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ORIGINAL ARTICLE

From extreme environments to fruit management: the antifungal potential of extremophilic yeasts to control *Penicillium expansum* on apple

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Abstract

Naganishia albida and *Papiliotrema wisconsinensis* strains isolated from extreme environments were tested as biocontrol agents (BCAs) against *Penicillium expansum* on apples and as inhibitors of patulin production through the biosynthesis of secondary metabolites. Each strain showed different capabilities in reducing *P. expansum* growth in in vitro and in vivo assays through different mechanisms of action. In particular, *P. wisconsinensis* strains showed the highest inhibition against the pathogen by producing VOCs. The strains displayed the highest amount of the main detected VOCs: 1-butanol, 3-methyl, propanoic acid, 2-methyl, butanoic acid, and phenylethyl alcohol. All the strains showed the same efficacy against *P. expansum* by producing non-VOCs in vitro. Conversely, in the in vivo assay, the cell strain suspensions completely inhibited rot symptoms and consequently the patulin production, probably due to the interaction between competition for nutrients and the activation of defence responses in the fruit. Conversely, yeast VOCs act at a metabolic level to modulate mycotoxin biosynthesis. In contrast, cell strain suspensions that include non-VOCs achieve broader protection through more direct antagonistic interactions. By FT-IR, the non-VOCs were mainly ascribed to a proteinaceous fraction and exopolysaccharides. This metabolic characterization emphasizes the importance of selecting BCAs based not only on their ability to inhibit fungal growth, but also on their impact on mycotoxin production.

Keywords *Naganishia* spp. · *Papiliotrema* spp. · Secondary metabolites · Patulin · Apple blue mould

Introduction

Apple orchards in Italy cover approximately 50,000 hectares of land, producing an annual output of about 2.5 million tons (FAO 2021). Apple fruit is a product highly susceptible to fungal pathogens, especially during the postharvest phase, where these pathogens can significantly impact fruit quality. *Penicillium expansum* is an aggressive fungal pathogen that causes blue mould on pome fruits mainly during postharvest storage (Luciano-Rosario et al. 2020). This pathogen is known to produce toxic secondary metabolites, such as patulin and citrinin, which pose significant health risks to consumers (Tannous et al. 2018). Patulin is a lactone known to cause nausea, gastrointestinal injuries, kidney damage, and even cancerous tumour growth, along with genetic mutations and developmental defects in embryos (European Commission 2006; Puel et al. 2010). Due to these health risks, the European Union has set stringent regulations for maximum allowable patulin mycotoxin levels in apple products. The limits are 50 µg/L for fruit juices and derived products, 25 µg/L for solid apple products, and 10 µg/L for baby foods, as specified in European Regulation 1881/2006 and Regulation EU 915/2023. The potential risk of patulin contamination highlights the importance of managing fungal infections, particularly on apples, which are among the most widely produced crops worldwide (FAO 2021). However, susceptibility to fungal diseases and toxin accumulation can vary based on apple cultivar and storage conditions (Spadaro et al. 2013). Traditionally, chemical fungicides have been used to control *P. expansum*;

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however, this approach faces challenges due to increasingly restrictive laws in the EU and the reduced efficacy of some fungicides over time (Spadaro et al. 2013). Given these limitations, relying solely on chemical treatments is not a sustainable strategy. Therefore, exploring alternative solutions, such as biological control methods, becomes crucial in reducing fungal contamination and ensuring food safety. In this context, biocontrol agents (BCAs) could represent a sustainable solution to control fruit storage pathogens. In particular, cold-adapted yeasts, such as isolates belonging to the *Naganishia*, *Holtermanniella*, and *Papiliotrema* genera, which are resilient to cold and freezing temperatures as well as high heat conditions (Sangorrín et al. 2014; Tapia-Vázquez et al. 2020; De Garcia et al. 2012), could be considered for fruit postharvest management. These yeasts possess unique adaptations that make them suitable for use in cold environments. In fact, during evolution, yeasts have undergone various physiological, biochemical, and molecular modifications to survive in cold environments, including the synthesis of cold-active enzymes and cryoprotectants such as cold shock proteins, antifreeze proteins, sugars, and polyols (Buzzini et al. 2012, 2018).

For these reasons, in the present study, non-pathogenic *Naganishia* and *Papiliotrema* spp. strains, isolated during winter from the Swedish coast and Algerian desert, were preliminarily tested as potential BCAs against *P. expansum*, also by inhibiting patulin accumulation in apples.

The biocontrol potential of the strains was investigated using in vitro and in vivo assays. More in detail, the efficacy of secondary metabolites (volatile and non-volatile) produced by extremophilic yeast strains against blue mould was investigated. Both metabolites were characterized by Solid Phase Microextraction Gas Chromatography–Mass Spectrometry (SPME-GC-MS) and Fourier Transform Infrared Spectroscopy (FT-IR), respectively. Additionally, the effect of VOCs and non-VOCs on patulin accumulation in apples was evaluated.

Materials and methods

Yeasts

Sixteen psychotropic and thermotropic yeast strains (Table 1), belonging to the mycological collection of Di4A-University of Udine, were isolated from the Algerian Desert and the Swedish Coast during the winter season of 2023 (Lucci et al. 2025). Purified strains were stored in a 10% glycerol solution at -80 °C until use. The yeasts were cultured on NYDA medium (8 g/L nutrient broth, 5 g/L yeast extract, 10 g/L dextrose, and 25 g/L technical agar) (Oxoid, UK) for 2 days at 25 °C. For the following experiments, yeast cells were collected and suspended in sterile distilled water (SDW) containing 0.05% (v/v) Tween 20, and the suspensions were adjusted to 1×10^8 cells/mL using a hemocytometer.

Fungal pathogen and fruit

The pathogenic fungal strain *P. expansum* PAT27 was provided by Professor D. Spadaro, University of Turin (Spadaro et al. 2013). This strain was selected due to its high level of virulence on apple cultivars (Cignola et al. 2024) and ability to produce patulin (Spadaro et al. 2013). The fungal pathogen was cultured on Czapek Dox Agar (39 g/L) (Oxoid, UK) and PDA (Potato Dextrose Agar, 39 g/L) (Oxoid, UK) plates for 5 days at 20 °C. For the following experiments, fungal conidia were collected and suspended in SDW containing 0.05% (v/v) Tween 20, and the suspension was adjusted to 1×10^5 conidia/mL using a hemocytometer.

Table 1 *Naganishia, Holtermanniella, Papiliotrema* spp. strains: origin and source of sampling, sample ID, and species

Origin	Sample ID	Identification	Source
Algerian Desert	RDSH_1	*Naganishia albida*	Desert rose
	S2R(4)	*Holtermanniella takashimae*	Rocks
	SP6GRAM(3)	*Holtermanniella takashimae*	Straw
Sweden Coast	SYP2	*Naganishia adeliensis*	Grass
	SYP1	*Naganishia adeliensis*	Grass
	S1R(1)	*Naganishia adeliensis*	Rocks
	SYP(20)	*Naganishia adeliensis*	Grass
	S5BSC(13)	*Papiliotrema laurentii*	Moss
	S6P1(17)	*Papiliotrema aurea*	Grass
	S2R2	*Papiliotrema tephrensis*	Rocks
	S2R9	*Papiliotrema fonsecae*	Rocks
	S5SOIL(4)	*Papiliotrema laurentii*	Soil
	S1BSC2_1	*Papiliotrema wisconsinensis*	Moss
	SP6_4	*Papiliotrema wisconsinensis*	Straw
	S6P2_4	*Papiliotrema wisconsinensis*	Grass
FVG Region	PASS1	*Papiliotrema flavescens*	Flower

Apples cv '*Pink Lady*' were harvested at the commercial ripe stage (15 °Brix) in an orchard located in Friuli-Venezia Giulia region (46°01′56.4″ N, 13°13′23.6″ E). Fruits were selected to be homogeneous in size, without any visible damage, and immediately used.

Isolation and molecular characterization of yeasts

The microbial isolates were cultured in flasks containing NYDB (NYDA without agar). The cultures were centrifuged at 3500×*g* for 30 min, and the supernatant was discarded. The cells were then frozen using liquid nitrogen and stored at -80 °C. Genomic DNA was extracted using 150 mg of each sample collected in sterile 2 mL tubes, following the protocol described by Lodhi et al. (1994). To characterize the cultures, the Internal Transcribed Spacer (ITS) nucleotide region was initially examined to molecularly identify the strains. A second *locus*, the D1/D2 domains of the large subunit (LSU) rRNA, was analyzed. The ITS region was amplified using the primers ITS1 (5'-TCCGT AGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGC TTATTATTGATATGC-3'), as described by White et al. (1990). The LSU region was amplified with the primers LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-A TCCTGAGGGAAACTTC-3') (Vilgalys and Hester 1990). PCR amplifications were performed using a MiniAmp Plus thermal cycler (Thermo Fisher Scientific, USA). For the ITS region, the cycle started with an initial denaturation at 94 °C for 2 min, followed by 40 cycles at 94 °C for 40 s, 55 °C for 40 s, and 72 °C for 1 min, with a final extension of 5 min at 72 °C. PCR conditions for amplifying partial LSU rDNA differed from ITS conditions only in the annealing temperature (58 °C) and cycle extension time (90 s per cycle), as reported by Stielow et al. (2015). PCR products were purified and sequenced by BMR Genomics (Padova, Italy). The obtained ITS and LSU sequences were aligned separately using Clustal Omega implemented in SeaView v.5.0.5 (Gouy et al. 2010). The alignments were concatenated to build a distance-based phylogenetic tree, which was constructed using the Neighbor-Joining (NJ) method with the Jukes–Cantor model as the distance metric. Bootstrap analysis was performed with 100 replicates to assess node support. Related sequences and those of the closest species were retrieved from GenBank and aligned with those analysed in this study. All the sequence information was reported in Supplementary Table S1.

In vitro assays

Yeasts isolated from extreme environments were tested by in vitro assays against the mycotoxigenic fungal pathogen *P. expansum* PAT27. To select the best potential BCAs, a dual culture assay was conducted as reported by Cignola et al. (2024). A fungal mycelial plug (6 mm in diameter), previously grown on Czapek agar, was placed 30 mm from the edge of a Petri plate containing PDA. Yeast cells were streaked 30 mm from the opposite edge of the Petri dish. Plates inoculated only with mycelial plugs were used as controls. Three plates for each strain were prepared and incubated at 20 °C for 5 d, and the experiment was conducted twice. Results were expressed as the percentage of inhibition (%) of the fungal colony growth using the equation (Dennis and Webster 1971):

$$\text{Inhibition } (\%) = \frac{\text{d1} - \text{d2}}{\text{d1}} \times 100$$

where d1 was the control fungal colony diameter and d2 was the diameter of the treated colony.

VOCs efficacy against *P. expansum* by in vitro assay

The *Naganishia albida* strain RDSH_1 and the *Papiliotrema wisconsinensis* strains S6P2_4, SP6_4 and S1BSC2_1 displayed the highest percentage of inhibition against *P. expansum* in the dual assay, and were therefore selected to verify the potential antifungal activity of VOCs through the double Petri dish assay (Di Francesco et al. 2015). The microbial VOCs were tested against the mycelial growth of the pathogen colony. For the experiment, NYDA plates were inoculated by spreading 100 µL of the antagonist cell suspension (10^8 cells/mL), as described in Di Francesco et al. (2017). After 48 h of incubation at 25 °C, the lid of each plate was replaced with the base of a PDA plate inoculated with a 6 mm diameter pathogen mycelial plug, previously grown on Czapek agar. The plates were sealed with a double layer of Parafilm and incubated at 20 °C for 5 d. Each sample consisted of three plates (replicates), with (treatment) or without (control) the antagonist. The experiment was conducted twice, and the colony diameter was measured using a digital caliper. The percentage of inhibition with respect to the control was calculated as reported above.

Non-VOCs efficacy against *P. expansum* by in vitro assay

The effectiveness of non-VOC compounds was evaluated in relation to the strains that displayed the highest percentage of inhibition against *P. expansum* in the dual assay, as previously reported. The non-VOCs assay was carried out using sterile cellophane layers (BIORAD, USA) positioned on PDA plates, on which 100 µL of each yeast suspension (1×10^8 cells/mL) was spread. Plates were incubated at 25 °C, and after 48 h, the cellophane layer was removed. A mycelial plug (6 mm in diameter) from a 7-day-old colony of *P. expansum* PAT27, grown on Czapek agar, was placed in the center of the plate (Di Francesco et al. 2020). Plates were incubated at 20 °C for 5 d. The sample unit consisted of three plates. The control was represented by plates inoculated with sterile water. The fungal colony diameter was measured using a digital caliper, and the experiment was conducted twice. The percentage of inhibition relative to the control was calculated as reported in paragraph 2.4.

VOCs analysis by SPME-GC-MS

Yeast VOCs production was analyzed using the method described by Di Francesco et al. (2024). Ten mL of NYDA were transferred into sterile 25 mL vials. The substrate was then inoculated with a suspension of each selected yeast strain (1×10^8 cells/mL). The vials were incubated at 25 °C for 2 d. After incubation, the VOCs were extracted and analyzed by a gas chromatograph (7890B Agilent Technologies, Santa Clara, USA) coupled with a mass spectrometer detector (5977 A, Agilent Technologies, Santa Clara, USA) equipped with an autosampler for SPME automatic injections (PAL RSI 85, CTC). A SPME fiber (DVB/CAR/PDMS) was selected and conditioned before use. The vials were heated at 40 °C for 5 min, then the fiber was exposed for 30 min at the same temperature. For the desorption of volatiles, the fiber was introduced into the injector at 250 °C for 5 min. Separation of the analytes was performed on a silica capillary column with an internal diameter of 0.50 mm and a polar stationary phase (DB-WAX, Agilent Technologies, Santa Clara, USA) featuring a film of 0.5 µm thickness. The GC oven temperature program was as follows: 40 °C for 3 min, increased to 220 °C at a constant rate of 4 °C min^{-1}; held at 220 °C for 1 min, increased to 250 °C at 10 °C min^{-1}; and held for 1 min. Helium served as the carrier gas with a constant column flow rate of 1.5 mL min^{-1} (Tulukoglu-Kunt et al. 2024). The transfer line temperature was kept constant at 250 °C. The ion source and quadrupole were adjusted to 230 °C and 150 °C, respectively. Upon exiting the column, compounds were ionized via electron impact at 70 eV and detected by a quadrupole mass spectrometer over a mass/charge ratio (m/z) range from 35 to 500. Chromatograms were processed and analyzed using Mass Hunter (Agilent, USA). Compound identification was performed by comparing the spectra with the NIST Standard Reference Database (NIST2014) and by calculating linear retention indices (LRI) under the same chromatographic conditions, injecting a C7–C30 n-alkane series (Supelco, Milan, Italy). VOCs content was reported as absolute peak area (Aprea et al. 2012). The sample unit comprised three replicates. The control consisted of vials with non-inoculated NYDA. The experiment was conducted once.

Non-VOCs analysis by FT-IR

Flasks containing 100 mL of NYDB medium (NYDA without agar) were inoculated with antagonist cell suspensions (1×10^8 cells/mL) and then incubated at 25 °C in a rotary shaker (250 rpm) for 48 h. After the incubation period, the liquid cultures were centrifuged at 5000×*g* for 20 min at 15 °C, and the supernatant was collected in sterile 2 mL tubes and stored at -20 °C. The control consisted of a growth medium without yeast inoculation. The supernatant was lyophilized and analyzed by FT-IR (Fourier Transform Infrared Spectroscopy) to obtain a rapid, non-destructive characterization of its main molecular components. Infrared spectra were recorded using a Bruker ALPHA series FT-IR spectrophotometer (Bruker, Ettlingen, Germany) equipped with a device for attenuated total reflectance (Diamond crystal). The spectra were collected from 4000 to 400 cm^{-1} and averaged over 100 scans (resolution = 4 cm^{-1}). Four spectra were measured for each sample at each sampling time.

In vivo assays

For the in vivo experiment, the two best strains for each in vitro experiment (VOCs and non-VOCs) were selected. To verify the VOCs' efficacy against blue mould, *P. wisconsinensis* strains SP6_4 and S1BSC2_1 were selected. One hundred mL of NYDA substrate was placed at the bottom of a sterile plastic box (26 × 16 × 8 cm, L×W×H. After drying, 500 µL of each strain suspension (1×10^8 cells/mL) was spread. The boxes were hermetically closed by Parafilm® and incubated at 25 °C. After 48 h, *Pink Lady* apples, previously surface sterilized with sodium hypochlorite (0.1% v/v) and rinsed with distilled water, were wounded (one wound per fruit) with a sterile needle (2 mm × 2 mm × 2 mm) and inoculated with 15 µL of *P. expansum* PAT27 conidial suspension (1×10^5 conidia/mL). After drying (1 h), 6 apples were placed on a sterile grid inside each previously inoculated box. Three boxes were prepared for each yeast strain. Fruits were incubated at 20 °C for 5 d at 80% R.H, after which pathogen incidence (%) and severity (mm) were measured. Apples not exposed to VOCs fumigation represented the control. The experiment was conducted twice.

The strains *N. albida* RDSH_1 and *P. wisconsinensis* S6P2_4 were selected based on their in vitro non-VOCs performance. Apples were prepared as described above. For this assay, fruits were artificially wounded at the equatorial part by using a sterile nail and inoculated with 15 µL of the yeast strain suspension (1×10^8 cells/mL). After drying (1 h), 20 µL of *P. expansum* PAT27 conidial suspension (1×10^5 conidia/mL) was inoculated into the same wound. Fruits were then incubated at 20 °C at 80% R.H. for 5 d, and the lesion diameter (mm) was measured. Apples inoculated with SDW, instead of yeast suspension, represented the control. The sample unit consisted of 10 apples per replicate (3) for each yeast. The experiment was conducted twice.

Penicillium expansum DNA quantification and patulin content in apples

The total fungal genomic DNA was extracted from lyophilized *Pink Lady* apple tissue (150 mg) using the protocol described by Lodhi et al. (1994). The DNA was extracted from apples treated with VOCs produced by *P. wisconsinensis* S6P(4) and S1BSC2(1), and from apples treated with non-VOCs produced by *N. albida* RDSH_1 and *P. wisconsinensis* S6P2_4, as well as from the respective untreated controls. Rotten fruit tissues were collected from the apples prepared for the experiment described in paragraph 2.7, after the incidence and severity measurements. Samples were collected using a sterile cork-borer (15 mm diameter and 10 mm in depth) at the site of the inoculation wound. *Penicillium expansum* PAT27 DNA was quantified using qPCR. PCR reaction mixtures contained 0.3 µM of each specific primer PatF-F and PatF-R (Tannous et al. 2015), 1× SsoFast™ EvaGreen® Supermix (Bio-Rad Inc, Hercules, CA, USA), molecular grade H_2O, and 2 µL of DNA (20 ng/µL). Cycling conditions were as follows: initial denaturation at 95 °C for 3 min; 40 cycles of 20 s at 95 °C; 20 s at 65 °C. In all positive samples, the amount of *P. expansum* DNA was reported as pg of *P. expansum* DNA/mg of apple tissue to normalize the data. To quantify pathogen DNA, a standard curve was prepared with 1:10 serial dilutions of fungal genomic DNA extracted from a pure culture of the PAT27 strain (quantified by using Qubit® 2.0 Fluorimeter). PCR mixtures and cycling conditions were performed as described above. The amount of fluorescence for each sample was measured at the end of each cycle and analyzed via CFX-Manager Software v. 2.0 (Bio-Rad Laboratories, Inc, Hercules, CA, USA). Each sample dilution and each control standard were replicated three times. The experiment was conducted twice.

Patulin was extracted and analyzed according to the AOAC 2000.02 methodology (AOAC 2002). One hundred and fifty grams of apple tissue from each experimental condition (Sect. 2.7), were collected as circular portions (20 mm diameter and 10 mm depth), at the artificial wound-inoculation sites. Collected tissues were stored at -20 °C and analyzed by Eurofins Laboratoire Contaminants Alimentaires in Nantes (France).

Statistical analysis

Data from all experiments were analyzed using one-way analysis of variance (ANOVA). Statistical comparison of the means was carried out using the Tukey test ($\alpha < 0.01$ and 0.05). All analyses were performed with the software MiniTab.16 (Minitab, Inc.).

Results

Isolation and molecular characterization of yeast strains

One hundred and seventeen colonies were morphologically identified as yeasts using an optical microscope and then selected and purified. All the purified cultures were characterized using molecular methods. The amplification of the ITS region yielded sequences of 620 nucleotides corresponding to *Papiliotrema* spp. for 9 of the isolates and of 500 nucleotides, corresponding to *Holtermanniella* spp., for 2 isolates. Sequences of 580 nucleotides corresponded to *Naganishia* spp. for 5 of the isolated strains. The remaining 101 strains belonged mainly to *Aureobasidium* (57%), *Vishniacozyma* (7%), and *Cystobasidium (*4%) (data not shown).

The strains were also analyzed using an additional target gene, LSU. Due to the variability of this *locus*, the sixteen strains were identified as reported in Table 1. The phylogenetic tree was constructed using the concatenated alignment of ITS and LSU loci, confirming that all isolated strains were closely related to the genera *Papiliotrema*, *Naganishia*, and *Holtermanniella* (Fig. 1), with each strain assigned to different species: respectively *P. laurentii, P. aurea, P. tephrensis, P. fonsecae, P. wisconsinensis*, and *P. flavescens*, *N. albida*, *N. adeliensis* and *Holtermanniella takashimae.*

In vitro assay: dual culture

Selected yeast strains were evaluated through various assays to determine their potential as BCAs. In the dual culture assay, all selected strains showed an average mycelial growth inhibition of *P. expansum* of 15%.

The strains that displayed the highest inhibition of PAT27 were *P. wisconsinensis* strains S6P2_4, SP6_4, and *N. albida* strain RDSH_1, with an average inhibition rate of 25% (Fig. 2).

In vitro efficacy of VOCs against *P. expansum*

The experiment was conducted to verify the efficacy of VOCs produced by the strains RDSH_1, S1BSC2_1, S6P2_4, and SP6_4, which showed the highest inhibition against *P. expansum* in the dual assay. Regarding VOCs production, Fig. 3 shows different antifungal effects depending on the microorganism. The strains S1BSC2_1 and SP6_4 were the most effective against the *P. expansum* PAT27. In

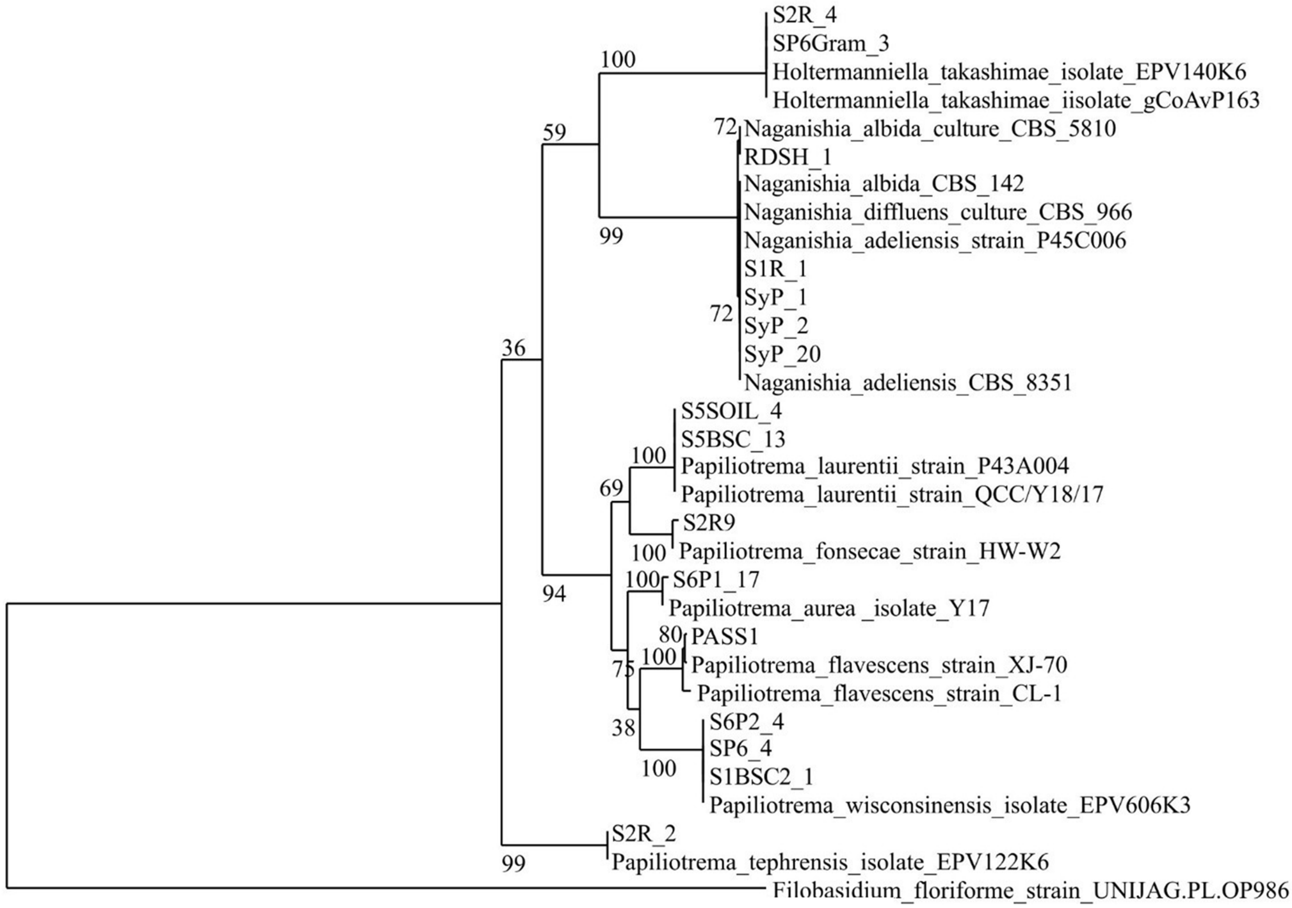


Fig. 1 Neighbor-Joining distance-based tree based on concatenated ITS and LSU rDNA sequences. Bootstrap values (based on 100 replicates) are shown at branch nodes. The tree was rooted with *Filobasidium floriforme* as an outgroup

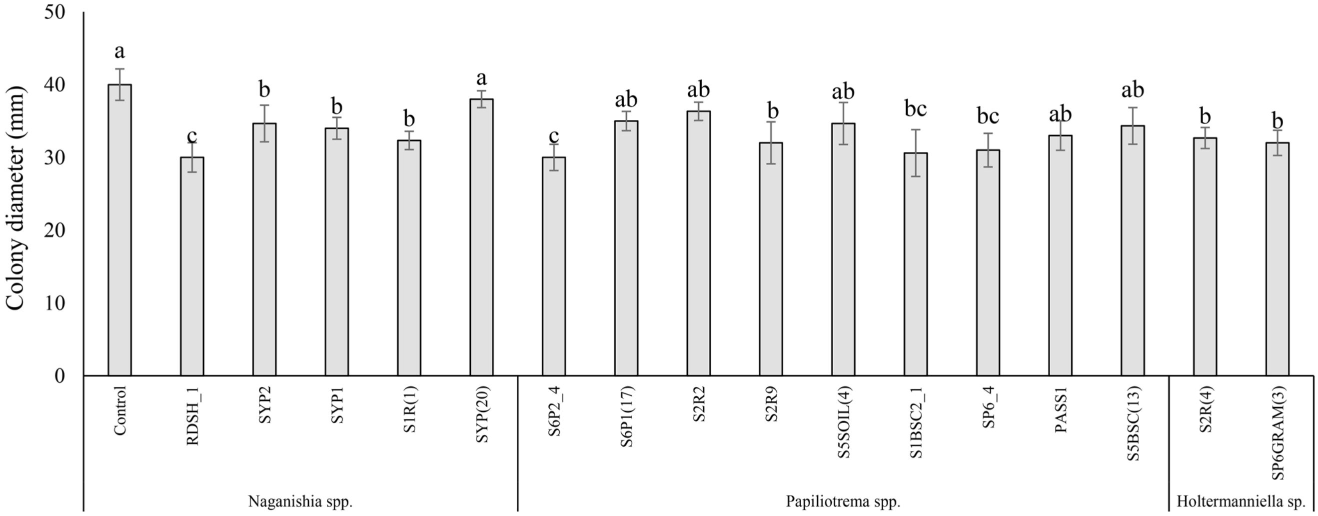


Fig. 2 Colony diameter of *Penicillium expansum* PAT27 in co-culture assay with the selected sixteen yeast strains belonging to *Naganishia, Papiliotrema* and *Holtermanniella* genera. Different letters indicate significant differences according to Tukey test (α < 0.01)

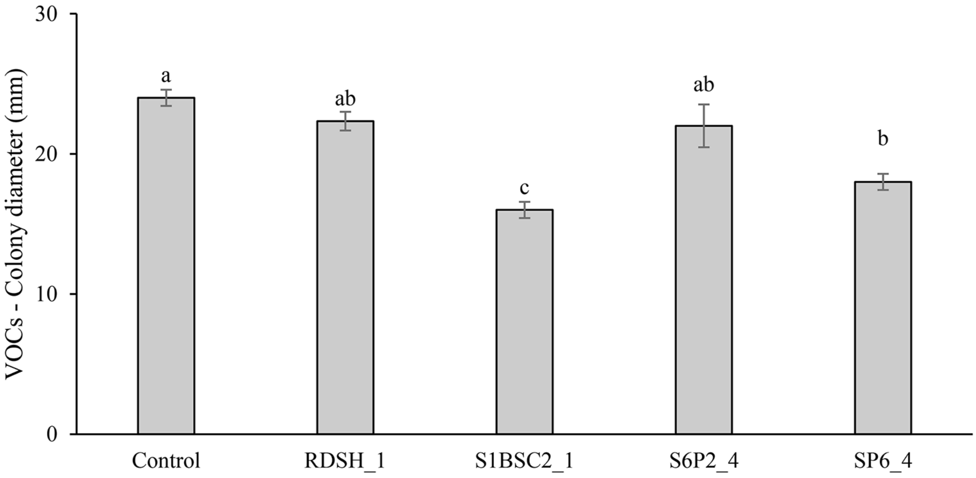


Fig. 3 In vitro efficacy of volatile organic compounds (VOCs) produced by *Papiliotrema wisconsinensis* and *Naganishia albida* against *Penicillium expansum* PAT27 mycelial colony growth. Data are the means of the lesions ± standard error. Different letters indicate significant differences according to Tukey test ($\alpha < 0.05$)

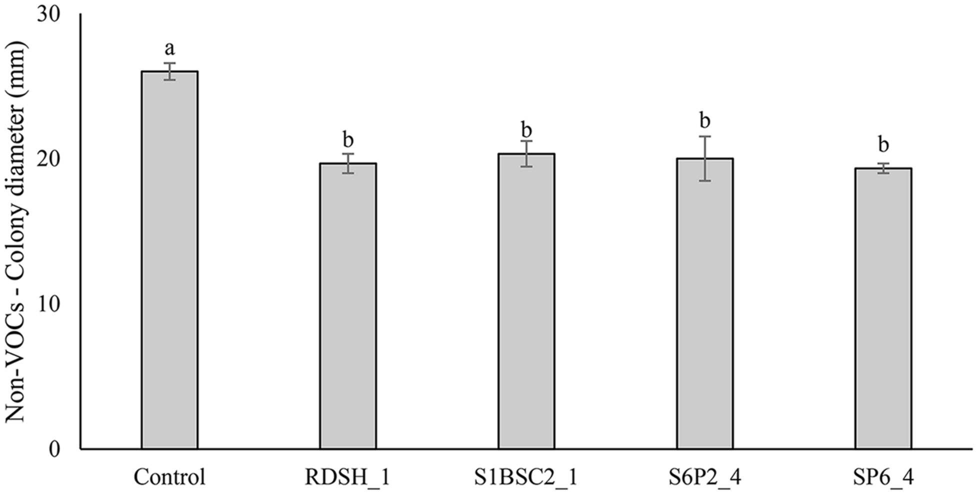


Fig. 4 In vitro efficacy of non-volatile organic compounds (non-VOCs) produced by *Papiliotrema wisconsinensis* and *Naganishia albida* against *Penicillium expansum* PAT27 mycelial colony growth. Data are the means of two independent experiments ± standard error. Different letters indicate significant differences according to Tukey test ($\alpha < 0.05$)

particular, *P. wisconsinensis* strains S1BSC2_1 and SP6_4 showed the highest inhibition against PAT27, resulting in a significant reduction of 33% and 25% in colony growth.

In vitro efficacy of non-VOCs against *Penicillium expansum*

The experiment was conducted to assess the antifungal activity of non-volatile metabolites produced by the four selected strains. As illustrated in Fig. 4, the inhibitory efficacy of the non-VOCs did not vary significantly among the yeast strains. Compared with the control, the inhibition rate was 23%.

VOCs analysis by SPME-GC-MS

Volatile metabolites produced by *Naganishia albida* (RDSH_1) and *P. wisconsinensis* (S6P2_4, SP6_4, S1BSC2_1) were analyzed by SPME/GC-MS. All the VOCs produced by the yeasts were reported in the Supplemental Table S2. The analysis of VOCs revealed distinct metabolite profiles for each of the four yeast strains, excluding those produced by the control (NYDA). This finding indicated considerable metabolic diversity among strains. *Naganishia albida* strain RDSH_1 exhibited the broadest spectrum of VOCs, producing 84 compounds, including alcohols (1-butanol, 1-heptanol, phenylethyl alcohol), esters (ethyl acetate), ketones (3-heptanone, 2-heptanone), acids (acetic acid, butanoic acid), and nitrogen-containing compounds (methylthiophosphonamidic acid, imidazole derivatives), aromatic and heterocyclic compounds (pyrazines, quinoxalines, and furans). *Papiliotrema wisconsinensis* strains S6P2_4 and SP6_4 displayed a narrow VOCs profile, with 24 and 19 compounds detected, respectively. These compounds suggested a limited but specific secondary metabolism, potentially indicative of specialized ecological or physiological function, and distinctbiosynthetic pathways. Conversely, *P. wisconsinensis* strain S1BSC2_1 demonstrated a different and rich VOCs profile, second only to RDSH_1, with over 100 compounds detected. This strain emitted a wide range of chemical classes, including alcohols (1-butanol, 1-hexanol), esters, ketones, furans (2-methylfuran, furfurylmethylamphetamine), acids (butanoic acid, propanoic acid), and aromatic compounds (benzaldehyde, phenylethyl alcohol). Its metabolic output included both common fermentation products and complex secondary metabolites, indicating robust metabolic capabilities. These findings highlighted the high metabolic plasticity among yeast strains, particularly the potential of the *P. wisconsinensis* strain S1BSC2_1 for applications requiring a broad VOCs profile. Among the detected VOCs, four were consistently produced by all the strains: 3-methyl-1-butanol, 2-methylpropanoic acid, butanoic acid, and phenylethyl alcohol. Despite their common presence, the relative abundance (AA, absolute area) of these compounds varied across the strains as reported in Table 2.

Non-VOCs analysis by FT-IR

The FT-IR spectroscopic measurements were carried out to determine the main non-volatile classes of compounds produced by the strains of *N. albida* and *P. wisconsinensis.* In Fig. 5, the infrared spectra of strains RDSH_1 (*N. albida*) and S6P2_4 (*P. wisconsinensis*) are reported. These strains previously showed high inhibition of *P. expansum* PAT27 mycelial growth in the non-VOCs assay (Fig. 4). Figure 5 also shows the spectra differences between the cultures of the two strains (blue line) and the control (NYDB, black line), which facilitate the interpretation of spectroscopic results. The positive peaks are related to the yeast's non-volatile metabolites, while the negative peaks should be attributed to the consumption of the culture medium. To the best of our knowledge, there are no data in the literature about the non-VOCs synthesized by *N. albida* and *P. wisconsinensis*. However, polysaccharides showed typical bands at 1626 (mixed with residual water), around 1450, 1404, around 1340, and in the spectral region between 1185 and 750 cm^{-1} (Breierova et al. 2005; Amiri et al. 2019) as shown in Fig. 5. Several bands indicate the composition of exopolysaccharides and the type of glycosidic bond: 980, 920, 866, and 818 cm^{-1} bands are attributed to mannan (Breierova et al. 2005), 1144 and 1032 cm^{-1} are attributed to glucans (Di Francesco et al. 2023), the 777 cm^{-1} to both glucans and galactans (Di Francesco et al. 2023); the 1184, 1132, around 1100, 1084, around 1050 and the 991 cm^{-1} to xylans (Kacurakova et al. 1999): these bands can also be associated with the presence of xylosides based on xylose, as in xylans. Finally, the 891 cm^{-1} is attributed to the β-anomeric conformation of glycosidic bonds and was detected only as a maximum in the difference spectra (Amiri et al. 2019; Yan et al. 2018). The presence of proteins in non-VOCs, which could be associated to lytic enzymes or to the peptidic fraction of polysaccharopeptides, was evidenced by the bands at 1586 (asymmetric stretching vibration of carboxylate in negatively charged amino acids, such as Asp and Glu), 1520 (amide II), 1404 (symmetric stretching vibration of carboxylate), 1296, 1258 and 1246 (amide III) cm^{-1} (Socrates 1998). The carbonylic group $C=O$ is typical of different classes of secondary metabolites such as aliphatic diketones, polyketides, and lactones. The band at around 1720 cm^{-1} appearing in the difference spectrum of both analyzed strains can be attributed to the stretching of the $C=O$ group present in all these classes of molecules. The enol form of diketones has a typical vibration near 1580 cm^{-1}, overlapped with the asymmetric stretching of carboxylates. The aromatic moiety of polyketides may show several bands in the 1620–1500 cm^{-1} spectral region (Socrates 1998).

In vivo assays

To evaluate the in vivo biocontrol efficacy of the selected yeast strains against *P. expansum* PAT27 on *Pink Lady* apples, two independent experiments were conducted.

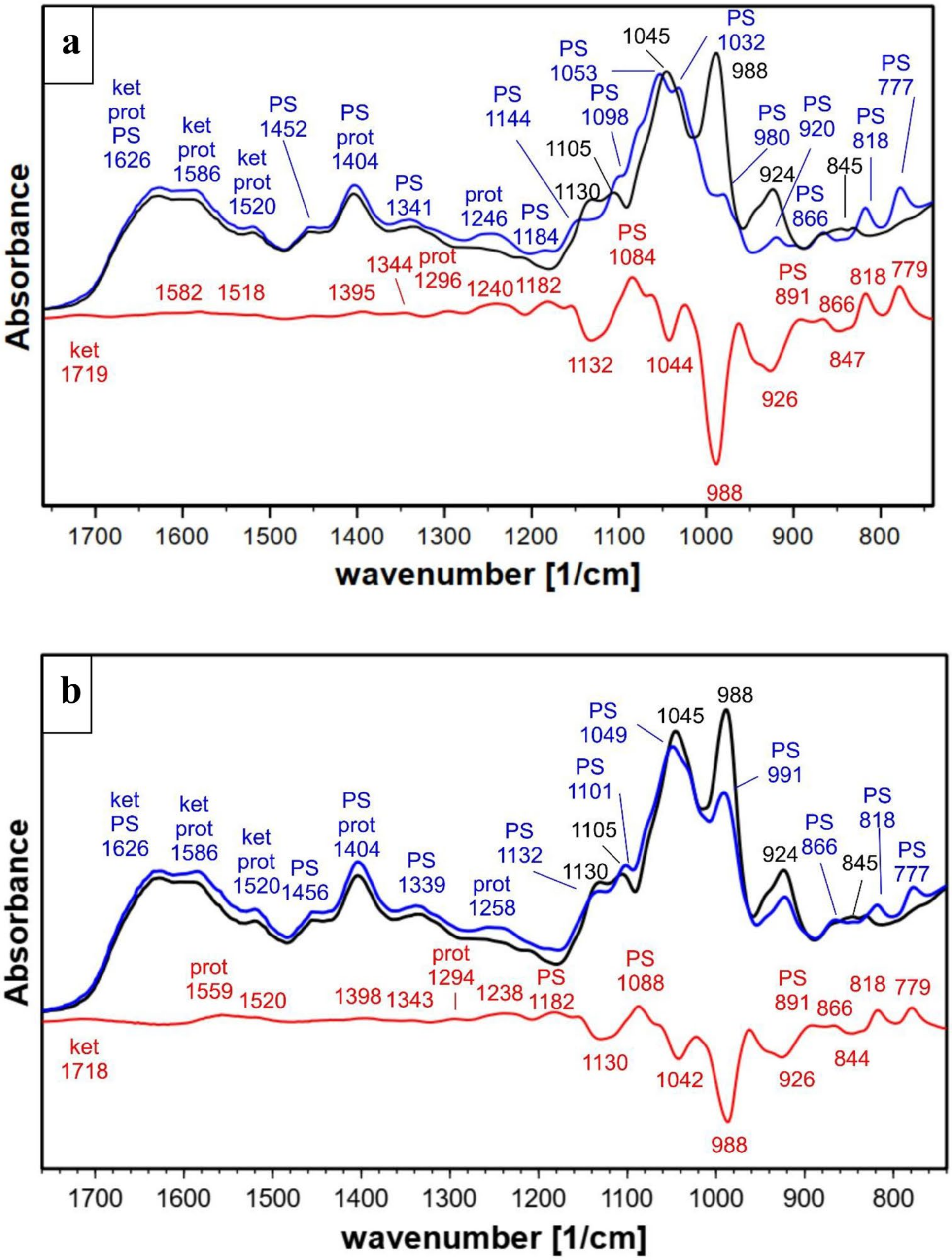
Papiliotrema wisconsinensis strains SP6_4 and S1BSC2_1 showed the highest VOCs-mediated inhibition of *P. expansum* growth in vitro, and were therefore used to biofumigate artificially inoculated apple fruits. Fruit exposed to the VOCs emitted by both strains did not exhibit a significant reduction in disease severity compared to the control (Table 3), except for a slight inhibition by the strain S1BSC2_1 (14.4%). Conversely, *N. albida* RDSH_1 and *P.*

Table 2 SPME/GC–MS analyses of volatile organic compounds (VOCs) secreted by the strains *Naganishia albida* (RDSH_1) and *Papiliotrema wisconsinensis* (S6P2_4, SP6_4, S1BSC2_1)

	Absolute Area (AA)				
	Strains				
Compound	RT	*N. albida* RDSH_1	*P. wisconsinensis S6P2_4*	*P. wisconsinensis SP6_4*	*P. wisconsinensis S1BSC2_1*
1-Butanol, 3-methyl	9.33	3.55E+06 c	1.02E+07 a	6.20E+06 b	6.05E+06 b
Propanoic acid, 2-methyl	20.21	3.50E+05 c	6.08E+05 b	2.22E+06 a	1.31E+06 a
Butanoic acid	21.92	1.24E+06 b	3.11E+06 a	4.02E+06 a	3.80E+06 a
Phenylethyl Alcohol	29.10	2.93E+05 c	7.96E+05 a	8.84E+05 a	4.60E+05 b

The values represented the average of the absolute areas (AA) of the common compounds (3-methyl-1-butanol, propanoic acid, 2-methylbutanoic acid, and phenylethyl alcohol) produced by all the strains ± standard error. Different letters represented statistically significant differences among the strains for each compound, as determined by Tukey test ($\alpha < 0.05$)

Fig. 5 (**a**) FT-IR/ATR spectra of control (black line), non-VOCs synthesized by RDSH_1 (blue lines), and their difference spectra (RDSH_1 – control, red line). (**b**) FT-IR/ATR spectra of control (black line), non-VOCs synthesized by S6P2_4 (blue lines), and their difference spectra (S6P2_4 – control, red line). The attribution of the bands is indicated as follows: ket: diketones and polyketides; prot: enzymes and peptides in polysaccharopeptides; PS: exopolysaccharides and polysaccharopeptides



wisconsinensis S6P2_4 cell suspensions completely inhibited *P. expansum* compared to the control (Table 3).

Penicillium expansum **DNA quantification and apple patulin analysis**

The DNA of *P. expansum* (PAT27) was quantified in *Pink Lady* apples exposed to volatile metabolites produced by the strains RDSH_1 and S6P2_4 and to the strain cell suspensions of SP6_4 and S1BSC2_1. Pathogen DNA was quantified by extrapolating from the standard curve the amount of *P. expansum* (PAT27) DNA in picograms (pg) and normalizing with the amount of sampled fruit tissue (mg).

In the VOCs treatment, both strains SP6_4 and S1BSC2_1 significantly reduced the pathogen DNA concentration to 5.92 and 6.23 pg/mg, respectively, compared to 19.2 pg/mg of the control. In the other assay, apples treated with strains RDSH_1 and S6P2_4 showed no detectable fungal DNA (0.00 pg/mg), indicating a complete suppression of pathogen colonization. In contrast, the untreated control exhibited a significantly higher DNA concentration (20.1 pg/mg), confirming an active fungal growth. These findings further confirmed the antifungal effect of yeasts metabolites at the molecular level, with a complete inhibition through the yeast cell suspensions and with VOCs leading to a significant reduction of *P. expansum* colonization

Table 3 In vivo efficacy of volatile organic compounds (VOCs) and cell suspensions by *Naganishia albida* (RDSH_1) and *Papiliotrema wisconsinensis* (S6P2_4, SP6_4, S1BSC2_1) strains

	Treatment	Disease severity (mm)
VOCs	Control	9.42 ± 0.73 a
	P. wisconsinensis SP6_4	9.22 ± 0.44 a
	P. wisconsinensis S1BSC2_1	8.06 ± 0.33 b
Cell suspension	Control	9.42 ± 0.97 a
	N. albida RDSH_1	0.00 ± 0.00 b
	P. wisconsinensis S6P2_4	0.00 ± 0.00 b

Values represented *Penicillium expansum* PAT27 severity (mm) (average of three replicates ± standard error). Different letters represented significant statistical differences among the treatments for each type of compound, as determined by Tukey test (α < 0.05)

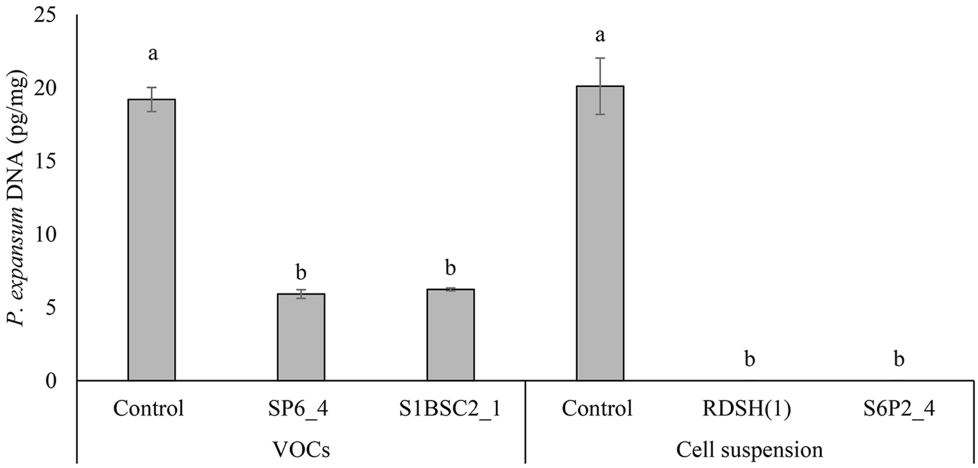


Fig. 6 DNA quantification of *Penicillium expansum* (pg/mg) on apple treated with volatile organic compounds (VOCs) and yeast cell suspensions by *Naganishia albida* RDSH(1) and *Papiliotrema winsconsiensis* S6P2_4, SP6_4, and S1BSC2_1. Each value was the average of three replicates ± standard error. Different letters represented significant differences among the treatments (strains) for each type of compound, as determined by Tukey test (α < 0.05)

Table 4 Effect volatile metabolites (VOCs) and cell suspensions by *Naganishia albida* RDSH_1, *Papiliotrema wisconsinensis* S6P2_4, and *P. wisconsinensis* SP6_4 and S1BSC2_ on apple patulin content (µg/kg)

	Treatment	Patulin (µg/kg)
VOCs	Control	840 ± 340
	P. wisconsinensis SP6_4	390 ± 160
	P. wisconsinensis S1BSC2_1	220 ± 88
Cell suspension	Control	3700 ± 1500
	N. albida RDSH_1	1200 ± 480
	P. wisconsinensis S6P2_4	33 ± 13

on fruits (Fig. 6). Through HPLC analysis, patulin content in treated apples was significantly reduced compared to the water control in both (Table 4). In particular, patulin levels in the non-VOCs control samples were higher than in the VOCs control, 3700 and 840 µg/kg, respectively. The diffusible metabolites produced by *P. wisconsinensis* S6P2_4 and *N. albida* RDSH_1 reduced the mycotoxin content by 99.1% and 67.5%, respectively, compared to the control. Regarding volatile compounds, *P. wisconsinensis* SP6_4 and S1BSC2_1 inhibited patulin production compared to the control by 50.5% and 73.8%, respectively.

Discussion

Naganishia albida and *P. wisconsinensis* strains demonstrated significant antagonistic activity against *P. expansum* by producing volatile and non-volatile metabolites. These findings align with previous reports that highlighted the antifungal potential of these microorganisms (Vidal et al. 2024). For instance, Miccoli et al. (2019) showed that *Papiliotrema terrestris* strain LS28 effectively reduced *P. expansum* infection and patulin contamination in apples during postharvest storage, supporting the relevance of basidiomycetous yeasts as promising BCAs. The current study expands upon this evidence by demonstrating similar antagonistic efficacy by yeasts strains isolated from extreme environments, further confirming their potential as eco-friendly alternatives to chemical fungicides. The role of volatile and diffusible compounds produced by the yeasts revealed important insights into their antagonistic mechanisms. In fact, both VOCs and non-VOCs contributed to the inhibition of *P. expansum* in in vitro assays. Inhibition mediated by the volatile fraction was more pronounced in *N. albida*, suggesting that strain-specific metabolic profiles may play a key role in antagonism. Nevertheless, in vivo experiments revealed that the *P. wisconsinensis* strains' cell suspensions were more effective in reducing both disease severity and fungal sporulation (data not reported).

The present study carefully analysed the volatilomes of individual yeast strains to evaluate the composition of a potential protective atmosphere that they create. At the same time, it should be considered that the yeast volatilome could interfere with the pathogen communication system and growth, as well as the host aroma and microbial community. However, most studies to date have focused on the unidirectional effect of the microbial VOCs produced by a single microbial entity in regard to another microbial species (Schulz-Bohm et al. 2015; Splivallo et al. 2015; Tilocca et al. 2020). The different results obtained between in vitro and in vivo assays suggested that VOCs exhibited stronger antifungal activity in contained environments. Their volatility and rapid diffusion may limit their efficacy in complex biological systems, such as in the presence of fruits (Di Francesco et al. 2024; Hood et al. 2010; de Paiva et al. 2016).

VOCs emitted by *N. albida* RDSH_1 and *P. wisconsinensis* strains SP6_4, S6P2_4, and S1BSC2_1 exhibited notable antifungal potential in vitro; conversely, the in vivo efficacy was less marked. A better understanding of the entire ecosystem in which microbial species interact, including a series of biotic and abiotic factors, would be necessary (Tilocca et al. 2020).

Although VOCs did not significantly reduce disease severity in artificially inoculated apples, they markedly inhibited patulin production. Specifically, SP6_4 and S1BSC2_1 significantly reduced patulin content, indicating that these VOCs interfered with *P. expansum* secondary metabolism rather than directly affecting colonization or virulence mechanisms. This dissociation between pathogen growth inhibition and mycotoxin suppression suggests a potential regulatory effect of VOCs on patulin biosynthetic pathways (Li et al. 2019). Similar outcomes were previously reported by some authors (Alasmar et al. 2020; Huang et al. 2021) who showed that volatile metabolites of various chemical classes, such as esters, alkanes, alcohols, terpenes, ketones, aldehydes, and aromatic hydrocarbons, inhibited *Penicillium* spp. growth and mycotoxin production. Compounds like phenylethyl alcohol and 3-methyl-1-butanol, detected across all yeast strains in this study, have been reported to suppress fungal sporulation and secondary metabolite production (Contarino et al. 2019; Bennett et al. 2012). Thus, while VOCs alone may not exhibit full biocontrol activity in vivo, their ability to suppress patulin synthesis could represent a valuable complementary strategy to reduce foodborne toxin risks in postharvest systems. In contrast, yeast cell suspension persisted longer at the infection site, providing more sustained protection (Zhao et al. 2022), likely due to the different mechanisms of action, such as competition for nutrients and space, antibiosis, and parasitism. In fact, through the presence of yeast cells and secondary metabolites, the exerted mechanisms of action included the production of lytic enzymes, biofilm formation, siderophores, and even direct mycoparasitism, all of which contribute to the long-term suppression of pathogens in vivo (Wu et al. 2024; Rodrigues et al. 2024). Secondary metabolites, such as VOCs and non-VOCs, could also enhance the physical and chemical properties of fruits, improving their storage (Di Francesco et al. 2020a; Dudas et al. 2026). The induced resistance is characterised by the activation of host defence mechanisms as described by Li et al. (2024) in blueberry fruits, where volatile metabolites produced by *Metschnikowia pulcherrima* strain T-2 increased the flavonoid content, such as quercetin, kaempferol, and myricetin, compared to the untreated fruits. A recent study reported that secondary metabolites produced by the strain *Meyerozyma caribbica* BBJ induced resistance in apple tissues, causing an up-regulation of defense-related genes coding for chitinase enzymes (Dudas et al. 2026).

FT-IR spectroscopic analysis has already proven to be suitable for a preliminary characterization of non-volatilemetabolites classes produced by antagonist bacteria (Di Francesco et al. 2023) or yeast-like fungi (Di Francesco et al. 2020b; Francesco et al. 2021; Oztekin et al. 2025). Due to the lack of a specific characterization of the secondary metabolites produced by *N. albida* and *P. wisconsinensis* strains, the identification of their non-volatile fractions was performed based on existing literature (Bills and Gloer 2016; Kiyama et al. 2018; Sandargo et al. 2019; Wu et al. 2022; Santra et al. 2023; Sum et al. 2023; Pinar et al. 2024; Wadhwa et al. 2024).

The FT-IR spectra of the non-volatile fractions (Fig. 5) are dominated by absorption bands typical of high-molecular-weight compounds, mainly proteinaceous material and polysaccharides. In particular, the spectral region between 1700 and 1500 $\mathrm{cm^{-1}}$ shows bands at 1626 $\mathrm{cm^{-1}}$ (amide I) and 1520 $\mathrm{cm^{-1}}$ (amide II), which are characteristic of peptide bonds and therefore indicate the presence of amide-containing compounds, such as proteins, peptides, or glycoproteins. Additional contributions in the amide III region (1300–1240 $\mathrm{cm^{-1}}$) further support the occurrence of proteinaceous components. This last spectral region showed the main differences between strain RDSH_1 (peak at 1246 $\mathrm{cm^{-1}}$) and strain S6P2_4 (peak at 1258 $\mathrm{cm^{-1}}$). Because the amide III band is influenced by protein conformation and molecular environment, this shift may reflect differences in the overall composition or structural organization of proteinaceous fractions between the two strains (Kiyama et al. 2018; Pinar et al. 2024). However, FT-IR analysis alone does not allow identification of specific proteins or enzymatic activities, and therefore these observations should be interpreted as indicative of compositional differences rather than evidence of particular functional biomolecules. The most intense and informative features occur in the carbohydrate fingerprint region (1260–800 cm⁻¹), where several diagnostic bands enable identification of various fungal polysaccharides (Fig. 5). Among these, exopolysaccharides are long-chain carbohydrates secreted by fungi as a protective barrier, possess notable antimicrobial properties, and are primarily composed of mannose, glucose, galactose, and xylose units (Pinar et al. 2024). Although the overall spectral profiles of the two strains are similar, clear differences are evident, particularly in the 1150–950 cm⁻¹ region (Fig. 5). Strain RDSH_1 exhibits characteristic bands of glucans (1144 and 1032 cm⁻¹) (Di Francesco et al. 2024) and mannans (980 cm⁻¹) (Breierova et al. 2005), which are absent in strain S6P2_4. In contrast, strain S6P2_4 shows distinctive bands of xylans (1132, 1084, and 991 cm⁻¹) (Kacurakova et al. 1999; Yao et al. 2016). These findings indicate strain-specific differences in the composition of exopolysaccharides, which may reflect distinct metabolic strategies and could be related to their biological activity, such as enhanced competition for space through more efficient biofilm formation by the yeast (Liu et al. 2025), or reduced pathogen adhesion capability (Oztekin et al. 2025). In particular, β-glucan- and mannan-rich yeast polysaccharides are widely reported as bioactive (e.g., antimicrobial and immunomodulatory), and differences in their relative abundance may result in distinct biological outcomes (Baek et al. 2024; Liu et al. 2021). It should be emphasized that FT-IR spectroscopy provides information on functional groups and compound classes, and therefore enables only a preliminary and comparative characterization of non-volatile metabolites rather than unequivocal identification of specific chemical structures. Based on these preliminary spectroscopic data, the composition of non-volatile metabolites could be further investigated by other analytical (chromatography), structural (NMR), and morphological (SEM) techniques (Oztekin et al. 2025).

A particularly relevant outcome of this study was the significant reduction in patulin accumulation in apples treated with the yeast strains' metabolites. This reduction occurred even in cases where pathogen colonization was not completely suppressed, highlighting a dissociation between fungal presence and mycotoxin biosynthesis. This finding was supported by molecular quantification of *P. expansum* DNA, which revealed that VOCs produced by strains SP6_4 and S1BSC2_1 significantly reduced fungal DNA levels compared to the untreated control. These reductions suggest that the pathogen may still be present to some extent. At the same time, patulin biosynthesis is considerably suppressed, suggesting that VOCs primarily interfere with secondary metabolism rather than fungal colonization. In contrast, RDSH_1 and S6P2_4 cell suspensions completely suppressed fungal colonization, with DNA levels below the detection limit. However, the reduction in patulin content was 67.6% for RDSH_1 and almost complete for S6P2_4. These data indicate that the cell suspensions achieved both pathogen inhibition and toxin suppression. Overall, the data highlight that application of the yeast in its current form may be more effective in vivo. The absence of detectable fungal DNA following this treatment suggests a fungicidal or at least strongly fungistatic effect of the strains (Rodrigues et al. 2024).

The reduction in patulin levels by the application of the yeast cell suspensions may also result from the interaction of multiple antagonistic mechanisms, such as competition for nutrients and space, and induction of host resistance (Castoria et al. 1997). In particular, competition is crucial since the availability of nutrients, space, and other physical resources is generally limited for the pathogen (Palmieri et al. 2022). Also, the rapid growth of a BCA allows it to occupy the niche, thus excluding the pathogen. This mechanism of action may involve biofilm formation and exopolysaccharide production by the microorganism (Klein and Kupper 2018).

In the present study, the combined analyses of pathogen DNA quantification and patulin content strongly supports the hypothesis that yeast VOCs can act at a metabolic level to modulate mycotoxin biosynthesis or lead to the formation of less toxic desoxypatulinic acid (Castoria et al. 2011). In contrast, non-VOCs achieve broader protection through more direct antagonistic interactions. Conversely, Zheng et al. (2017) reported that the antagonistic yeast *Rhodosporidium kratochvilovae* LS11 stimulated patulin biosynthesis by *P. expansum* in apple wounds, although the overall contamination of apples was reduced.

This distinction emphasizes the importance of selecting BCA strains based not only on their ability to inhibit fungal growth, but also on their impact on mycotoxin production, both of which are essential for effective postharvest biocontrol strategies (Wu et al. 2024). These findings are especially significant, given the established health risks associated with patulin exposure and the strict regulatory limits imposed by food safety authorities. The reduction in patulin levels could be attributed to a combination of factors, including the suppression of *P. expansum* metabolism, competition for nutrients, and possibly direct biodegradation or adsorption of the toxin by yeast cells, a phenomenon that has been previously observed in other yeast species such as *S. cerevisiae* and *Rhodotorula mucilaginosa* (Zhao et al. 2022; Hassan et al. 2021). The observed biocontrol effects likely result from multiple mechanisms, including the production of inhibitory metabolites, competition for space and nutrients, and possible modification of the microenvironment, such as pH alteration or oxygen depletion. Yang et al. (2025) demonstrated that *S. cerevisiae* effectively reduced patulin contamination in apple juice by physical adsorption and enzymatic degradation. The study also highlighted the yeast's tolerance to high patulin concentrations and its ability to grow well under such conditions, supporting the idea that yeast-mediated inhibition involves competition for nutrients and production of inhibitory compounds. Also, Coelho et al. (2008) found that *Pichia ohmeri* and *S. cerevisiae* degraded over 83% and 96% of patulin, respectively, in apple juice. These multifaceted strategies are advantageous in postharvest settings, where microbial competition is dynamic and environmental conditions fluctuate. The current results demonstrate that these yeasts possess desirable characteristics for practical application as BCAs. From an applied perspective, the use of *N. albida* and *P. wisconsinensis* could represent a sustainable and effective alternative to synthetic fungicides in managing *P. expansum* infections during apple storage. Their dual action, both as inhibitors of fungal disease and as reducers of patulin levels, offers an alternative strategy to chemical fungicides. Future research should evaluate the performance of these yeasts under extended storage duration and within a synergistic application system. Also, the interactions with native fruit microbiota will be crucial for real-world implementation. Overall, this study contributes valuable evidence supporting the integration of extremophile yeasts into postharvest disease management programs and opens the door to further innovation in the biological control of mycotoxigenic pathogens.

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Data availability Data will be made available on request.

Declarations

Ethical approval Not applicable.

Conflict of interests Authors declare no conflict of interests.

References

Alasmar R, Ul-Hassan Z, Zeidan R, Al-Thani R, Al-Shamary N, Alnaimi H, Migheli Q, Jaoua S (2020) Isolation of a novel *Kluyveromyces marxianus* strain QKM-4 and evidence of its volatilome production and binding potentialities in the biocontrol of toxigenic fungi and their mycotoxins. ACS Omega 5:17637–17645. https://doi.org/10.1021/acsomega.0c02124

Amiri S, Mokarram RR, Khiabani MS, Bari MR, Khaledabad MA (2019) Exopolysaccharides production by *Lactobacillus acidophilus* LA5 and *Bifidobacterium animalis subsp. lactis* BB12: Optimization of fermentation variables and characterization of structure and bioactivities. Int J Biol Macromol 123:752–765. https://doi.org/10.1016/j.ijbiomac.2018.11.084

AOAC (2002) Patulin in clear and cloudy apple juices and apple puree. Liquid chromatographic

Aprea E, Corollaro ML, Betta E, Endrizzi I, Demattè ML, Biasioli F et al (2012) Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. Food Res Int 49:677–686. https://doi.org/10.1016/j.foodres.2012.09.023

Baek KR, Lee HJ, Jeong KH, Park BR, Kim SJ, Seo SO (2024) Parabiotic immunomodulatory activity of yeast cell wall polysaccharides from *Saccharomyces cerevisiae* and *S. boulardii* J Funct Foods 123:106577 https://doi.org/10.1016/j.jff.2024.106577

Bennett R, Hung S, Le S (2012) Fungal and bacterial volatile organic compounds: an overview and their role as ecological signaling agent. In: Hock (ed) Fungal Associations The Mycota IXB. Springer-, Berlin Heidelberg. https://doi.org/10.1007/978-3-642-30826-0_18

Bills GF, Gloer JB (2016) Biologically active secondary metabolites from the fungi. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.FUNK-0009-2016. 4 FUNK-0009-2016

Breierová E, Hromádková Z, Stratilová E, Sasinková V, Ebringerová A (2005) Effect of salt stress on the production and properties of extracellular polysaccharides produced by *Cryptococcus laurentii*. Z Fur Naturforschung C 60:444–450

Buzzini P, Branda E, Goretti M, Turchetti B (2012) Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. FEMS Microbiol Ecol Nov 82:217–241. https://doi.org/10.1111/j.1574-6941.2012.01348.x

Buzzini P, Turchetti B, Yurkov A (2018) Extremophilic yeasts: the toughest yeasts around? Yeast 35:487–497. https://doi.org/10.1002/yea.3314

Castoria R, De Curtis F, Lima G, De Cicco V (1997) β-1,3-glucanase activity of two saprophytic yeasts and possible mode of action as biocontrol agents against postharvest diseases. Postharvest Biol Technol 12:293–300

Castoria R, Mannina L, Durán-Patrón R, Maffei F, Sobolev AP, De Felice DV, Pinedo-Rivilla C, Ritieni A, Ferracane R, Wright SAI (2011) Conversion of the Mycotoxin Patulin to the Less Toxic Desoxypatulinic Acid by the Biocontrol Yeast Rhodosporidium kratochvilovae Strain LS11. J Agric Food Chem 59:11571–11578

Cignola R, Zucchinali S, Firrao G, Di Francesco A (2024) Aspects of the biocontrol activity of *Aureobasidium* spp. strain against

Penicillium expansum of apple. Ann Appl Biol 12892. https://doi.org/10.1111/aab.12892

Coelho AR, Celli MG, Sataque E, Hoffmann FL, Pagnocca FC, Garcia S, Sabino M, Harada KI, Wosiacki G, Hirooka EY (2008) Patulin biodegradation using *Pichia ohmeri* and *Saccharomyces cerevisiae.* World Mycotox. J 1:325–331. https://doi.org/10.3920/WMJ2008.1040

Contarino R, Brighina S, Fallico B, Cirvilleri G, Parafati L, Restuccia C (2019) Volatile organic compounds (VOCs) produced by biocontrol yeasts. Food Microbiol 82:70–74. https://doi.org/10.1016/j.fm.2019.01.008

De Garcia V, Brizzio S, van Broock MR (2012) Yeasts from glacial ice of Patagonian Andes, Argentina. FEMS Microbiol Ecol. 82:540–550. https://doi.org/10.1111/j.1574-6941.2012.01470.x

Dennis C, Webster J (1971) Antagonistic properties of species–groups of *Trichoderma*: I. Production of non–volatile antibiotics. Soil Biol Biochem 3:89–96. https://doi.org/10.1016/S0007-1536(71)80077-3

De Paiva E, Serradilla MJ, Ruiz-Moyano S, Córdoba MG, Villalobos MC, Casquete R, Hernández A (2016) Combined effect of antagonistic yeast and modified atmosphere to control *Penicillium expansum* infection in sweet cherries cv. Ambrunés Postharvest Biol Technol 124:51–58. https://doi.org/10.1016/j.ijfoodmicro.2016.10.033

Di Francesco A, Di Foggia M, Baraldi E (2020a) *Aureobasidium pullulans* volatile organic compounds as alternative postharvest method to control brown rot of stone fruits. Food Microbiol 87. https://doi.org/10.1016/j.fm.2019.103395

Di Francesco A, Di Foggia M, Corbetta M, Baldo D, Ratti C, Baraldi E (2021) Biocontrol activity and plant growth promotion exerted by *Aureobasidium pullulans*, strains. J Plant Growth Reg 40:1233–1244. https://doi.org/10.1007/s00344-020-10184-3

Di Francesco A, Di Foggia M, Zajc A, Gunde-Cimerman N, Baraldi E (2020b) Study of the efficacy of *Aureobasidium* strains belonging to three different species: *A. pullulans, A. subglaciale* and *A. melanogenum* against *Botrytis cinerea* of tomato. Ann Appl Biol 177:266–275. https://doi.org/10.1111/aab.12627

Di Francesco A, Jabeen F, Di Foggia M, Zanon C, Cignola R, Sadallah A, Tugnoli V, Ermacora P, Martini M (2023) Study of the efficacy of bacterial antagonists against *Cadophora luteo-olivacea* of kiwifruit. Biol Control 180:105199. https://doi.org/10.1016/j.biocontrol.2023.105199

Di Francesco A, Jabeen F, Vall-llaura N, Moret E, Martini M, Torres R, Ermacora P, Teixidó N (2024) Pseudomonas synxantha volatile organic compounds: Efficacy against Cadophora luteo-olivacea and Botrytis cinerea of kiwifruit. Front Plant Sci 15:1398014. https://doi.org/10.3389/fpls.2024.1398014

Di Francesco A, Milella F, Mari M, Roberti R (2017) A preliminary investigation into *Aureobasidium pullulans* as a potential biocontrol agent against *Phytophthora infestans* of tomato. Biol Control 114:126–133. https://doi.org/10.1016/j.biocontrol.2017.08.010

Di Francesco A, Ugolini L, Lazzeri L, Mari M (2015) Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. Biol Control 81:8–14. https://doi.org/10.1016/j.biocontrol.2014.10.004

Dudaš T, Celano G, Budakov D, Cotugno P, Grahovac M, Incerti O, Stojšin V, De Angelis M, Ippolito A, Sanzani SM (2026) Efficacy and mode of action of the biocontrol yeast *Meyerozyma caribbica* BBJ against blue mould and patulin production by *Penicillium expansum*. Int J Food Microbiol 444:111442. https://doi.org/10.1016/j.ijfoodmicro.2025.111442

European Commission (2006) Regulation (EC) No 1881/2006 of the European Parliament and of the Council of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. European Union

FAO (2021) Food and Agriculture Organization of the United Nations – Agricultural Statistics. FAO

Gouy M, Guindon S, Gascuel O (2010) Sea view version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221–224. https://doi.org/10.1093/molbev/msp259

Hassan ZU, Al Thani R, Atia FA, Alsafran M, Migheli Q, Jaoua S (2021) Application of yeasts and yeast derivatives for the biological control of toxigenic fungi and their toxic metabolites. Env Technol Innov 23:101447. https://doi.org/10.1016/j.eti.2021.101447

Hood JR, Burton D, Wilkinson JM, Cavanagh HMA (2010) Antifungal activity of Leptospermum petersonii oil volatiles against Aspergillus spp. in vitro and in vivo. J Antimicrob Chem 65:285–288. https://doi.org/10.1093/jac/dkp400

Huang C, Zhang L, Johansen PG, Petersen MA, Arneborg N, Jespersen L (2021) *Debaryomyces hansenii* strains isolated from Danish cheese brines act as biocontrol agents to inhibit germination and growth of contaminating molds. Front Microbiol 12:662785. https://doi.org/10.3389/fmicb.2021.662785

Kačuráková M, Wellner N, Ebringerová A, Hromádková Z, Wilson RH, Belton PS (1999) Characterisation of xylan-type polysaccharides and associated cell wall components by FT-IR and FT-Raman spectroscopies. Food Hydrocol 13:35–41. https://doi.org/10.1016/S0268-005X(98)00067-8

Kiyama R, Furutani Y, Kawaguchi K, Nakanishi T (2018) Genome sequence of the cauliflower mushroom *Sparassis crispa* (Hanabiratake) and its association with beneficial usage. Sci Rep 8:16053. https://doi.org/10.1038/s41598-018-34415-6

Klein MN, Kupper KC (2018) Biofilm production by *Aureobasidium pullulans* improves biocontrol against sour rot in citrus. Food Microbiol 69:1–10

Li B, Chen Y, Zong Y, Shang Y, Zhang Z, Xu X, Wang X, Long M, Tian S (2019) Dissection of patulin biosynthesis, spatial control and regulation mechanism in *Penicillium expansum*. Env Microbiol 21:508–520. https://doi.org/10.1111/1462-2920.14542

Liu Y, Wu Q, Wu X, Algharib SA, Gong F, Hu J, Luo W, Zhou M, Pan Y, Yan YY, Wang Y (2021) Structure, preparation, modification, and bioactivities of β-glucan and mannan from yeast cell wall: A review. Int J Biol Macromol 173:445–456. https://doi.org/10.1016/j.ijbiomac.2021.01.125

Liu Y, Yan H, Yao J, Yan Y, Zhou J, Shi W (2025) Bioprospection of *Metschnikowia pulcherrima* as biocontrol agents against gray mold on grapes with their potential modes of action. Int J Food Microbiol 442:111383. https://doi.org/10.1016/j.ijfoodmicro.2025.111383

Li Z, Liu Q, Wu C, Yuan Y, Ma Z, Chang R, Wang Y (2024) *Metschnikowia Pulcherrima* yeast T-2 VOCs enhances postharvest blueberry fruit resistance to *Botrytis cinerea* by activating flavonoid metabolic pathways. LWT 201:116112. https://doi.org/10.1016/j.lwt.2024.116112

Lodhi MA, Ye GN, Weeden NF, Reisch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13. https://doi.org/10.1007/BF02668658

Lucci M, Khomutovska N, Firrao G, Di Francesco A (2025) Strains of *Aureobasidium pullulans* from extreme environments: new potential biocontrol agents? Microorganisms 13(11):2596. https://doi.org/10.3390/microorganisms13112596

Luciano-Rosario D, Keller NP, Jurick WM (2020) *Penicillium expansum*: biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. Mol Plant Pathol 21:1391–1404. https://doi.org/10.1111/mpp.12990

Miccoli C (2019) Biological control against *Penicillium expansum*: Influence of *Papiliotrema terrestris* LS28 on extracellular pH and patulin biosynthesis, and development of molecular-genetics tools for the validation of biocontrol mechanisms. University of Molise Institutional Repository. https://hdl.handle.net/11695/91487

Oztekin S, Dikmetas DN, Karbancıoglu-Guler F (2025) A novel exopolysaccharide from cold-adapted yeast *Rhodotorula glutinis*, along with structural, rheological, antioxidant, and antibiofilm properties. Biomass Conv Bioref 15:1507–1523. https://doi.org/10.1007/s13399-023-05208-3

Palmieri D, Ianiri G, Del Grosso C, Barone G, De Curtis F, Castoria R, Lima G (2022) Advances and perspectives in the use of biocontrol agents against fungal plant diseases. Horticulturae 8:577

Pinar O, Rodriguez-Couto S (2024) Biologically active secondary metabolites from white-rot fungi. Front Chem 12:1363354. https://doi.org/10.3389/fchem.2024.1363354

Puel O, Galtier P, Oswald IP (2010) Biosynthesis and toxicological effects of patulin. Toxins 2:613–631. https://doi.org/10.3390/toxins2040613

Rodrigues PL, Galvão JR, Maia BK, Oliveira S et al (2024) Mechanisms of biocontrolling action of yeasts in postharvest fruit: a review. Rev Gestão Soc Amb 18:e09718. https://doi.org/10.24857/rgsa.v18n11-080

Sandargo B, Chepkiruia C, Chenga T, Chaverra-Muñoza L, Thongbaia B, Stadler M, Hüttel S (2019) Biological and chemical diversity go hand in hand: basidiomycota as source of new pharmaceuticals and agrochemicals. Biotech Adv 37:107344. https://doi.org/10.1016/j.biotechadv.2019.01.011

Sangorrín MP, Lopes CA, Vero S, Wisniewski M (2014) Cold-adapted yeasts as biocontrol agents: biodiversity, adaptation strategies and biocontrol potential. In: Buzzini P, Margesin R (eds) Cold-adapted Yeasts. Springer, Berlin Heidelberg. https://doi.org/10.1007/978-3-642-39681-6_20

Santra HK, Banerjee D (2023) Antifungal activity of volatile and non-volatile metabolites of endophytes of *Chloranthus elatior* Sw. Front Plant Sci 14:1156323. https://doi.org/10.3389/fpls.2023.1156323

Schulz-Bohm K, Zweers H, de Boer W, Garbeva P (2015) A fragrant neighborhood: volatile mediated bacterial interactions in soil. Front Microbiol 6:1212. https://doi.org/10.3389/fmicb.2015.01212

Socrates G (1998) Infrared characteristic group frequencies. Tables and Charts. 2nd edition. John Wiley and Sons, Chichester, UK

Spadaro D, Lorè A, Garibaldi A, Gullino ML (2013) A new strain of *Metschnikowia fructicola* for postharvest control of *Penicillium expansum* and patulin accumulation on four cultivars of apple. Postharvest Biol Technol 75:18. https://doi.org/10.1016/j.postharvbio.2012.08.001

Splivallo R, Deveau A, Valdez N, Kirchhoff N, Frey-Klett P, Karlovsky P (2015) Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. Environ Microbiol 17:2647–2660. https://doi.org/10.1111/1462-2920.12521

Stielow JB, Levesque CA, Seifert KA, Meyer W, Iriny L et al (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia 35:242–263. https://doi.org/10.3767/003158515X689135

Sum WC, Ebada SS, Matasyoh JC, Stadler M (2023) Recent progress in the evaluation of secondary metabolites from Basidiomycota. Curr Res Biotech 6:100155. https://doi.org/10.1016/j.crbiot.2023.100155

Tannous J, Atoui A, El Khoury A, Kantar S, Chdid N, Oswald IP, Puel O, Lteif R (2015) Development of a real-time PCR assay for *Penicillium expansum* quantification and patulin estimation in apples. Food Microbiol 50:28–37. https://doi.org/10.1016/j.fm.2015.03.001

Tannous J, Keller NP, Atoui A, El Khoury A, Lteif R, Oswald IP, Puel O (2018) Secondary metabolism in *Penicillium expansum*: Emphasis on recent advances in patulin research. Crit Rev Food Sci Nutr 58:2082–2098. https://doi.org/10.1080/10408398.2017.1305945

Tapia-Vázquez I, Sánchez-Cruz R, Arroyo-Domínguez M, Lira-Ruan V, Sánchez-Reyes A, Sánchez-Carbente MDR, Padilla-Chacón D, Batista-García RA, Folch-Mallol JL (2020) Isolation and characterization of psychrophilic and psychrotolerant plant-growth promoting microorganisms from a high-altitude volcano crater in Mexico. Microbiol Res 231:126394. https://doi.org/10.1016/j.micres.2019.126394

Tilocca B, Cao A, Migheli Q (2020) Scent of a killer: microbial volatilome and its role in the biological control of plant pathogens. Front Microbiol 11:41. https://doi.org/10.3389/fmicb.2020.00041

Tulukoglu-Kunt KS, Moret E, Khomutovska N, Stenberg J, Di Francesco A (2024) Efficacy of volatile organic compounds by *Aureobasidium pullulans* immobilized on hydrogel spheres against strawberry fungal pathogens. J Sci Food Agr 104:8380–8386

Vidal A, Castro P, Navarro F, Parada R, Mendoza L, Cotoras M (2024) Effect of volatile compounds emitted by an endophytic yeast isolated from the endemic plant *Echinopsis chiloensis* against *Botrytis cinerea*. Horticulturae 10:1005. https://doi.org/10.3390/horticulturae10091005

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Naganishia* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wadhwa K, Kapoor N, Kaura H, Abu-Seer EA, Tariq M, Siddiqui, Yadav VK, Niazi P, Kumar P, Alghamdi S (2024) A comprehensive review of the diversity of fungal secondary metabolites and their emerging applications in healthcare and environment. Mycobiol 52:335. https://doi.org/10.1080/12298093.2024.2416736

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: PCR protocols. Guide Meth Appl 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Wu F, Wang H, Lin Y, Feng S, Li X (2024) Biocontrol mechanisms of antagonistic yeasts on postharvest fruits and vegetables and the approaches to enhance the biocontrol potential of antagonistic yeasts. Int J Food Microbiol 414:111038. https://doi.org/10.1016/j.ijfoodmicro.2024.111038

Wu J, Yang X, Duan Y, Wang P, Q J, Gao JM, Liu C (2022) Biosynthesis of sesquiterpenes in basidiomycetes: a review. J Fungi 8:913. https://doi.org/10.3390/jof8090913

Yang C, Hu C, Huang L, Peng B (2025) Mechanism of patulin biodegradation by a reductase from *Saccharomyces cerevisiae* and its potential application to apple juice. Food Res Int 176:116066. https://doi.org/10.1016/j.foodres.2025.116066

Yan JK, Ding ZC, Gao XL, Wang YY, Yang Y, Wu D, Zhang HN (2018) Comparative study of physicochemical properties and bioactivity of *Hericium erinaceus* polysaccharides at different solvent extractions. Carb Polym 193:373–382. https://doi.org/10.1016/j.carbpol.2018.04.019

Yao L, Zhu LP, Xu XY, Tan LL, Sadilek M, Fan H, Hu B, Shen XT, Yang J, Qiao B, Yang S (2016) Discovery of novel xylosides in co-culture of basidiomycetes *Trametes versicolor* and *Ganoderma applanatum* by integrated metabolomics and bioinformatics. Sci Rep 6:33237. https://doi.org/10.1038/srep332347

Zhao X, Zhou J, Tian R, Liu Y (2022) Microbial volatile organic compounds: antifungal mechanisms, applications, and challenges. Front Microbiol 13:922450. https://doi.org/10.3389/fmicb.2022.922450

Zheng X, Yang Q, Zhang X, Apaliya MT, Ianiri G, Zhang H, Castoria R (2017) Biocontrol Agents increase the specific rate of patulin production by *Penicillium expansum* but decrease the disease and total patulin contamination of apples. Front Microbiol 8:1240