Patulin inhibition of specific apple microbiome members uncovers *Hanseniaspora uvarum* as a potential biocontrol agent

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ABSTRACT

Penicillium expansum is a major postharvest pathogen of apples, causing loss in fruits through tissue damage, as well as in apple products due to contamination with the mycotoxin patulin. During infections, patulin is a cultivar-dependent virulence factor that facilitates apple lesion development. Patulin also has characterized antimicrobial activity and is important for inhibiting other competitive phytopathogens, but the role of this inhibitory activity has not been investigated in the context of the apple microbiome. In our current study, we isolated 68 apple microbiota and characterized their susceptibility to *P. expansum* extracts. We found Gram-negative bacteria and Basidiomycete yeast to demonstrate largely patulin-specific growth

inhibition compared to Gram-positive and Ascomycete isolates. From co-cultures, we identified a *Hanseniaspora* and *Gluconobacter* pairing that reduced *P. expansum* biomass and found that *Hanseniaspora uvarum* alone is sufficient to reduce apple disease progression *in vivo*. We investigated possible mechanisms of *H. uvarum* biocontrol activity and found modest inhibition on apple puree plates, as well as a trend toward lower patulin levels at the wound site. Active biocontrol activity required live yeast, which also were effective in controlling *Botrytis cinerea* apple infections. Lastly, we explored the breadth of *H. uvarum* biocontrol activity with over 30 *H. uvarum* isolates and found consistent inhibition of *P. expansum* apple disease.

Keywords: *Penicillium expansum*, patulin, apple microbiome, biocontrol, *Hanseniaspora uvarum*

INTRODUCTION

Apples are an important crop grown globally with over 95 million tons produced worldwide in 2022, which represents a nearly 15% increase from five years prior (FAO). Nutritionally, they are an important source of vitamins and antioxidants, especially for children because they consume considerably more apple products than adults on average (Biedrzycka and Amarowicz 2008; O'Neil *et al*. 2015). Apple products extend beyond apple ciders, juices, sauces, and leathers, because apple juice is used as the base ingredient for many juice products. A particularly versatile aspect of apples is their storage capacity, allowing for long-term storage and long-distance exportation (Małachowska and Tomala 2023). Unfortunately, postharvest pathogens present a significant disease threat to stored apples by causing physical damage to fruits, as well as contaminating downstream products with mycotoxins (Patriarca 2019). Patulin is the most prominent mycotoxin found in postharvest spoiled apples and downstream products, including pasteurized and fermented products (Moake *et al*. 2005). The acute and chronic toxicity of patulin

has been demonstrated across many studies, which has prompted many nations to monitor patulin levels in apple products (Mahato *et al*. 2021).

Penicillium expansum is the postharvest pathogen responsible for the majority of patulin contamination in apples (Luciano-Rosario *et al*. 2020). Patulin, however, is not a prerequisite for successful *P. expansum* apple infections as patulin deletion mutants are still virulent (Ballester *et al*. 2015). Further investigation into the role of patulin in virulence concluded that it expedites lesion development in an apple cultivar-specific manner (Snini *et al*. 2016). Patulin is also well-described as an antimicrobial compound (Singh 1967; Wallen *et al*. 1980; Iwahashi *et al*. 2006; Vidal *et al*. 2019) and was once considered a possible treatment for the common cold (Report of the Patulin Clinical Trials Committee 2004). Bridging these activities of patulin, a recent report demonstrated that patulin inhibits spore germination of other fungal apple pathogens, thus putatively providing a fitness advantage to *P. expansum* during apple colonization (Bartholomew *et al*. 2022). Furthermore, a recent study of stored apples showed the microbiome of apples contaminated with patulin displayed very different bacterial and fungal communities than patulin-free apples (Al Riachy *et al*. 2024). Together, this suggests that a primary role of patulin during apple disease development is in mediating the composition of the apple microbiome.

The microbiome of host fruit is a promising source for effective biocontrol strains (Janisiewicz and Korsten 2002; Droby and Wisniewski 2018). For example, the bacterium *Pseudomonas syringae* and the yeast *Sporobolomyces roseus,* both isolated from fruit trees, were found to antagonize lesion development on apples by *P. expansum* (Janisiewicz and Bors 1995). Additionally, some species of antagonistic yeast and lactic acid bacteria decreased patulin contamination of apples inoculated with *P. expansum*, suggesting their potential usefulness as a management strategy (Hatab *et al*. 2012; Chen *et al*. 2017; Zheng *et al*. 2017). The objective of this study was to elucidate the ecological interactions between patulin producing and non-producing strains of *P. expansum* and the apple host microbiome. We isolated and identified

bacteria and yeast from the apple microbiome and examined their susceptibilities to *P. expansum* extracts. Trends from our studies showed that Gram-negative bacteria and Basidiomycete yeast were, in general, more sensitive to extracts from the patulin producing *P. expansum* strain than Gram-positive bacteria or Ascomycete yeast. We found that the Ascomycete yeast, *Hanseniaspora uvarum*, consistently inhibited lesion development on apples by *P. expansum* and a second fungal pathogen of apples, *Botrytis cinerea.* Antagonistic activity required living yeast rather than their secreted products. Furthermore, effective biocontrol properties were found in 32 additional isolates of *H. uvarum* collected from a broad range of environments, suggesting conserved endogenous biocontrol abilities of this species.

MATERIALS AND METHODS

Apple microbiota isolation

Nine apples were collected from three Wisconsin orchards, representing 8 cultivars total. Apple cultivar (cv.) 'Releika' was collected from West Madison Agricultural Research Station and is the only cultivar represented by two apples, with one taken from the tree (RelT) and the other taken from the ground (RelG). All other apple cultivars were only collected from the orchard floor to capture microbiota that *P. expansum* is likely to naturally encounter as infections on fruits still attached to trees is rare. Apple cv. 'Blushing Golden Delicious,' 'Golden Delicious,' 'Cortland,' 'Empire,' and 'Jonafree' were collected from an orchard near Cottage Grove, Wisconsin. Lastly, Apple cv. 'Blondee' and 'Honeycrisp' were collected from an orchard near Chilton, Wisconsin. Apple cores were removed, and the remaining tissue sliced into large pieces, which were placed in equal weight phosphate-buffer saline (PBS, pH 7.4) and homogenized using an immersion hand blender (Fisher brand™ 150 Handheld Homogenizer Motor, Pittsburgh, PA, USA). Homogenates were serially diluted in PBS and plated onto CPGY agar medium (0.1% casamino

acids, 1% peptone, 0.5% glucose and 0.1% yeast extract) and incubated for two days at 25°C. Individual colony forming units (CFUs) were selected and subcultured for five passages. Glycerol stocks (25% glycerol final) from liquid cultures of each apple microbe were made and stored at -80°C.

Maintenance of microbial strains

P. expansum Pe-21 is an apple isolate from Israel (Barad *et al*. 2014; Tannous *et al*. 2020) and is the background strain used throughout the study. The wild type strain used in this study is TJT14.1 (Δ*ku*70::six-site) (Tannous *et al*. 2018) and the in-cluster patulin transcription factor *patL* deletion strain is TJLE3.1 (Δ*ku70*::six-site, Δ*patL*::*βrec/hyg*). *P. expansum* strains, as well as apple-isolated *Botrytis cinerea* Bc8000 (Bartholomew *et al*. 2022), were inoculated from glycerol stocks on glucose minimal media (GMM) (Shimizu and Keller 2001) and incubated at 25°C for 5 days before spores were harvested using 0.01% (*v/v*) Tween-20®.

Apple microbiota (Table S1) were activated on CPGY plates from glycerol stocks. Liquid cultures for assays were inoculated from CPGY plates into liquid CPGY and incubated at 25°C at 225 rpm. After overnight incubation (approximately 18 hours), 1 ml was transferred to a 1.5 ml tube, centrifuged at 9000 *xg* for 2 minutes, and washed with 1 ml PBS. Other isolates of *H. uvarum* received from the Hittinger lab (UW-Madison) were maintained as glycerol stocks and cultured on CPGY identically to apple microbiota.

For both fungal pathogen spore suspensions and apple microbiota cell suspensions, cell concentrations were measured with a Cellometer® K2 (Nexcelom Bioscience LLC, Lawrence, Massachusetts, USA).

Apple microbiota amplicon sequencing

From overnight cultures, 1 ml of each isolate was subjected to a phenol-chloroform genomic DNA (gDNA) extraction. Each isolate was amplified using universal 16S primers: 27F

(5’- AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’- GGTTACCTTGTTACGACTT-3’) (Weisburg *et al*. 1991); as well as universal ITS region primers ITS1 (5’- TCCGTAGGTGAACCTGCGG-3’) and ITS4 (5’- TCCTCCGCTTATTGATATGC-3’) (White *et al*. 1990). Each PCR product was purified with a PCR clean-up kit (QIAquick® PCR purification kit, Qiagen, Hilden, Germany) and submitted for Sanger sequencing at UW-Madison Biotechnology Center. Sequence data were queried on NCBI BLASTN, and genus-level identification was assigned based on highest percent identity (Table S1). Isolates whose sequence data matched to multiple genera, suggesting either incomplete purification in the stocks or external DNA contamination, were omitted from further study.

We found *Hanseniaspora* and *Gluconobacter* spp. were co-isolated. Sequence data returned for isolate RelG19 contained both an ITS sequence that matched to *Hanseniaspora* and a 16S sequence that matched to *Gluconobacter*. We passaged this mixture five more times on CPGY amended with either tetracycline (15 µg/ml) or cycloheximide (100 µg/ml) to select for *Hanseniaspora* and *Gluconobacter*, respectively. We further sequenced these isolates using the ITS1 and NL4 (5’- GGTCCGTGTTTCAAGACGG-3’) amplicon to determine the D1-D2 region of the 18S gene (Mannarelli and Kurtzman 1998), which allowed us to identify our isolate as *H. uvarum*.

All sequence data are available through NCBI accession numbers: bacterial isolates’ 16S data under PP663190-PP663234, and yeast isolates’ ITS data under PP700265-PP700287 (Table S1).

Patulin biosynthesis deletion and secondary metabolite extractions

The patulin cluster-specific transcription factor gene *patL* was deleted via homologous recombination using a hygromycin B-resistance marker gene, and secondary metabolites were extracted as previously described (Dor *et al*. 2024). Briefly, wild type (TJT14.1) and Δ*patL*

(TJLE3.1) strains were extracted from point-inoculated potato dextrose agar (PDA) plates with ethyl acetate.

Apple microbiota challenge with *P. expansum* extracts

Purified apple microbiota were grown in the presence of extracts from *P. expansum* wild type or Δ*patL*, and the optical densities of extract-containing cultures were normalized to solvent control conditions as described previously (Dor *et al*. 2024). Briefly, each *P. expansum* strain was pointed inoculated with 10 µl containing 500,000 spores onto the center of 200 potato dextrose agar (PDA) plates. After two weeks of growth in the dark at 25°C, the media and fungus were removed from the plates, pooled into glass beakers per strain, and homogenized with ethyl acetate. Each extraction was repeated, and the extracts pooled for like strains. For antimicrobial assays, the extracts were dissolved in methanol and serially diluted in CPGY to determine a concentration that would yield quantifiable growth inhibition across the apple microbiota. We chose 125 µg/ml as a final concentration to assess our microbiome collection. Antimicrobial assays were carried out in 96-well plates, shaking at 25°C.

Co-cultures of *P. expansum* and apple microbiota

Apple microbiota were selected based on their co-isolation from apples (Table S1). One thousand *P. expansum* spores (10 µl of 10^5 spores/ml in 0.01% Tween-20 suspension) or 10 µl 0.01% Tween-20 (control) were inoculated into 3 ml of CPGY in triplicate within three different 12-well plates to correspond with each destructively-sampled timepoint. After 36 hours of incubation at 25°C, 1000 cells of each apple isolate (10 µl of each 10^5 cells/ml PBS suspension for 20 µl total) or 20 µl of PBS were added to each well.

After an additional 36 hours, the Day 3 plate was processed as follows: For *P. expansum* treatments, floating mycelial mats were transferred to a pre-weighed 1.5 ml tube and freeze-dried. After two days of lyophilization, the dry weight was measured. The remaining culture was

transferred to a 50 ml conical tube and vortexed before an aliquot was removed and mixed with glycerol for a final 25% glycerol stock of each culture. The remaining culture in the 50 ml conical was directly stored at -80°C. This process was repeated two days later for 5-day timepoints, followed by a subsequent two days for the 7-day timepoints.

To quantify the abundances of each apple microbiome member from the co-cultures, each glycerol stock was removed from -80°C to thaw on ice. Each sample was serially diluted in PBS and 10 µl of each dilution was spot plated onto two types of CPGY plates in triplicate: CPGY + tetracycline [15 µg/ml] and CPGY + cycloheximide [100 µg/ml] for yeast and bacterial CFU counts, respectively. Plates were incubated at 25°C until CFUs were countable.

To analyze the metabolome of the co-culture and quantify patulin concentrations, the 50 ml conical tubes of each culture were lyophilized and extracted twice with 15 ml methanol, filtered into a pre-weighed scintillation vial, and left to dry completely under a fume hood at room temperature. Dry weights were taken to calculate 1 mg/ml concentrations of each sample in methanol for running on ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). We analyzed the extracts using a Thermo Scientific Q Exactive Orbitrap mass spectrometer coupled to a Vanquish UHPLC operated in positive and negative ionization modes. A Waters XBridge BEH-C18 column (2.1 × 100 mm, 1.7 µm) was used with LCMS-grade acetonitrile+0.05% formic acid (organic phase) and water+0.05% formic acid (aqueous phase) as solvents at a flow rate of 0.2 ml/min. The screening gradient method for the samples was as follows: Starting at 10% organic for 5 min, followed by a linear increase to 90% organic over 20 min, another linear increase to 98% organic for 2 min, holding at 98% organic for 5 min, decreasing back to 10% organic for 3 min, and holding at 10% organic for the final 2 min, for a total of 37 min. A volume of 10 µl of each sample was injected into the system for analysis. Patulin peak intensity was measured in negative ionization mode at 153.0199 *m/z*, and co-

cultures with the *P. expansum* Δ*patL* strain confirmed the retention time for the peak analysis (Figure S1).

Apple infection experiments

Apple cv. 'Golden Delicious' purchased from a local market in Madison, WI were used for all experiments. The apples were surface sterilized with 2% bleach followed by a sterile ddH_2O rinse, then wounded at 2 mm depth with a sterile wooden toothpick. Wounds were in pairs around the equator of the apple, with a maximum of 12 wounds per apple (6 pairs). After allowing the wounds to dry in the biosafety cabinet for 15 minutes, they were injected with either 5 µl of PBS or 5 µl of a $2x10^6$ cells/ml apple microbe cell suspension (10,000 cells per wound). After an additional 15 minutes, wounds were injected with 5 µl of a $2x10^5$ spores/ml *P. expansum* spore suspension (1000 spores per wound). Apples were then placed in plastic tubs with closed lids and stored at 25°C throughout the experiment. Tubs contained approximately 200 ml of ddH_2O in a beaker to maintain humidity. Each apple represented a biological replicate, and each apple also received three wounds injected with PBS followed by *P. expansum* as a control per apple. By day 5, if the *P. expansum* only wounds failed to reach 5 mm in diameter, these apples were omitted from the replicates. Each biocontrol experiment quantified had at least three apple replicates, each with three wounds of each condition. The same treatment was performed with *B. cinerea* infections.

For the *Hanseniaspora* + *Gluconobacter* treated apples, the original RelG19 isolate was used as it contained both microbes. For the cell-free supernatant (CFS) treatment, a CPGY culture of *H. uvarum* was grown overnight, then a 1 ml aliquot was filtered through a 0.2 µm syringe filter. Finally, the heat-killed *H. uvarum* experiment was treated with 5 µl of a $2x10^6$ cells/ml *H. uvarum* suspension that had been incubated at 65°C for 12 minutes prior (non-viability confirmed by plating on CPGY).

For the other *H. uvarum* isolates (Table S2), the same procedure was carried out. Each experiment included the *H. uvarum* apple isolate as a control. Each isolate included in the study was tested at least twice, in triplicate.

In vitro H. uvarum and *P. expansum* interaction plate assays

For the confrontation experiments, 1000 *P. expansum* spores in 10 µl and/or 10,000 *H. uvarum* cells in 10 µl were inoculated 2 cm apart on CPGY, PDA and apple puree agar media (APAM) plates in triplicate. APAM plates were prepared with 'Golden Delicious' apples according to Baert *et al.* (Baert *et al*. 2007). Plates were incubated at 25°C for 7 days, then imaged, and the mycelial area was quantified on ImageJ (Schneider *et al*. 2012).

For the co-inoculation experiments, the three pairings consisted of either 5 µl of PBS + 1000 *P. expansum* spores in 5 µl; 10,000 *H. uvarum* cells in 5 µl + 1000 *P. expansum* spores in 5 µl; or 5 µl *H. uvarum* CFS + 1000 *P. expansum* spores in 5 µl. Each pairing was vortexed and inoculated into the center of CPGY and APAM plates, in triplicate. After 7 days at 25°C, images were taken, and mycelial area was quantified using ImageJ (Schneider *et al*. 2012).

Patulin and *P. expansum* burden quantification from apple infections

Three apples were wounded 12 times: 6 wounds were treated with PBS followed by 1000 *P. expansum* spores and 6 wounds treated with 10,000 *H. uvarum* cells followed by 1000 *P. expansum* spores. After 7 days of incubation at 25°C, lesions were excised in two different ways: 1) entire lesions were excised with a scalpel with approximately 1 mm margin outside the lesion; 2) core samples centered at the wound site (radius = 6 mm; height = 24 mm; volume ~ 2.71 ml). Wounds with the same treatment from the same apple were pooled into pre-weighed 50 ml conical tubes. A volume of PBS equal to the mass of the lesion tissue was added before homogenizing with an immersion hand blender (Fisher brand™ 150 Handheld Homogenizer Motor, Pittsburgh, PA, USA). The homogenate was then frozen at -80 °C overnight and then lyophilized. The dried

material was vortexed, and an equivalent mass was transferred to new 50 ml conical tubes for methanol extractions as described above.

For patulin detection, analytical reversed-phase high-performance liquid chromatography (HPLC) was performed using a Gilson 322 pump system and a Gilson Verity 1741 UV-VIS detector (Gilson, Middleton, WI, USA) equipped with an Eclipse Plus C18 column (5 µm, 19 × 250 mm) (Agilent, Santa Clara, CA, USA). A gradient solvent system using 0.1% formic acid in methanol (organic phase) and 0.1% formic acid water (aqueous phase) was run as follows: 5% to 10% organic for 15 min, followed by a linear increase to 100% organic for 5 min, 100% organic isocratic elution for 15 min, then a linear decrease to 5% organic for 1 min with a final 5% organic hold for 4 min, for a 40 min total. A patulin peak was detected at ~11.63 min of retention time (UV detection = 276 nm and flow rate = 1 ml/min). The patulin standard was prepared starting from 500 µg/mL to 1 mg/ml using HPLC-grade methanol. Area under the curve was recorded for each concentration to establish a patulin standard curve. The biocontrol sample extracts were dissolved to 10 mg/ml in HPLC-grade methanol and analyzed on the HPLC with 20 µl injection. The level of patulin detected in each sample was determined based on the standard curve.

Statistical and graphing methods

All statistical tests were performed on Prism® GraphPad (v. 10.2.2), and test specifics are listed in the figure legends. All graphs were also created using GraphPad except the UpsetPlot in Figure 1, which was graphed using SRplot, an online graphing tool (Tang *et al*. 2023).

RESULTS

Isolation and identification of apple microbiome members reveals culturable bacterial and yeast diversity. To test *P. expansum* interactions with ecologically relevant microorganisms, we purified bacterial and yeast isolates from eight apple cultivars collected from 3 Wisconsin

orchards. In total, 68 isolates were purified, and 16S rRNA or ITS amplicons were sequenced by Sanger sequencing, which resulted in the identification of 21 genera. Among the 45 bacterial isolates, *Rhodococcus* (22.22%) followed by *Gluconobacter* (15.56%) and *Curtobacterium* (13.33%), were the most dominant of the fourteen genera identified (Figure 1A). From the 23 yeast isolates, we identified *Metschnikowia* (30.43%), *Hanseniaspora* (30.43%) and *Pichia* (17.39%) as the top three most dominant of 7 genera identified (Figure 1B). 'Golden Delicious' harbored the most diverse culturable microbiome with nine genera identified, six of which were unique to this cultivar (Figure 1C). Of note, *Gluconobacter, Curtobacterium* and *Rhodococcus* were the only bacterial genera isolated from three different apple cultivars, and *Hanseniaspora* and *Pichia* were the only yeast genera isolated from multiple apple cultivars.

Figure 1. Isolation and identification of apple microbiome members reveals culturable bacterial and yeast diversity. A) Relative abundance of bacterial isolates (n = 45) categorized by genus identification. **B)** Relative abundance of yeast isolates (n = 23) categorized by genus identification. **C)** UpSet Plot with all isolates categorized by cultivar of origin. The left-, x-axis indicates total number of genera isolated from each respective apple cultivar, and the right-, y-axis indicates from which cultivar each genus was isolated by a black filled-in circle.

Patulin-specific inhibition of Gram-negative bacteria and Basidiomycete yeast by *P. expansum* extracts. To gain an initial view of how patulin could impact apple microbiome composition, growth of each of the 68 microbes was compared in extracts either from wild type (WT) *P. expansum* or in extracts from a patulin synthesis mutant (Δ*patL*). All Gram-negative bacterial isolates but only two Gram-positive isolates, *Curtobacterium* and *Rhodococcus,* appeared to be more susceptible to WT extracts than to Δ*patL* extracts (Figure 2A). Examination

of growth patterns of yeast showed that, in general, Basidiomycete yeast isolates were more sensitive to WT extracts than Ascomycete yeast isolates (Figure 2B).

Figure 2. Patulin-specific inhibition of Gram-negative bacteria and Basidiomycete yeast by *P. expansum* extracts. A) Inhibition of bacterial isolates divided into Gram-negative and Gram-positive. **B)** Inhibition of yeast isolates divided into Basidiomycetes and Ascomycetes. All isolates were exposed to 125 µg/ml *P. expansum* extract, as well as a solvent control. Percent inhibition calculated with OD_{600} as inhibition compared to solvent control treatments. Parenthetical values represent the number of isolates tested for each genus, with the individual data points showing the replicate values for each isolate. Two-way ANOVA followed by Šídák's multiple comparisons test was performed using GraphPad Prism version 10.2.2 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

***Hanseniaspora* and *Gluconobacter* apple isolates reduce *P. expansum* growth in co-culture.** While fungal extracts may indicate antibiotic activity of patulin, the biologically-relevant concentration during interactions with other microorganisms may be lower, thus it is important to investigate the role of these metabolites during direct interactions (Drott *et al*. 2019). We chose three bacteria and yeast pairs that were isolated from the same apple to create co-cultures with *P. expansum.* Overall, the apple microbiota were less abundant when co-cultured with *P. expansum* compared to the microbiota alone condition (Figure S2). We did detect patulin-specific inhibition of our *Asaia* isolate in co-culture (Figure S2F), but this did not hold true for our other Gram-negative *Gluconobacter* isolate (Figure S2B). Taken together with our extract data, these results suggest that patulin does have an inhibitory role, but other factors are certainly involved during interactions. When we focused on the impact of *P. expansum* during co-cultures,

Hanseniaspora and *Gluconobacter* isolates significantly reduced *P. expansum* biomass throughout the course of the experiment (Figure 3A). Co-cultures containing *Papiliotrema* and *Curtobacterium* isolates also reduced *P. expansum* biomass at day 3, but *P. expansum* caught up to monoculture biomass by the next timepoint (Figure 3B). *Pichia* and *Asaia* co-cultures failed to reduce *P. expansum* biomass (Figure 3C). From the same co-cultures, we assessed patulin concentrations and report the five-day timepoint that indicated significantly higher patulin in the *Papiliotrema* + *Curtobacterium* and *Pichia* + *Asaia* treatments (Figure 3D). In contrast, co-cultures with *Hanseniaspora* and *Gluconobacter* isolates did not reach patulin levels higher than the *P. expansum* monoculture conditions. These data suggest that the *Hanseniaspora* and *Gluconobacter* co-culture may be suppressing patulin accumulation, either through biosynthesis inhibition and/or degradation.

Figure 3. *Hanseniaspora* and *Gluconobacter* isolates significantly reduce *P. expansum* biomass in co-culture while suppressing patulin accumulation. *P. expansum* dry mycelial biomass after co-culturing with **A)** *Hanseniaspora* and *Gluconobacter* isolates, **B)** *Papiliotrema* and *Curtobacterium*, **C)** *Pichia* and *Asaia*. **D)** Signal intensity of patulin (153.0199 *m/z* in negative ionization mode on UHPLC-MS/MS) in monoculture (black) compared to co-cultures at five days. Multiple unpaired t tests (A-C) and a one-way ANOVA followed by Tukey's multiple comparison test (D) were performed using GraphPad Prism version 10.2.2 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

***Hanseniaspora* reduces *P. expansum* apple disease.** We hypothesized that one or both *Hanseniaspora* and *Gluconobacter* isolates could show biocontrol properties in controlling blue mold disease given their inhibitory phenotypes from the co-cultures. We directly tested biocontrol

activity in apples by treating wounds with both microbes, as well as individually. Both *Hanseniaspora* and *Gluconobacter*, either in mixed culture or alone, decreased lesion diameter by *P. expansum* (Figure 4A). Interestingly, *Hanseniaspora* alone when added to *P. expansum* infections significantly reduced lesion development to a greater extent than the co-culture or *Gluconobacter* alone treatment conditions (Figure 4B and C). Additional sequencing of the 18S gene of the *Hanseniaspora* isolate allowed us to identify it as *H. uvarum.*

Figure 4. *Hanseniaspora* isolate alone is sufficient to reduce *P. expansum*-mediated apple disease. A) Quantification of apple disease development on wounds with *P. expansum* versus with treatments of *Hanseniaspora* and *Gluconobacter* separately and together. **B)** Disease reduction percentages of co- or mono-treatments normalized to *P. expansum* only wounds. **C)** Representative apple infection images at day 7 post-inoculations. A two-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 10.2.2 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

Diffusible compounds are insufficient for *P. expansum* apple disease protection, requiring live *H. uvarum* cell treatment. Recently, it was reported that the biocontrol activity of a *Hanseniaspora osmophila* isolate against *Botrytis cinerea* and *P. expansum* on grapes is through inhibitory volatile compounds (Delgado *et al*. 2021). This *H. osmophila* isolate was also shown to inhibit phytopathogens, including *P. expansum*, through secreted compounds *in vitro* (Olivera *et al*. 2021). To determine if *H. uvarum* secretes compound(s) that contribute to *P. expansum* apple lesion reduction, lesion size was compared between treatments with *H. uvarum* and *H. uvarum* cell-free supernatants derived from *H. uvarum* grown in casamino acid-peptone-glucose-yeast (CPGY) media. Only intact *H. uvarum* caused lesion reduction (Figure 5A). Considering that *H.*

uvarum is likely to produce different metabolites when cultured on laboratory media compared to apples, we next carried out *in vitro* confrontation experiments in which *P. expansum* and *H. uvarum* were grown on two different lab media or on pureed apples. Only on apple puree agar media (APAM) was a reduction of *P. expansum* growth detected (Figure 5B), suggesting that apple-derived nutrients are important for *H. uvarum* biocontrol activity. To test the hypothesis that APAM plates are necessary for biocontrol activity *in vitro*, we tested conditions akin to the apple infection assays where we co-inoculated CPGY or APAM plates with *P. expansum* spores alone or mixed with either *H. uvarum* cells or cell-free supernatants from CPGY. We again observed significant *P. expansum* growth reductions only when cultured on APAM (Figure 5C). Together, our APAM results suggest that secreted compounds by *H. uvarum* impart only a modest inhibitory impact on *P. expansum* and require an apple-like environment. Lastly, we tested whether live *H. uvarum* yeast are necessary to inhibit *P. expansum* disease, and we found that heat-killed yeast were unable to reduce lesion development (Figure 5D). From these data we conclude that while modest biocontrol activity was observed on APAM plates, live *H. uvarum* cells are required to effectively protect apples from *P. expansum* infections.

Figure 5. Live *H. uvarum* is required to reduce *P. expansum* apple disease. A) *P. expansum* apple infections treated with cell-free supernatant (CFS). **B)** Confrontation assays with *P. expansum* with 2 cm apart inoculation of vehicle control (PBS) or *H. uvarum* on casamino acid-peptone-glucose-yeast extract (CPGY), potato dextrose agar (PDA) or apple puree agar media (APAM). **C)** *P. expansum* spores mixed with PBS, *H. uvarum* or *H. uvarum* CFS and co-inoculated on CPGY or APAM plates. **D)** *P. expansum* apple infections treated with either *H. uvarum* or heat-killed *H. uvarum*. All mycelial area measurements were seven days post inoculations. Two-way ANOVA followed by Dunnett's multiple comparisons test (A and D), multiple unpaired t tests (B) and a two-way ANOVA followed by Tukey's multiple comparisons test (C) were performed using

GraphPad Prism version 10.2.2 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

Patulin concentrations trend lower around the infection wound site. Given the importance of patulin for *P. expansum* virulence (Snini *et al*. 2016), we compared patulin levels of apples inoculated with *P. expansum* alone or co-inoculated with *H. uvarum*. We hypothesized *H. uvarum* would degrade patulin given the suppression of patulin accumulation we observed in our co-cultures (Figure 3D). When we excised the entirety of each lesion, normalized to equal dry mass of each and quantified patulin, no differences were observed (Figure 6A). From the same lesions, we also observed no difference in *P. expansum* burden in the wounds (Figure 6B). These data indicate *H. uvarum* does not reduce patulin contamination overall in apple infections. However, we were curious whether patulin degradation was occurring at the wound site from a mechanistic perspective. Thus, we extracted same-sized core samples from the lesions centered around the wound site and found patulin levels were still not different, although it appeared there was a trend towards more reduction in the *H. uvarum*-treated samples (Figure 6C). Core samples also showed equivalent *P. expansum* CFUs between treatments (Figure 6D). Together, these findings suggest that *H. uvarum* does not reduce patulin accumulation in the total lesion, but it may decrease patulin in the localized area of infection.

Figure 6. Patulin levels trend lower at core samples centered at wound site. A) Entire lesion of *P. expansum* alone or *P. expansum* with *H. uvarum* treatment excised and patulin levels quantified by high performance liquid chromatography (HPLC). **B)** *P. expansum* burden within the entire lesion samples. **C)** Core lesion samples for HPLC analysis of patulin. **D)** *P. expansum*

burden from core samples. Unpaired t tests were performed using GraphPad Prism version 10.2.2 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

***H. uvarum* isolates broadly reduce apple disease.** We hypothesized that our *H. uvarum* apple isolate was effective at reducing apple disease due to its isolation origin and that other *H. uvarum* isolates from different origins would be less effective. As such, we obtained 32 other isolates of *H. uvarum* originating from various source materials (Table S2) and assessed their abilities to reduce *P. expansum* apple disease (Figure 7A). All *H. uvarum* isolates reduced apple disease development and did not support the hypothesis that apple isolates would be more effective antagonists of an apple pathogen. We further tested whether *H. uvarum* isolates could reduce apple infections by the phytopathogen *Botrytis cinerea* and found that the yeast reduced lesion development by *B. cinerea* similarly to *P. expansum* (Figure 7B). These findings suggest that *H. uvarum* is generally capable of inhibiting fungal-mediated apple disease.

Figure 7. *H. uvarum* isolates broadly inhibit apple disease caused by *P. expansum* and *B. cinerea*. A) Experimental average day 7 reduction percentages of 32 *H. uvarum* isolates alongside our *H. uvarum* isolate, with source material of isolation listed on the y-axis. **B)** Disease reduction in *H. uvarum*-treated wounds compared to *P. expansum* and *B. cinerea* infections alone. A two-way ANOVA followed by Tukey's multiple comparison test was performed using GraphPad Prism version 10.2.2 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

DISCUSSION

Our study investigated culturable apple microbiota and their tolerances to *Penicillium expansum* extracts. We discovered that Gram-negative bacteria and Basidiomycete yeast isolates were sensitive to patulin. By moving into co-culture experiments, we found a *Hanseniaspora* and *Gluconobacter* pairing that reduced *P. expansum* biomass and suppressed patulin accumulation. Follow-up experiments using this *Hanseniaspora uvarum* isolate demonstrated that it alone was sufficient to reduce apple disease caused by *P. expansum* and *B. cinerea*. The biocontrol activity of *H. uvarum* was not specific to our isolate, nor did it appear to be primarily due to diffusible compound production. We found a trend toward patulin level decrease at the *H. uvarum*-treated wound site, so this may be one possible mechanism of action. However, patulin is known to not be required for successful *P. expansum* apple infections, and biocontrol of *B. cinerea* is independent of patulin reduction, thus we conclude *H. uvarum* is generally well-adapted to compete in the apple niche against these phytopathogens.

With the emergence of apple microbiome studies, there is potential to approach postharvest disease management within the context of pathogen-host microbiome interactions (Droby and Wisniewski 2018; Droby *et al*. 2022). Mycotoxin production is an important aspect of postharvest diseases, and there is a dearth of information on how these compounds impact host microbiomes. As such, we investigated *P. expansum* and culturable apple host microbiota interactions in the context of the mycotoxin patulin inhibition. In our apple microbiota collection, we found approximately 51% of bacterial isolates belonged to Proteobacteria, followed by ~47% belonging to Actinobacteria and ~2% Firmicutes. Metagenomic studies on apple microbiomes also report these phyla as the dominant bacterial taxa (Wassermann *et al*. 2019; Abdelfattah *et al*. 2021). Specifically, *Gluconobacter*, *Curtobacterium*, *Pantoea* and *Sphingomonas* are genera commonly associated with apple microbiomes (Wassermann *et al*. 2019; Abdelfattah *et al*. 2020; Al Riachy *et al*. 2021; Abdelfattah *et al*. 2021; Zhimo *et al*. 2022), which are the dominant genera isolated from multiple apple cultivars (Figure 1A and 1C). Of note, we found a dominance of

Rhodococcus that were isolated from three apple cultivars and composed ~22% of all bacterial isolates (Figure 1A and 1C). *Rhodococcus* has been reported in the apple microbiome (Abdelfattah *et al*. 2021), but it does not appear often or as the core microbiome in studies thus far, which indicates our selection process may have skewed toward culture conditions amenable to *Rhodococcus* purification. As for the yeast genera we isolated, Ascomycetes were dominant with *Metschnikowia*, *Hanseniaspora* and *Pichia* accounting for the majority of isolates (Figure 1B). The dominance of Ascomycota is supported by metagenomic studies on apple microbiome (Abdelfattah *et al*. 2020; Al Riachy *et al*. 2021; Abdelfattah *et al*. 2021; Zhimo *et al*. 2022). Although we only have three Basidiomycete isolates, the presence of these genera is also supported by the same studies. Further investigations into these Basidiomycete yeast may yield important information about apple microbiome stability and/or management practices. In fact, *Papiliotrema* species have been characterized as biocontrol agents against postharvest pathogens including *P. expansum* (Ianiri *et al*. 2024; Li *et al*. 2024). It should be noted that our apple microbiome sampling was narrower than other studies that utilize sequence information as we required pure cultures of each isolate. We also chose to sample a limited number of apples with a wider diversity of apple cultivar source, most of which were taken from orchard floors to represent a variety of microbes that *P. expansum* may naturally encounter as a postharvest pathogen.

One interesting observation from our study was that the apple microbiota displayed a striking pattern of Gram-negative bacteria and Basidiomycete yeast isolates exhibiting patulin-specific inhibition (Figure 2). Other secondary metabolites present in the Δ*patL* extract are still inhibitory to Gram-positive bacterial isolates, including roquefortine C (Kopp and Rehm 1979). The fact that Gram-negative bacterial isolates are significantly more tolerant when patulin is absent suggests that a selective pressure to detoxify patulin may exist. In fact, bacterial lactonases typically involved in degrading lactone quorum sensing molecules are capable of degrading patulin (Dor *et al*. 2021). Patulin also interferes with quorum sensing by competitively

binding to receptor proteins, thereby inhibiting biofilm production (Vijayababu *et al*. 2018). Therefore, there is at least pressure from both *P. expansum* and Gram-negative bacteria centered around patulin.

Our co-cultures of *P. expansum* and apple microbiota suggested the *Hanseniaspora* and *Gluconobacter* pairing did not induce patulin production as much as the other two co-culture treatments (Figure 3C). Patulin levels from this co-culture may be lower due to the decreased *P. expansum* biomass (Figure 3A). It is also possible that patulin production was equivalent to the other co-culture treatments, but *Hanseniaspora* and/or *Gluconobacter* were able to degrade patulin. *Hanseniaspora uvarum* has been shown to decrease *Aspergillus flavus* aflatoxin production (Tejero *et al*. 2021), as well as detoxify aflatoxin and ochratoxin (Gómez-Albarrán *et al*. 2021). *Gluconobacter oxydans* has also been shown to degrade patulin into less toxic ascladiol (Ricelli *et al*. 2007). We did not find features corresponding to the reported patulin degradation products such as ascladiol or deoxypatulinic acid (Liu *et al*. 2023) in UHPLC-MS/MS analysis of the co-cultures (data not shown); however, we note that our patulin signal intensities were low, and any smaller peaks relating to these byproducts would be difficult to detect with the experimental methods used in this work.

The inhibitory activity against *P. expansum* we detected during co-cultures translated into biocontrol activity during apple infections (Figure 4). We are not the first to document the ability of *Hanseniaspora* or *Gluconobacter* to inhibit *P. expansum*, which makes our co-isolation of these genera from the apple microbiome supportive of their co-residence in nature. Indeed, the pairing of *H. osmophila* and *G. cerinus* as a biocontrol consortium has been characterized as a strong inhibitor of *P. expansum* during grape disease development (Delgado *et al*. 2021). During *in vitro* studies, though, *H. osmophila* alone was the strongest inhibitor of *P. expansum* vegetative growth (Delgado *et al*. 2021; Olivera *et al*. 2021). These two previous studies demonstrated the biocontrol activity from their isolates to act through diffusible compounds, which was not the case for our *in*

vitro experiments aside from a modest effect on apple puree media (Figure 5). We then hypothesized that reducing patulin production may have been the mechanism of *H. uvarum* biocontrol activity, but our analysis from apple infections failed to indicate this as a mechanism, although core samples from around the wound site did suggest a trend to lower patulin levels (Figure 6C).

In conclusion, we purified and characterized an *H. uvarum* isolate that exhibited consistent inhibition of apple disease by *P. expansum* and *B. cinerea*. Extending this beyond our apple isolate, we found that all tested *H. uvarum* isolates were able to reduce *P. expansum* blue mold disease progression (Figure 7). These data suggest that *H. uvarum* is generally able to outcompete these phytopathogens in the apple wound niche, which may be due to nutrient and/or space limitation that has been shown to occur in several other biocontrol systems (Rabosto *et al*. 2006; Liu *et al*. 2013; Di Francesco *et al*. 2017). *Hanseniaspora* species are often isolated from fruits (Vadkertiová *et al*. 2012; van Wyk *et al*. 2024), especially grapes where they are involved in spontaneous fermentation (Bezerra-Bussoli *et al*. 2013; Medina *et al*. 2013; Grangeteau *et al*. 2015). *Hanseniaspora* species have also been identified previously as members of apple microbiomes (Abdelfattah *et al*. 2021; Vankova *et al*. 2021). Thus, they are adapted to growth within the fruit carposphere and can compete for nutrients with other microbes, including *P. expansum*. It may be that other mechanisms such as eliciting the apple immune response, as has been shown with the biocontrol *Yarrowia lipolytica* (Zhang *et al*. 2017), or modulating microbiome activity, as was the case with a *Trichoderma* biocontrol to treat *Fusarium* wilt disease (Tao *et al*. 2023), are contributing to *H. uvarum*-mediated apple disease inhibition as well. It is also possible that *H. uvarum* metabolite production could play a role in inhibiting *P. expansum* growth.

Overall, the goal of our study was to investigate the interactions between *P. expansum* and its host microbiota to identify potential regional biocontrol agents. In our screen, we also uncovered a possible specific impact of patulin to inhibit Gram-negative bacteria and

Basidiomycete yeast, a topic to explore in the future. These findings expand a view of patulin as a colonization factor for *P. expansum*, adding to the recent characterization of patulin as a competitive germination inhibitor of other phytopathogenic fungi (Bartholomew *et al*. 2022). Given the general toxicity of patulin (Singh 1967), it likely benefits the producing fungus in several aspects; however, the fact that non-plant pathogenic fungi also produce patulin (Draughon and Ayres 1980), evokes the question of what benefit a soil-dwelling fungus, for instance, may gain from this toxin. We posit that inhibition of neighboring Gram-negative bacteria or Basidiomycete yeast may be one benefit yet to be characterized. Investigating the microbiome of host fruit also opens new opportunities to understand important biological interactions between pathogenic fungi and their host microbiomes, which can provide insight into the best biocontrol selection and management strategies (Droby and Wisniewski 2018; Droby *et al*. 2022).

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Correction for: "Patulin Inhibition of Specific Apple Microbiome Members Uncovers *Hanseniaspora uvarum* as a Potential Biocontrol Agent"

In the article, "Patulin Inhibition of Specific Apple Microbiome Members Uncovers *Hanseniaspora uvarum* as a Potential Biocontrol Agent" by Eagan et al., the authors would like to amend the publication to add details about the *H. uvarum* isolates tested in Fig. 7A, citations, and two authors who suggested and designed the experiment in Fig. 7A.

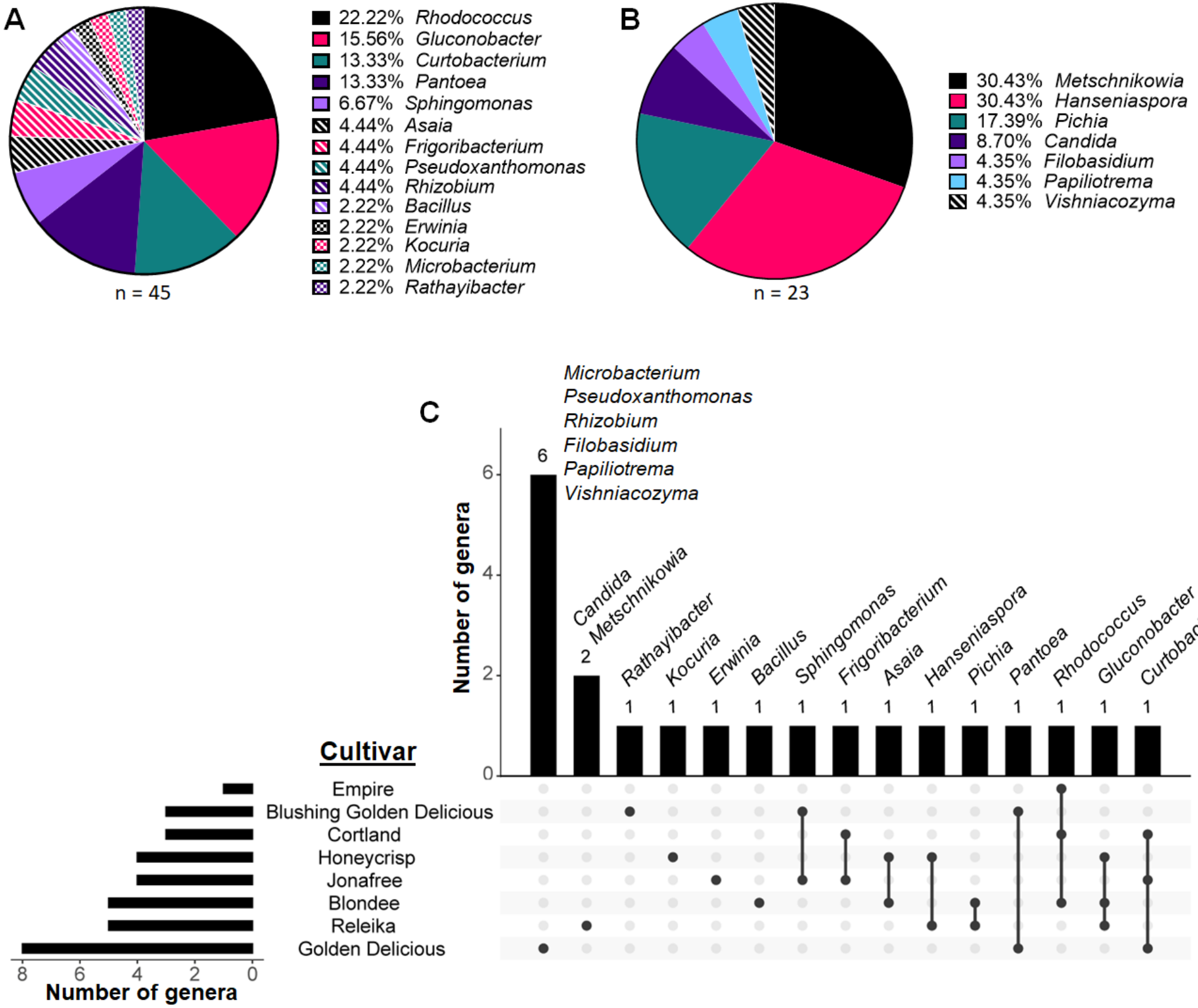
In the Materials and Methods, we stated that "Other isolates of *H. uvarum* received from the Hittinger lab (UW-Madison) were maintained as glycerol stocks and cultured on CPGY identically to apple microbiota." However, this section should be revised as:
"*H. uvarum* has been previously isolated from a variety of substrates using standard yeast enrichment methods (Spurley et al. 2022; Sylvester et al. 2015). Isolates for this study were specifically chosen to represent a range of environments that include fruits and the non-fruit environments of leaves, fungus, and soil. Supplementary Table S2 has been corrected to document strain sources. *H. uvarum* isolates were routinely maintained on yeast peptone dextrose (YPD), but prior to experiments, they were grown on CPGY identically to apple microbiota."

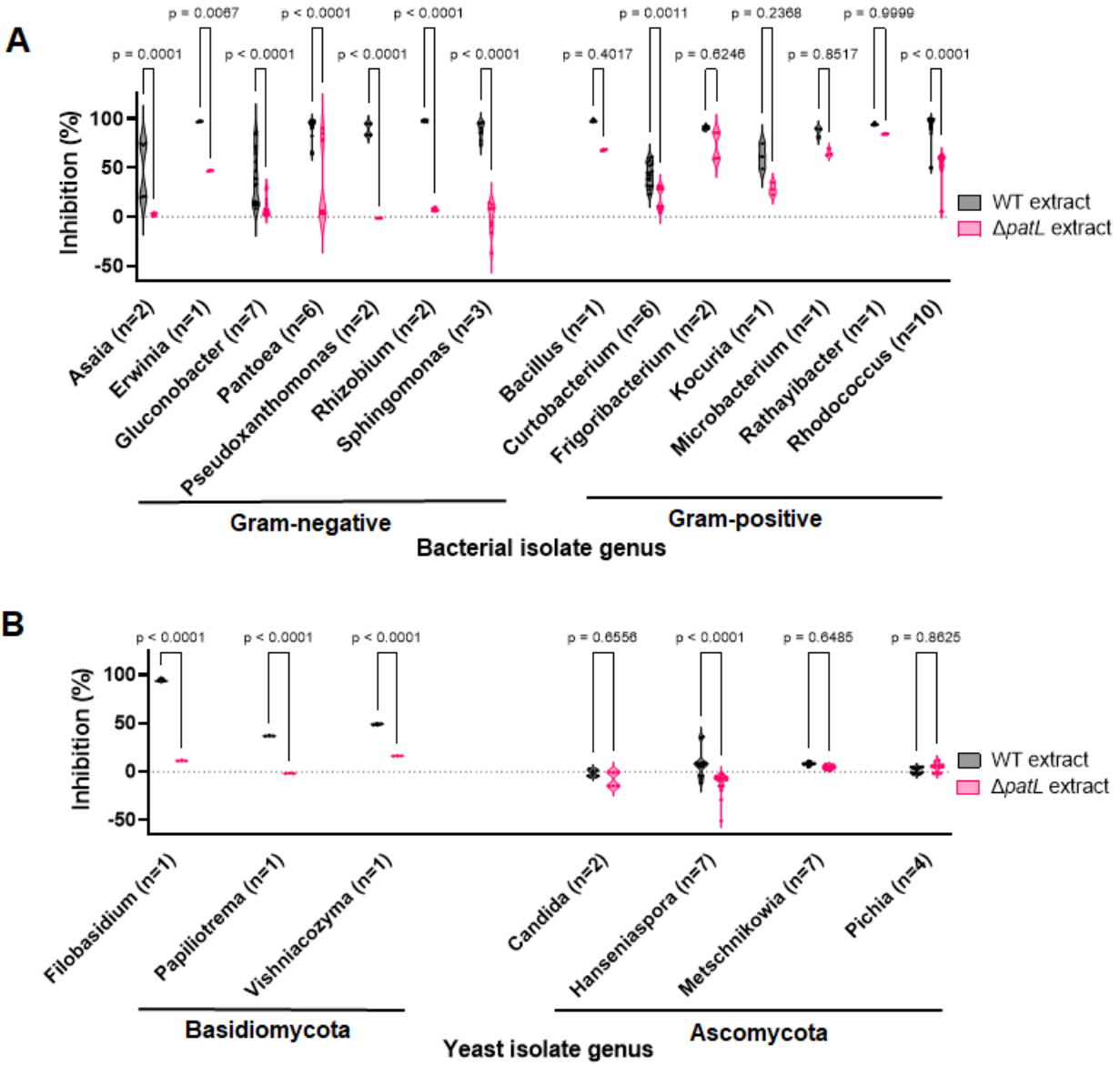
The revised author list should be, "Justin L. Eagan, Evan R. Digman, Martijn den Boon, Roberto Regalado, Mira S. A. Rawa, Linda C. Horianopoulos, Chris Todd Hittinger, Christina M. Hull, and Nancy P. Keller" with affiliations for L.C.H. and C.T.H. of "Laboratory of Genetics, DOE Great Lakes Bioenergy Research Center, J. F. Crow Institute for the Study of Evolution, Center for Genomic Science Innovation, Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, WI 53726, U.S.A.". The author contribution statement should be revised to add, "L.C.H and C.T.H. designed the experiments in Figure 7A by curating and selecting a set of yeast strains to test the hypothesis. All authors have approved the corrected manuscript." Funding should be revised to add, "Research in the Hittinger Lab is funded by the National Science Foundation (DEB-2110403), USDA National Institute of Food and Agriculture (Hatch Project 7005101), and in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE–SC0018409)." Accordingly, the corrected Acknowledgments should read, "We thank the growers and orchards that allowed us to enter their property and collect samples and Wayne Jurick for providing *B. cinerea* Bc8000."

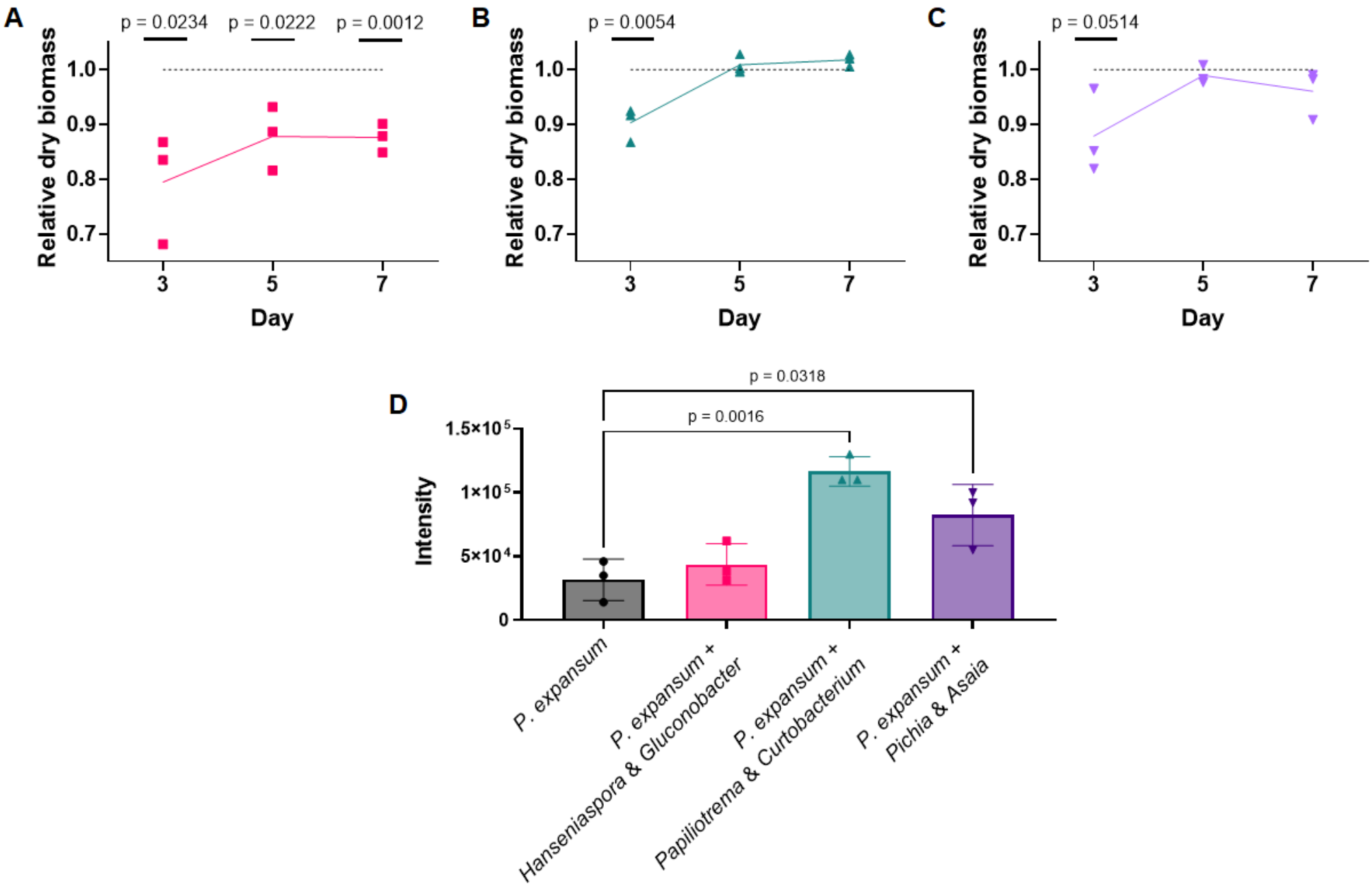
The Literature Cited should be modified to include two additional citations:

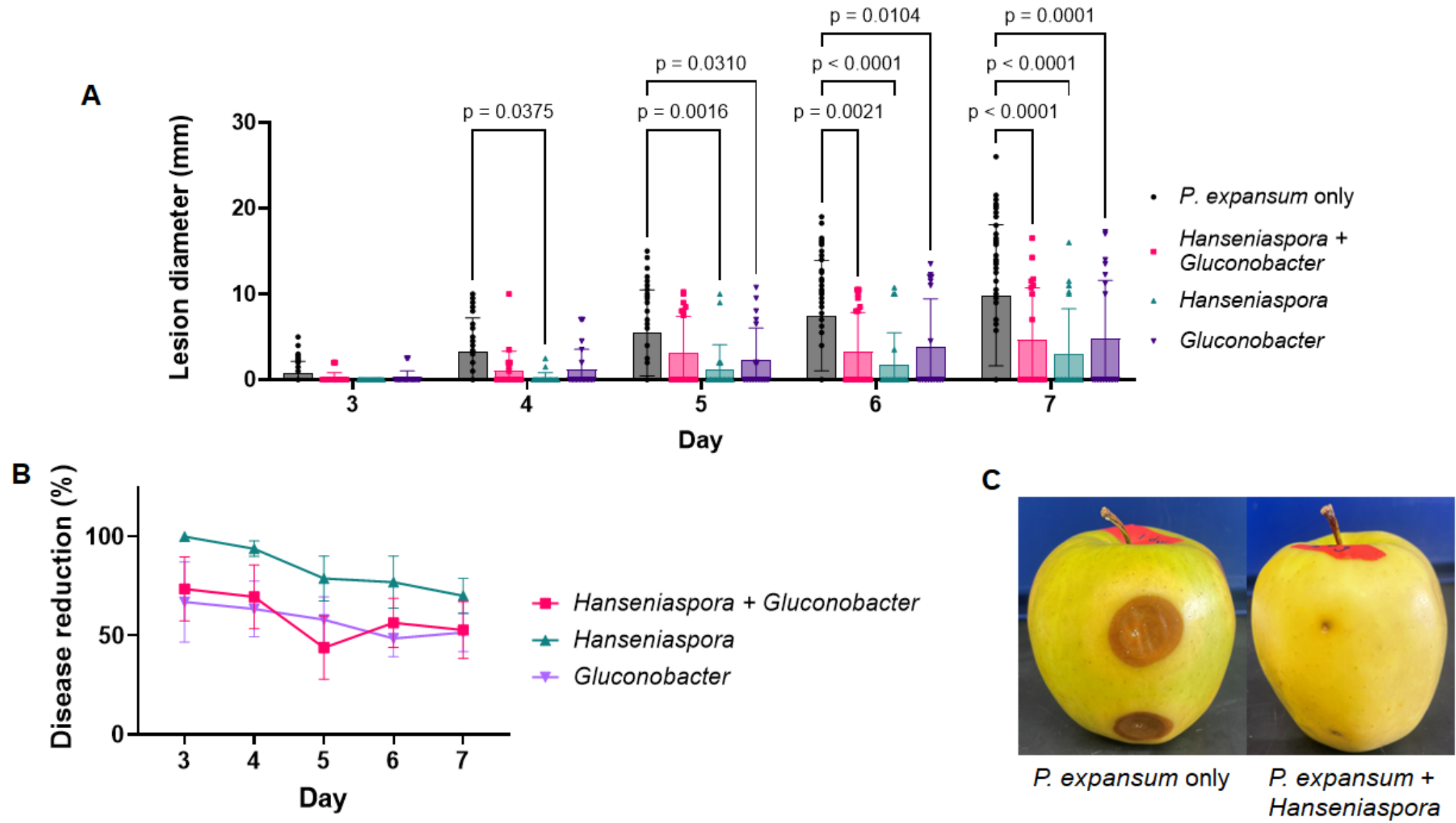
Spurley, W. J., Fisher, K. J., Langdon, Q. K., Buh, K. V., Jarzyna, M., Haase, M. A. B., Sylvester, K., Moriarty, R. V., Rodriguez, D., Sheddan, A., Wright, S., Sorlie, L., Hulfachor, A. B., Opulente, D. A., and Hittinger, C. T. 2022. Substrate, temperature, and geographical patterns among nearly 2000 natural yeast isolates. Yeast 39:55-68.

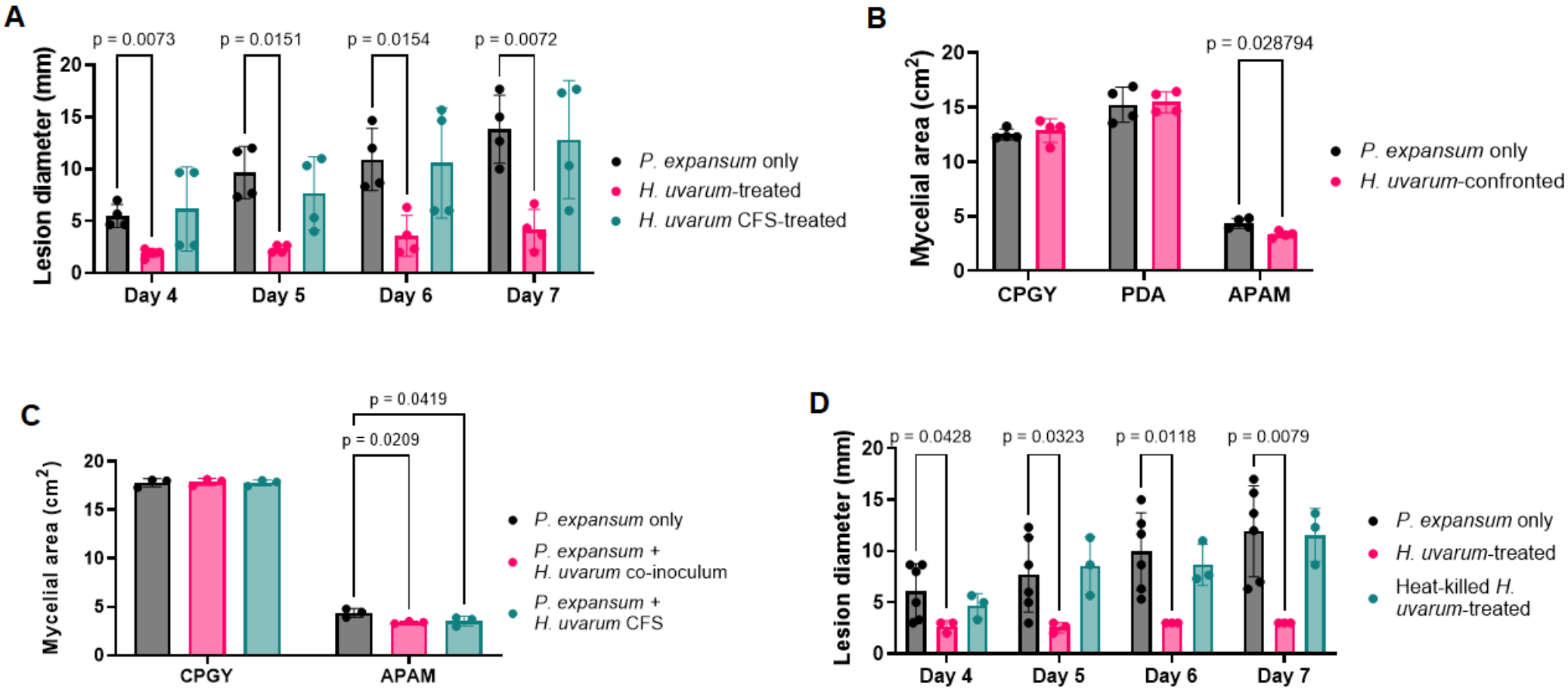
Sylvester, K., Wang, Q. M., James, B., Mendez, R., Hulfachor, A. B., and Hittinger, C. T. 2015. Temperature and host preferences drive the diversification of *Saccharomyces* and other yeasts: a survey and the discovery of eight new yeast species. FEMS Yeast Res 15:1–16.

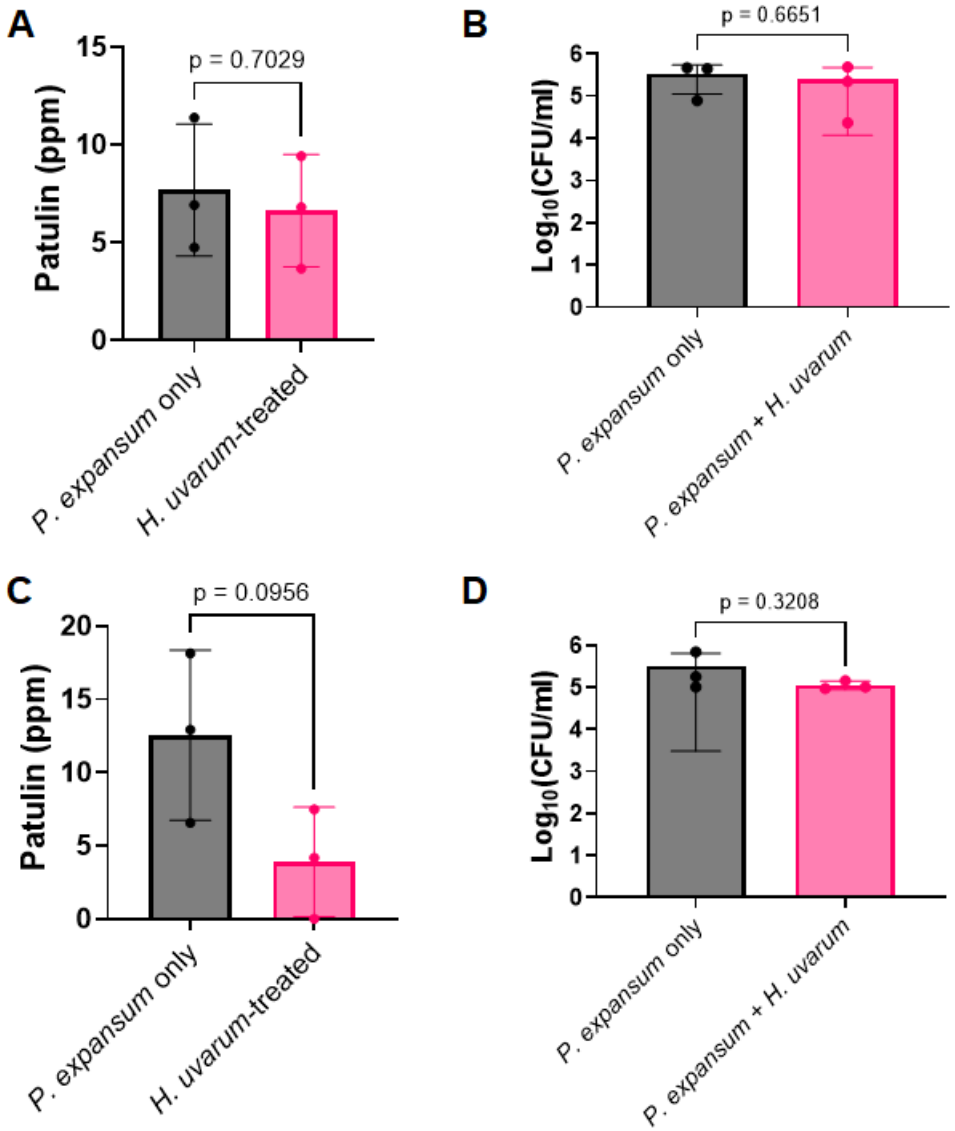

A
22.22% Rhodococcus
15.56% Gluconobacter
13.33% Curtobacterium
13.33% Pantoea
6.67% Sphingomonas
4.44% Asaia
4.44% Frigoribacterium
4.44% Pseudoxanthomonas
4.44% Rhizobium
2.22% Bacillus
2.22% Erwinia
2.22% Kocuria
2.22% Microbacterium
2.22% Rathayibacter
n = 45
B
30.43% Metschnikowia
30.43% Hanseniaspora
17.39% Pichia
8.70% Candida
4.35% Filobasidium
4.35% Papiliotrema
4.35% Vishniacozyma
n = 23
C
Microbacterium
Pseudoxanthomonas
Rhizobium
Filobasidium
Papiliotrema
Vishniacozyma
Number of genera
Candida
Metschnikowia
Rathayibacter
Kocuria
Erwinia
Bacillus
Sphingomonas
Frigoribacterium
Asaia
Hanseniaspora
Pichia
Pantoea
Rhodococcus
Gluconobacter
Curtobacterium
Cultivar
Empire
Blushing Golden Delicious
Cortland
Honeycrisp
Jonafree
Blondee
Releika
Golden Delicious
Number of genera

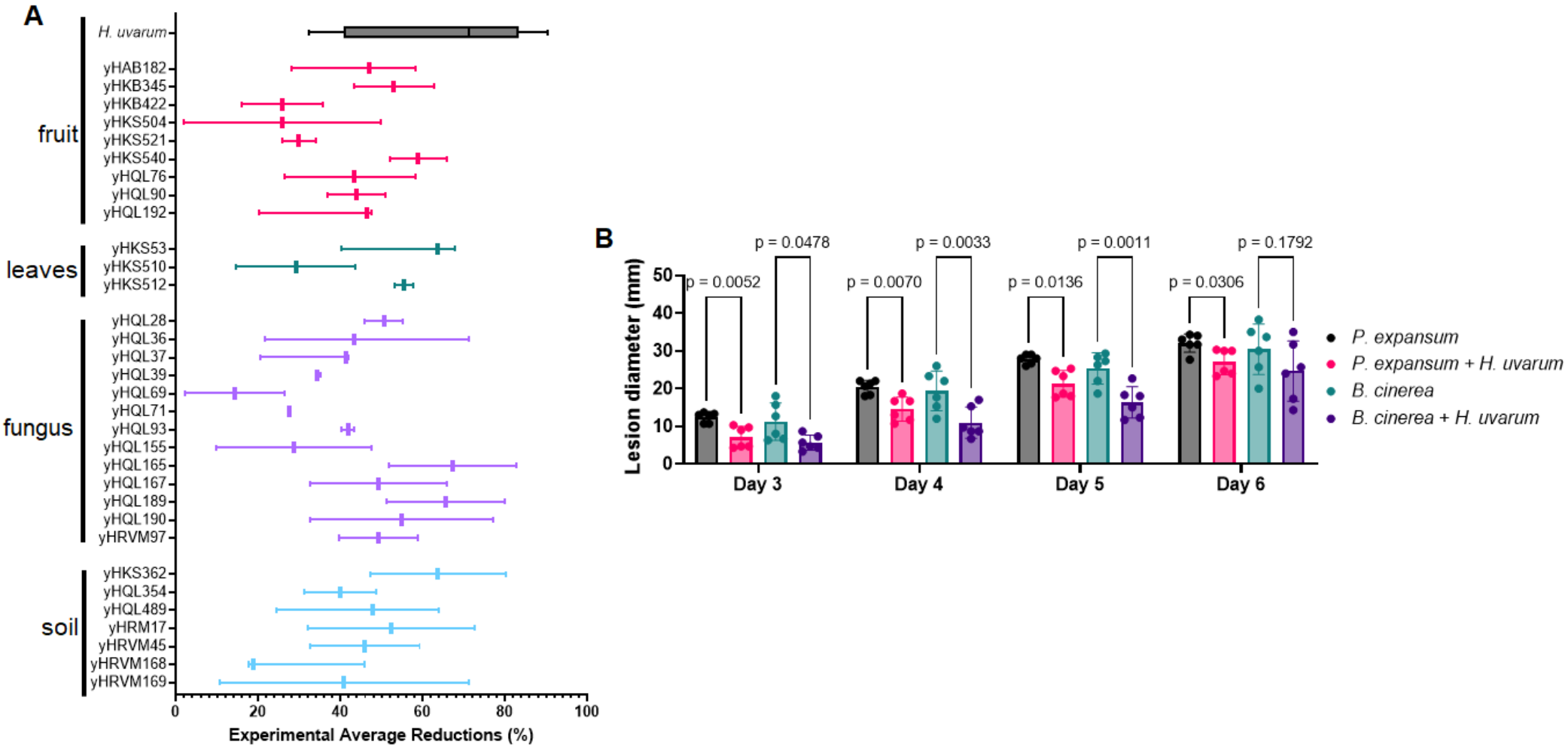

A
p = 0.0001
p = 0.0067
p < 0.0001
p < 0.0001
p < 0.0001
p < 0.0001
p = 0.4017
p = 0.0011
p = 0.6246
p = 0.2368
p = 0.8517
p = 0.9999
p < 0.0001
Inhibition (%)
100
50
0
-50
WT extract
ΔpatL extract
Asaia (n=2)
Erwinia (n=1)
Gluconobacter (n=7)
Pantoea (n=6)
Pseudoxanthomonas (n=2)
Rhizobium (n=2)
Sphingomonas (n=3)
Bacillus (n=1)
Curtobacterium (n=6)
Frigoribacterium (n=2)
Kocuria (n=1)
Microbacterium (n=1)
Rathayibacter (n=1)
Rhodococcus (n=10)
Gram-negative
Gram-positive
Bacterial isolate genus
B
p < 0.0001
p < 0.0001
p < 0.0001
p = 0.6556
p < 0.0001
p = 0.6485
p = 0.8625
Inhibition (%)
100
50
0
-50
WT extract
ΔpatL extract
Filobasidium (n=1)
Papiliotrema (n=1)
Vishniacozyma (n=1)
Candida (n=2)
Hanseniaspora (n=7)
Metschnikowia (n=7)
Pichia (n=4)
Basidiomycota
Ascomycota
Yeast isolate genus


A
p = 0.0234
p = 0.0222
p = 0.0012
Relative dry biomass
1.0
0.9
0.8
0.7
3
5
7
Day
B
p = 0.0054
Relative dry biomass
1.0
0.9
0.8
0.7
3
5
7
Day
C
p = 0.0514
Relative dry biomass
1.0
0.9
0.8
0.7
3
5
7
Day
D
p = 0.0318
p = 0.0016
Intensity
1.5×10^5
1×10^5
5×10^4
0
P. expansum
P. expansum + Hanseniaspora & Gluconobacter
P. expansum + Papiliotrema & Curtobacterium
P. expansum + Pichia & Asaia


A
Lesion diameter (mm)
30
20
10
0
p = 0.0375
p = 0.0310
p = 0.0016
p = 0.0104
p < 0.0001
p = 0.0021
p = 0.0001
p < 0.0001
p < 0.0001
3
4
5
6
7
Day
P. expansum only
Hanseniaspora + Gluconobacter
Hanseniaspora
Gluconobacter
B
Disease reduction (%)
100
50
0
3
4
5
6
7
Day
Hanseniaspora + Gluconobacter
Hanseniaspora
Gluconobacter
C
P. expansum only
P. expansum + Hanseniaspora


A
p = 0.0073
p = 0.0151
p = 0.0154
p = 0.0072
Lesion diameter (mm)
0
5
10
15
20
Day 4
Day 5
Day 6
Day 7
P. expansum only
H. uvarum-treated
H. uvarum CFS-treated
B
p = 0.028794
Mycelial area (cm2)
0
5
10
15
20
CPGY
PDA
APAM
P. expansum only
H. uvarum-confronted
C
p = 0.0419
p = 0.0209
Mycelial area (cm2)
0
5
10
15
20
CPGY
APAM
P. expansum only
P. expansum +
H. uvarum co-inoculum
P. expansum +
H. uvarum CFS
D
p = 0.0428
p = 0.0323
p = 0.0118
p = 0.0079
Lesion diameter (mm)
0
5
10
15
20
Day 4
Day 5
Day 6
Day 7
P. expansum only
H. uvarum-treated
Heat-killed H.
uvarum-treated


A
Patulin (ppm)
15
10
5
0
p = 0.7029
P. expansum only
H. uvarum-treated
B
Log10(CFU/ml)
6
5
4
3
2
1
0
p = 0.6651
P. expansum only
P. expansum + H. uvarum
C
Patulin (ppm)
20
15
10
5
0
p = 0.0956
P. expansum only
H. uvarum-treated
D
Log10(CFU/ml)
6
5
4
3
2
1
0
p = 0.3208
P. expansum only
P. expansum + H. uvarum


A
H. uvarum
yHAB182
yHKB345
yHKB422
yHKS504
yHKS521
yHKS540
yHQL76
yHQL90
yHQL192
fruit
yHKS53
yHKS510
yHKS512
leaves
yHQL28
yHQL36
yHQL37
yHQL39
yHQL69
yHQL71
yHQL93
yHQL155
yHQL165
yHQL167
yHQL189
yHQL190
yHRVM97
fungus
yHKS362
yHQL354
yHQL489
yHRM17
yHRVM45
yHRVM168
yHRVM169
soil
0
20
40
60
80
100
Experimental Average Reductions (%)
B
Lesion diameter (mm)
0
10
20
30
40
50
p = 0.0052
p = 0.0478
p = 0.0070
p = 0.0033
p = 0.0136
p = 0.0011
p = 0.0306
p = 0.1792
Day 3
Day 4
Day 5
Day 6
P. expansum
P. expansum + H. uvarum
B. cinerea
B. cinerea + H. uvarum