmicb.2018.02072.

Oppong-Danquah, E., Budnicka, P., Blümel, M., Tasdemir, D., 2020. Design of fungal co-cultivation based on comparative metabolomics and bioactivity for discovery of marine fungal agrochemicals. Mar. Drugs 18 (73). https://doi.org/10.3390/md18020073.

Oren-Young, L., Llorens, E., Bi, K., Zhang, M., Sharon, A., 2021. *Botrytis cinerea* methyl isocitrate lyase mediates oxidative stress tolerance and programmed cell death by modulating cellular succinate levels. Fungal Genet. Biol. 146, 103484 https://doi.org/10.1016/j.fgb.2020.103484.

Oro, L., Feliziani, E., Ciani, M., Romanazzi, G., Comitini, F., 2018. Volatile organic compounds from *Wickerhamomyces anomalus, Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* inhibit growth of decay causing fungi and control postharvest diseases of strawberries. Int. J. Food Microbiol. 265, 18–22. https://doi.org/10.1016/j.ijfoodmicro.2017.10.027.

Oztekin, S., Karbancioglu-Guler, F., 2021. Bioprospection of *Metschnikowia* sp. isolates as biocontrol agents against postharvest fungal decays on lemons with their potential modes of action. Postharvest Biol. Technol. 181, 111634 https://doi.org/10.1016/j.postharvbio.2021.111634.

Pang, Z., Chong, J., Zhou, G., Anderson De Lima Morais, D., Chang, L., Barrette, M., Gauthier, C., Jacques, P.‐E., Li, S., Xia, J., 2021. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Res. 49. https://doi.org/10.1093/nar/gkab382.

Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2015. Biocontrol ability and action mechanism of food-isolated yeast strains against *Botrytis cinerea* causing post-harvest bunch rot of table grape. Food Microbiol. 47, 85–92. https://doi.org/10.1016/j.fm.2014.11.013.

Pawlikowska, E., James, S.A., Breierova, E., Antolak, H., Kregiel, D., 2019. Biocontrol capability of local *Metschnikowia* sp. isolates. Antonie van Leeuwenhoek. Int. J. Gen. Mol. Microbiol. 112, 1425–1445. https://doi.org/10.1007/s10482-019-01272-w.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M.S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V., Anfora, G., 2017. A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot. 97, 70–84. https://doi.org/10.1016/j.cropro.2016.11.025.

Pohl, C.H., Kock, J.L.F., Thibane, V.S., 2011. Antifungal free fatty acids: A review. Sci. against Microb. Pathog. Curr. Res. Technol. Adv. 3, 61–71.

Pokorny, A., Smilanick, J., Xiao, C.L., Farrar, J.J., Shrestha, A., 2016. Determination of fungicide resistance in *Botrytis cinerea* from strawberry in the Central Coast region of California. Plant Heal. Prog. 17, 30–34. https://doi.org/10.1094/PHP-RS-15-0053.

Powell, D.N., Swimm, A., Sonowal, R., Bretin, A., Gewirtz, A.T., Jones, R.M., Kalman, D., 2020. Indoles from the commensal microbiota act via the AHR and IL-10 to tune the cellular composition of the colonic epithelium during aging. Proc. Natl. Acad. Sci. U. S. A. 117, 21519–21526. https://doi.org/10.1073/pnas.2003004117.

Price, N.P., Bischoff, K.M., Leathers, T.D., Cossé, A.A., Manitchotpisit, P., 2017. Polyols, not sugars, determine the structural diversity of anti-streptococcal liamocins produced by *Aureobasidium pullulans* strain NRRL 50380. J. Antibiot. (Tokyo) 70, 136–141. https://doi.org/10.1038/ja.2016.92.

Qin, G., Zong, Y., Chen, Q., Hua, D., Tian, S., 2010. Inhibitory effect of boron against *Botrytis cinerea* on table grapes and its possible mechanisms of action. Int. J. Food Microbiol. 138, 145–150. https://doi.org/10.1016/j.ijfoodmicro.2009.12.018.

Qiu, M., Wang, Y., Sun, L., Deng, Q., Zhao, J., 2021. Fatty acids and oxylipins as antifungal and anti-mycotoxin agents in food: A review. Toxins (Basel). 13, 852. https://doi.org/10.3390/toxins13120852.

Raspor, P., Miklič-Milek, D., Avbelj, M., Čadež, N., 2010. Biocontrol of grey mould disease on grape caused by *Botrytis cinerea* with autochthonous wine yeasts. Food Technol. Biotechnol. 48, 336–343.

Reis, E.M., Zanatta, M., Carmona, M., Menten, J.O.M., Reis, E.M., Zanatta, M., Carmona, M., Menten, J.O.M., 2015. Relationship between IC50 determined in vitro/in vivo and the fungicide rate used in the field. Summa Phytopathol. 41, 49–53. https://doi.org/10.1590/0100-5405/1997.

Ribes, S., Fuentes, A., Talens, P., Barat, J.M., 2017. Application of cinnamon bark emulsions to protect strawberry jam from fungi. Leb. und-Technologie 265–272. https://doi.org/10.1016/j.lwt.2016.12.047.

Roca-Couso, R., Flores-Félix, J.D., Rivas, R., 2021. Mechanisms of action of microbial biocontrol agents against *Botrytis cinerea*. J. Fungi 7, 1045. https://doi.org/10.3390/jof7121045.

Rodrigues, M.P., Tomaz, D.C., Ângelo de Souza, L., Onofre, T.S., Aquiles de Menezes, W., Almeida-Silva, J., Suarez-Fontes, A.M., Rogéria de Almeida, M., Manoel da Silva, A., Bressan, G.C., Vannier-Santos, M.A., Rangel Fietto, J.L., Teixeira, R.R., 2019. Synthesis of cinnamic acid derivatives and leishmanicidal activity against *Leishmania braziliensis*. Eur. J. Med. Chem. 183, 111688 https://doi.org/10.1016/j.ejmech.2019.111688.

Roullier-Gall, C., David, V., Hemmler, D., Schmitt-Kopplin, P., Alexandre, H., 2020. Exploring yeast interactions through metabolic profiling. Sci. Rep. 10, 1–10. https://doi.org/10.1038/s41598-020-63182-6.

Ruiz-Moyano, S., Martín, A., Villalobos, M.C., Calle, A., Serradilla, M.J., Córdoba, M.G., Hernández, A., 2016. Yeasts isolated from figs (*Ficus carica* L.) as biocontrol agents of postharvest fruit diseases. Food Microbiol. 57, 45–53. https://doi.org/10.1016/j.fm.2016.01.003.

Sailwal, M., Das, A.J., Gazara, R.K., Dasgupta, D., Bhaskar, T., Hazra, S., Ghosh, D., 2020. Connecting the dots: Advances in modern metabolomics and its application in yeast system. Biotechnol. Adv. 44, 107616 https://doi.org/10.1016/j.biotechadv.2020.107616.

Santos, A., Sánchez, A., Marquina, D., 2004. Yeasts as biological agents to control *Botrytis cinerea*. Microbiol. Res. 159, 331–338. https://doi.org/10.1016/j.micres.2004.07.001.

Saravanakumar, D., Ciavorella, A., Spadaro, D., Garibaldi, A., Gullino, M.L., 2008. *Metschnikowia pulcherrima* strain MACH1 outcompetes *Botrytis cinerea*, *Alternaria alternata* and *Penicillium expansum* in apples through iron depletion. Postharvest Biol. Technol. 49, 121–128. https://doi.org/10.1016/j.postharvbio.2007.11.006.

Schoonbeek, H.J., Jacquat-Bovet, A.C., Mascher, F., Métraux, J.P., 2007. Oxalate-degrading bacteria can protect *Arabidopsis thaliana* and crop plants against *Botrytis cinerea*. Mol. Plant-Microbe Interact. 20, 1535–1544. https://doi.org/10.1094/MPMI-20-12-1535.

Schrimpe-Rutledge, A.C., Codreanu, S.G., Sherrod, S.D., Mclean, J.A., 2016. Untargeted metabolomics strategies-challenges and emerging directions. J. Am. Soc. Mass Spectrom. 27, 1897–1905. https://doi.org/10.1007/s13361-016-1469-y.

Siedler, S., Balti, R., Neves, A.R., 2019. Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. Curr. Opin. Biotechnol. 56, 138–146. https://doi.org/10.1016/j.copbio.2018.11.015.

Sipiczki, M., Parafati, L., Vazquez, F., Wu, X., Yue, J., Sui, Y., Wisniewski, M., Droby, S., Piombo, E., Wu, X., Yue, J., 2020. Genome sequence, assembly, and characterization of the antagonistic yeast *Candida oleophila* used as a biocontrol agent against post-harvest diseases. Front. Microbiol. 11, 295. https://doi.org/10.3389/fmicb.2020.00295.

Sjögren, J., Magnusson, J., Broberg, A., Schnürer, J., Kenne, L., 2003. Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* MiLAB 14. Appl. Environ. Microbiol. 69, 7554–7557. https://doi.org/10.1128/AEM.69.12.7554-7557.2003.

Sova, M., 2012. Antioxidant and antimicrobial activities of cinnamic acid derivatives. Mini-Reviews Med. Chem. 12, 749–767. https://doi.org/10.2174/138955712801264792.

Spadaro, D., Droby, S., 2016. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. Trends Food Sci. Technol. 47, 39–49. https://doi.org/10.1016/j.tifs.2015.11.003.

Spadaro, D., Vola, R., Piano, S., Gullino, M.L., 2002. Mechanisms of action and efficacy of four isolates of the yeast *Metschnikowia pulcherrima* active against postharvest pathogens on apples. Postharvest Biol. Technol. 24, 123–134. https://doi.org/10.1016/S0925-5214(01)00172-7.

Swimm, A., Giver, C.R., DeFilipp, Z., Rangaraju, S., Sharma, A., Ulezko Antonova, A., Sonowal, R., Capaldo, C., Powell, D., Qayed, M., Kalman, D., Waller, E.K., 2018. Indoles derived from intestinal microbiota act via type I interferon signaling to limit graft-versus-host disease. Blood 132, 2506–2519. https://doi.org/10.1182/blood-2018-03-838193.

Takesako, K., Ikai, K., Haruna, F., 1991. Aureobasidins, new antifungal antibiotics. J. Antibiot. (Tokyo) 44, 919–924. https://doi.org/10.7164/antibiotics.44.919.

Tretter, L., Patocs, A., Chinopoulos, C., 2016. Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. Biochim. Biophys. Acta - Bioenerg. 1857, 1086–1101. https://doi.org/10.1016/j.bbabio.2016.03.012.

Tsitsigiannis, D.I., Keller, N.P., 2007. Oxylipins as developmental and host-fungal communication signals. Trends Microbiol. 15, 109–118. https://doi.org/10.1016/j.tim.2007.01.005.

Turska, M., Rutyna, R., Paluszkiewicz, M., Terlecka, P., Dobrowolski, A., Pelak, J., Turski, M.P., Muszyńska, B., Dabrowski, W., Kocki, T., Plech, T., 2019. Presence of kynurenic acid in alcoholic beverages – Is this good news, or bad news? Med. Hypotheses 122, 200–205. https://doi.org/10.1016/j.mehy.2018.11.003.

Vargas-Tah, A., Gosset, G., 2015. Production of cinnamic and p-hydroxycinnamic acids in engineered microbes. Front. Bioeng. Biotechnol. 3 https://doi.org/10.3389/fbioe.2015.00116.

Walker, A., Leroux, P., 2015. Management of resistance in grapevine gray mold in France. In: Ishii, I., Hollomon, D.W. (Eds.), Fungicide Resistance in Plant Pathogens. Springer. https://doi.org/10.1007/978-4-431-55642-8.

Wang, Y., Sun, Y., Wang, J., Zhou, M., Wang, M., Feng, J., 2019. Antifungal activity and action mechanism of the natural product cinnamic acid against *Sclerotinia sclerotiorum* 103, 944–950. https://doi.org/10.1094/PDIS-08-18-1355-RE.

Wang, Y., Qiao, Y., Zhang, M., Ma, Z., Xue, Y., Mi, Q., Wang, A., Feng, J., 2021. Potential value of small-molecule organic acids for the control of postharvest gray mold caused by *Botrytis cinerea*. Pestic. Biochem. Physiol. 177, 104884 https://doi.org/10.1016/j.pestbp.2021.104884.

Wang, J., Wang, W., Xiong, H., Song, D., Cao, X., 2020. Natural phenolic derivatives based on piperine scaffold as potential antifungal agents. BMC Chem. 14, 1–12. https://doi.org/10.1186/s13065-020-00676-4.

Wróbel-Kwiatkowska, M., Turski, W., Kocki, T., Rakicka-Pustułka, M., Rymowicz, W., 2020. An efficient method for production of kynurenic acid by *Yarrowia lipolytica*. Yeast 37, 541–547. https://doi.org/10.1002/yea.3469.

Wu, H.S., Liu, Y.D., Zhao, G.M., Chen, X.Q., Yang, X.N., Zhou, X.D., 2011. Succinic acid inhibited growth and pathogenicity of in vitro soil-borne fungus *Fusarium oxysporum* f. sp. niveum. Acta Agric. Scand. Sect. B Soil Plant Sci. 61, 404–409. https://doi.org/10.1080/09064710.2010.496737.

Yılmaz, C., Gökmen, V., 2019. Kinetic evaluation of the formation of tryptophan derivatives in the kynurenine pathway during wort fermentation using *Saccharomyces pastorianus* and *Saccharomyces cerevisiae*. Food Chem. 297 https://doi.org/10.1016/j.foodchem.2019.124975.

Yoo, J.A., Lim, Y.M., Yoon, M.H., 2016. Production and antifungal effect of 3-phenyllactic acid (PLA) by lactic acid bacteria. J. Appl. Biol. Chem. 59, 173–178. https://doi.org/10.3839/jabc.2016.032.

Zain, M.E., Awaad, A.S., Razak, A.A., Maitland, D.J., Khamis, N.E., Sakhawy, M.A., 2009. Secondary metabolites of *Aureobasidium pullulans* isolated from Egyptian soil and their biological activity. J. Appl. Sci. Res. 5, 1582–1591.

Zhang, H., Mahunu, G., Castoria, R., Yang, Q., Apaliya, M., 2018. Recent developments in the enhancement of some postharvest biocontrol agents with unconventional chemicals compounds. Trends Food Sci. Technol. 78, 180–187. https://doi.org/10.1016/j.tifs.2018.06.002.

Zhang, Z., Qin, G., Li, B., Tian, S., 2015. Effect of cinnamic acid for controlling gray mold on table grape and its possible mechanisms of action. Curr. Microbiol. 71, 396–402. https://doi.org/10.1007/S00284-015-0863-1.

Zhao, W., Wisniewski, M., Wang, W., Liu, J., Liu, Y., 2014. Heat-induced oxidative injury contributes to inhibition *of Botrytis cinerea* spore germination and growth. World J. Microbiol. Biotechnol. 30, 951–957. https://doi.org/10.1007/s11274-013-1513-z.

Zook, M., Hammerschmidt, R., 1997. Origin of the thiazole ring of camalexin, a phytoalexin from *Arabidopsis thaliana*. Plant Physiol. 113, 463–468. https://doi.org/10.1104/pp.113.2.463.

Zou, C.G., Xu, Y.F., Liu, W.J., Zhou, W., Tao, N., Tu, H.H., Huang, X.W., Yang, J.K., Zhang, K.Q., 2010. Expression of a serine protease gene prCIs up-regulated by oxidative stress in the fungus *Clonostachys rosea*: Implications for fungal survival. PLoS One 5, 1–8. https://doi.org/10.1371/journal.pone.0013386.