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# Postharvest yeast treatments control strawberry rots and shift the fruit microbiome

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ABSTRACT

Strawberries are highly perishable fruit and managing postharvest decay remains a major challenge. Biological control agents have gained increasing attention as sustainable alternatives to synthetic fungicides. However, their effects on the fruit-associated microbial communities as well as on quality parameters have not been widely investigated. This study explored the use of antagonistic yeasts as a sustainable strategy to control postharvest decay on strawberries, focusing on the selection of effective strains and on evaluating the impact of their use on fruit quality and microbiome. Epiphytic and endophytic yeasts were screened for their biocontrol activity on naturally infected fruit. The most effective strains, identified as *Aureobasidium pullulans* and *Metschnikowia pulcherrima*, were further tested for their efficacy. The applied yeasts significantly reduced gray mold incidence and severity compared to the untreated control after 10 days of cold storage and two days of shelf-life, showing results comparable to those of a commercial M. *fructicola*-based biofungicide. None of the treatments, however, had any significant effect on key fruit quality parameters, such as firmness, total soluble solids and titratable acidity. Metabarcoding analysis revealed that yeast application significantly affected the fruit microbiome, reshaping both fungal and bacterial communities. The antagonistic strains generally successfully colonized the fruit surface and reduced *Botrytis* abundance. Furthermore, they promoted the development of beneficial fungal and bacterial taxa, particularly *Achromobacter*. These findings suggest that mechanism of action of yeast biocontrol agents extends beyond direct pathogen inhibition, fostering interactions with resident microbial communities and contributing to the establishment of a beneficial fruit microbiome to prevent disease development.

## 1. Introduction

Postharvest diseases, primarily caused by fungal pathogens, are a major constraint to the quality, shelf-life and marketability of fresh fruit. Strawberries (*Fragaria* × *ananassa*) are among the most widely consumed berries worldwide, valued for their flavour, bright colour and as a source of nutrients, vitamins and health promoting antioxidants (Giampieri et al., 2012). However, they are particularly perishable due to their soft texture, high moisture content, lack of protective skin and susceptibility to mechanical damage. Consequently, wounds occurring during harvesting, postharvest handling or transportation greatly facilitate fungal infections, leading to rapid decay and limiting their commercialization (Feliziani and Romanazzi, 2016; Romanazzi et al., 2019). *Botrytis cinerea*, the causal agent of gray mold, is considered the most important pathogen affecting strawberries. Infections mainly occur in the field on stolons, leaves and fruit and can remain latent until storage, where high relative humidity and low temperatures slow down the fruit defence responses, promoting disease development. Other fungal genera such as *Rhizopus*, *Mucor*, *Penicillium* and *Colletotrichum* are also commonly associated with postharvest decay (Feliziani and Romanazzi, 2016).

Disease management is currently based on preventive treatments

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with synthetic fungicides, primarily targeting *B. cinerea*. However, their use is not permitted in organic farming, whereas in integrated management systems only preharvest applications are allowed (Romanazzi et al., 2019). Additionally, growing concerns about pesticide residues, environmental impact and development of resistant pathogen strains have driven the search for more sustainable alternatives. Several approaches have been explored for postharvest disease control on fruit, particularly on strawberries, including physical treatments (Nigro et al., 2000), inducers of resistance (Rajestary et al., 2023; Romanazzi et al., 2013), essential oils and natural compounds (Buonsenso et al., 2023; Pansera et al., 2025), and edible coatings (Vu et al., 2011). Biological control based on microbial antagonists has emerged as a promising tool to reduce chemical inputs. Yeasts and yeast-like fungi are particularly suitable for this purpose, as they combine biocontrol efficacy features with a high tolerance to environmental stresses, such as low water activity and fluctuations in temperature, pH and humidity (Zhang et al., 2011). Their adaptability to the sugar-rich, osmotically challenging fruit surfaces further supports their colonization and persistence (Piombo et al., 2018). Moreover, unlike filamentous fungi, yeasts do not produce allergenic spores or mycotoxins harmful to humans (Droby et al., 2016; Spadaro and Droby, 2016).

In recent years, it has become generally recognized that developing effective disease management strategies requires considering the multitrophic interactions taking place in the postharvest system, that includes the fruit, the pathogen, the biocontrol agents (BCAs) and the fruit-associated microbiome (Droby et al., 2016, 2009; Wisniewski and Droby, 2019; Zhimo et al., 2025). This holistic perspective has been supported by advances in microbiome research, which have highlighted the central role of microbial communities in plant health, productivity and resilience (Berg et al., 2017; Kusstatscher et al., 2020). Fruit, in particular, harbour an abundant and complex microbiota, which was shown to play a key role in maintaining postharvest quality, suppressing diseases and extending shelf-life (Droby and Wisniewski, 2018; Kusstatscher et al., 2020).

These communities can be shaped by multiple factors, including tissue type, spatial distribution on the fruit surface, storage conditions and management practices (Droby and Wisniewski, 2018). Particularly, postharvest treatments such as ozone, hot water dipping, waxing and the use of essential oils have been shown to alter the diversity and abundance of microbial taxa (Abdelfattah et al., 2020; Remolif et al., 2024a; Wassermann et al., 2019; Zhang et al. 2022).

Although several microorganisms have shown efficacy to control postharvest diseases, their ability to integrate within the native fruit-associated communities and modify their composition, influencing the abundance and diversity of other taxa, is still poorly understood (Kusstatscher et al., 2020; Massart et al., 2015). A deeper understanding of these interactions is essential to elucidate how changes induced by the application of BCAs can inhibit the development of fungal pathogens and contribute to an effective disease management.

This study aimed (i) to evaluate the efficacy of several yeasts to control postharvest decay of strawberries through *in vivo* trials, and (ii) to investigate the impact of the selected strains on fruit quality and on the composition of the strawberry-associated microbiome.

## 2. Materials and methods

### 2.1. Microorganisms

Epiphytic and endophytic yeasts were included in this study. Epiphytes were previously isolated from the carposphere of various fruit species (Table 1) and maintained in the collection of the Department of Agricultural, Forest and Food Sciences, University of Turin and in the Turin University Culture Collection (TUCC). The strains were preserved at −80 °C and reactivated prior to use by triple streaking on Malt Extract Agar (MEA; VWR International, Leuven, Belgium) plates.

Endophytic yeasts were isolated from strawberries 'Alba', 'Melissa', 'Rosetta' and 'Clery' (Table 1). Fruit were visually inspected to ensure the absence of rots or physical injuries and were first washed under running tap water to remove surface debris. Surface sterilization was performed by sequential dipping in 70% ethanol for 1 min, 2% sodium hypochlorite for 2 min and 70% ethanol for 30 s. Subsequently, strawberries were rinsed three times in sterile water and let dry under a laminar flow hood on sterile paper. The effectiveness of surface sterilization was verified by plating the final rinse water onto the same media used for endophyte isolation. Surface-sterilized fruit were then transferred into sterile Stomacher filter bags, added with sterile Ringer solution (Merck, Darmstadt, Germany) and homogenized using a Stomacher blender (IUL Instruments, Barcelona, Spain). The resulting filtered homogenate was serially diluted and plated onto Petri dishes containing Rose Bengal Chloramphenicol Agar (RBCA, Scharlab, Lodi, Italy) and MEA, both supplemented with streptomycin sulphate (50 mg/L) to inhibit bacterial growth. Plates were incubated at 25 °C for 5–7 days. Colonies showing yeast morphology were sub-cultured onto new MEA plates to obtain pure cultures. Isolated endophytic yeasts were preserved at −80°C in the collection of the University of Turin.

**Table 1**
Epiphytic and endophytic yeast strains used in this study.

| Epiphytes | |
|---|---|
| Strain | Isolation source |
| AP1 | *Malus* x *domestica* cv. Fuji |
| AP2 (TUCC00001040) | *Malus* x *domestica* cv. Ambrosia |
| AP47 (TUCC00000499) | *Malus* x *domestica* cv. Golden Delicious |
| CAR1 | *Malus* x *domestica* cv. Fuji |
| MS (TUCC00000498) | *Malus* x *domestica* cv. Golden Delicious |
| PL5 (TUCC00000491) | *Prunus domestica* cv. Angeleno |
| Endophytes | |
| Strain | **Isolation source** |
| AL1B | *Fragaria* x *ananassa* cv. Alba |
| FR4A (TUCC00000658) | *Fragaria* x *ananassa* cv. Clery |
| FR9A | *Fragaria* x *ananassa* cv. Clery |
| FR9B | *Fragaria* x *ananassa* cv. Clery |
| FR9C | *Fragaria* x *ananassa* cv. Clery |
| MEL1A | *Fragaria* x *ananassa* cv. Melissa |
| ROS2C | *Fragaria* x *ananassa* cv. Rossetta |
| ROS2D | *Fragaria* x *ananassa* cv. Rossetta |

### 2.2. Screening trials

Two screening trials were carried out to assess the potential antagonistic activity of the yeast strains. Strawberries 'Clery', free from decay and physical injuries, were used to conduct the experiments. The antagonistic activity of the yeasts was evaluated against naturally occurring infections, therefore fruit were not surface-disinfected. Each treatment group included three replicates, with each replicate containing 250 g of strawberries.

Six epiphytes and eight endophytes were used for the first and the second trial, respectively. Yeast strains were cultured in Erlenmeyer flasks containing 30 mL of Yeast extract-Peptone-Dextrose broth (YPD; 10 g/L yeast extract, 20 g/L peptone, 20 g/L d(+)-glucose) and incubated on a rotary shaker at 100 rpm at 25 ± 1 °C. After 48 h, yeast cells were harvested by centrifugation at 4000 x g for 10 min, washed and resuspended in sterile Ringer solution. Cell concentration was determined using a haemocytometer and adjusted to $1 \times 10^8$ cells/mL. The commercial biofungicide Noli® (Koppert, The Netherlands), based on *Metschnikowia fructicola* strain NRRL Y-27328, was included in the trials and prepared following the manufacturer label.

Strawberries were treated by spraying the yeast suspensions on their surface and air dried. Fruit sprayed with sterile water served as untreated control. Fruit were maintained at 1 ± 1 °C in cold storage under normal atmosphere and 90% relative humidity (RH). Disease incidence was assessed after 10 days and expressed as percentage of decayed fruit per treatment.

### 2.3. Identification of the most effective yeasts

The most effective strains in the screening trials were selected for further analyses and molecularly identified. Genomic DNA was extracted from liquid cultures obtained growing the strains for 48 h in YPD broth at 25°C. Cell suspensions were centrifuged at 4000 x g for 10 min, the supernatant was discarded and the cell pellets were washed twice with sterile distilled water to remove any residual medium. DNA extraction from the pellets was performed using the E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-Tek, Norcross, GA, USA), following the manufacturer instructions. The D1/D2 domains of the LSU rRNA gene were amplified using the primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3'; Kurtzman and Robnett, 1998). PCR reactions were performed in 25 μL, containing 2.5 μL of 10x Qiagen PCR Buffer, 1.4 μL of $MgCl_2$ (25 mM), 1.4 μL of dNTPs (10 mM), 0.5 μL of each primer (10 μM), 0.2 μL of Taq DNA Polymerase (Qiagen, Hilden, Germany) and 1 μL of template DNA (20 ng). Amplifications were carried out under the following conditions: initial denaturation at 94 °C for 3 min, followed by 34 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were analysed by electrophoresis on 1% agarose gel (VWR Life Science AMRESCO® biochemicals) stained with GelRed™ and sequenced in both directions by Macrogen Europe (Milan, Italy). Consensus sequences were generated using Geneious v11.1.5 (Biomatters, Auckland, New Zealand) and identified by comparison with reference sequences in the NCBI database using the BLASTn algorithm. All sequences obtained in this study have been deposited in GenBank.

### 2.4. Biocontrol efficacy trial

The selected yeast strains were further tested on naturally infected strawberries cv. Clery, selected for uniform size and absence of visible defects. To minimize variability due to batch differences, fruit used for phytopathological survey, quality and microbiome analyses were taken from the same batch and randomly assigned to treatments. Yeast cell suspensions were prepared according to the same procedure described for the screening trials. Each treatment group included five replicates, with each replicate consisting of a fruit packaging box containing 250 g of strawberries. Treatments were applied by spraying the cell suspensions onto the fruit surface, followed by air-drying. Fruit sprayed with sterile water served as untreated controls. Strawberries were stored for 10 days at 1 ± 1 °C under normal atmosphere and 90% RH, followed by a shelf-life period of 2 days at 20 ± 1 °C to simulate retail conditions. Disease incidence and severity were assessed at 4, 7 and 10 days of cold storage as well as after shelf-life. Disease incidence was expressed as the percentage of decayed fruit. Weighted disease severity was calculated using the McKinney index (McKinney, 1923), which expresses the weighted average of severity as a percentage of the maximum possible disease level. Severity scores were assigned based on an empirical scale ranging from 0 to 4, where: 0 = healthy fruit; 1 = 1–25% of the fruit surface infected; 2 = 26–50%; 3 = 51–75% and 4 = 76–100%. These scores were then used to calculate the McKinney index according to the following formula:

$$\textit{McKinney index}\ (\%) = \frac{\sum (d \times f)}{N \times D} \times 100$$

where $d$ is the severity class, $f$ is its frequency, $N$ is the total number of fruit assessed (both healthy and rotted) and $D$ is the maximum value of disease severity occurring on the empirical scale.

### 2.5. Fruit quality analyses

Quality analyses were performed by measuring firmness, total soluble solids and titratable acidity at harvest, after 10 days of cold storage and after 2 days of shelf-life, to evaluate the effect of the treatments on strawberry quality. Analyses were conducted on three replicates of 10 strawberries per treatment.

Firmness (N) was determined using a Fruit Texture Analyzer (FTA, Turoni, Italy), equipped with a 3 mm probe. Measurements were taken along the equatorial region of each fruit. For titratable acidity and total soluble solids determination, juice was extracted from the same strawberries used for firmness analysis using a juice extractor. Total soluble solids were measured at 20°C using a digital refractometer (NR151, Rose Scientific Ltd, Canada) and results were expressed in °Brix. Titratable acidity was determined by titration of 6 g of strawberry juice, diluted with 50 mL of distilled water, using a 0.1 M NaOH solution. The endpoint was reached at pH 8.2, measured with a Phenomenal® pH 5000 L pH-meter (VWR International, Leuven, Belgium). Titratable acidity was expressed as percentage of citric acid, calculated with the following equation:

$$\frac{(V_{NaOH} \times 0.0064)}{6} x \quad 100$$

where 0.0064 represents the citric acid factor and 6 the amount (g) of juice used for the titration.

### 2.6. Statistical analyses

Statistical analyses for disease incidence, McKinney index and quality parameters were performed using RStudio version 4.3.1 (R Core Team, 2023). Data were subjected to one-way analysis of variance (ANOVA) after verifying normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. A log transformation was applied to data that did not meet the assumption of normality. Statistical significance was assessed at $p < 0.05$ level. Tukey's HSD test was used for post-hoc pairwise comparisons among treatments.

### 2.7. Microbiome sampling, DNA extraction and sequencing

Microbiome sampling was carried out at harvest and after 10 days of cold storage, to evaluate the impact of the applied treatments on the fruit microbial communities. Four replicates, each consisting of 250 g of strawberries, were sampled per treatment. Each replicate was mixed with 100 mL of sterile Ringer solution and homogenized using a Stomacher blender. The resulting homogenate was transferred into sterile 50 mL tubes and sonicated for 10 min. Samples were then centrifuged at 6000 × g for 20 min and the supernatant was discarded. DNA was extracted from the resulting pellet using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), following the manufacturer protocol with minor modifications. Specifically, the 10-minute vortex step was replaced by a 30-minute agitation with a TissueLyser (Qiagen). Moreover, the agitation, centrifugation and supernatant recovery steps were repeated after the addition of 500 μL of lysis buffer SL2 (Macherey-Nagel GmbH & Co. KG, Düren, Germany) to the same PowerBeads Pro tubes.

Extracted DNA was quantified using the Qubit dsDNA HS Assay Kit (ThermoFisher, Waltham, USA) and normalized to 5 ng/μL. PCR were conducted to amplify the bacterial 16S rRNA region using primers 341 f (5'-CCTACGGGNGGCWGCAG-3'; Klindworth et al., 2013) and 805r (5'-GACTACHVGGGTATCTAATCC-3'; Herlemann et al., 2011), and the fungal internal transcribed spacer 2 (ITS2) region using primers ITS3_kyo2 (5′-GATGAAGAACGYAGYRAA-3′; Toju et al., 2012) and ITS4ngs (5′-TTCCTSCGCTTATTGATATGC-3′; Tedersoo et al., 2014). In both cases, standard Illumina overhang adapter sequences were added to the locus-specific primers. PCR reactions were carried out with an initial denaturation at 95°C for 3 min, followed by 25 cycles of denaturation (95°C for 30 s), annealing (55°C for 30 s) and extension (72°C for 30 s), with a final extension at 72°C for 10 min. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter Genomics) and indexed according to the Illumina Sequencing Library

Preparation protocol (Illumina, San Diego, USA).

Amplicons were quantified using the Qubit dsDNA HS Assay Kit, diluted to 4 nM, denatured with 0.2 N NaOH, and spiked with 20% (v/v) PhiX. The pooled libraries, including PhiX, were adjusted to a final concentration of 8 pM. Paired-end sequencing (2 × 250 bp) was performed on the MiSeq platform using the MiSeq Reagent Kit v2 (Illumina, San Diego, USA), following the manufacturer's standard protocol.

### 2.8. Bioinformatics

Sequencing data analysis was carried out separately for fungal and bacterial microbiomes using the QIIME2 suite (Bolyen et al., 2019). Adapter sequences were trimmed with the Cutadapt plugin (Martin, 2011) and the resulting sequences were filtered and denoised with the DADA2 plugin (Callahan et al., 2016), both run using default parameters.

Generated Amplicon Sequence Variants (ASVs) were taxonomically classified using two Naïve-Bayes predictors trained on the UNITE v10.0 (Abarenkov et al., 2024) and on SILVA v138 (Quast et al., 2012) for fungi and bacteria, respectively, with a minimum confidence threshold of 0.9. Host sequences were removed, and features with a prevalence lower than 10% were discarded to exclude probable contaminants. Raw genus-level composition data were visualized as bar plots generated with Matplotlib v3.8.2 (Hunter, 2007).

Alpha diversity was estimated by Shannon index, number of observed features and Pielou evenness after sample normalization with the SRS (Scaling with Ranked Subsampling) method implemented in the s2-SRS plugin (Heidrich et al., 2021). The normalization depth was set to 2000 reads for fungi and 780 reads for bacteria, as a compromise between sequencing depth and the number of samples retained across time points and treatments. Statistical significance was evaluated by Kruskal–Wallis tests followed by Dunn's post hoc tests, applying Benjamini–Hochberg correction for false discovery rate (FDR), with a rejection threshold set at 0.05.

Beta diversity was computed using the Robust Aitchison distance. Sample normalization and metric calculation were performed with DEICODE (Martino et al., 2019), using the same normalization depths as for alpha diversity. Significant differences in variance were detected with a Permutational Analysis of Variance (PERMANOVA) as integrated in plugin Adonis (Anderson, 2001; Oksanen et al., 2001), with significance threshold set at 0.05. Pairwise differences in centroid variance and dispersion were determined by means of pairwise PERMANOVA and Permutational Dispersion Analysis (PERMDISP), respectively. Dimensionality reduction with the Principal Coordinate Analysis (PCoA) and representation of beta diversity was performed using the PCoA plugin (Halko et al., 2011).

Differential abundance of genera between the untreated control and yeasts treatments at the end of storage was assessed using Analysis of Composition of Microbiomes with Bias Correction (ANCOM-BC; Lin and Peddada, 2020). The analysis was performed with a sample feature count cutoff of 400 for bacteria and 8000 for fungi, as well as a prevalence cutoff of 0.5 for both taxa. Statistical significance threshold was set at 0.05 after Benjamini-Hochberg correction.

## 3. Results

### 3.1. Screening trials and identification of the most effective yeasts

The efficacy of yeast treatments in reducing postharvest decay on strawberries, predominantly caused by gray mold, was evaluated after 10 days of storage at 1 ± 1 °C (Table 2). The tested yeasts exhibited varying levels of efficacy. Among the epiphytes, three strains (PL5, MS and AL27) showed gray mold incidence values ranging from 19.0% to 26.6%, significantly lower than the untreated control (40.1%). Notably, PL5 and MS treatments resulted in the lowest values (19.0% and 19.5%, respectively), statistically comparable to those observed for strawberries treated with the formulated *M. fructicola* NRRL Y-27328 (25.2%).

**Table 2**
Gray mold incidence (%) on strawberries treated with epiphytic (A) and endophytic (B) yeasts stored at 1 ± 1°C for 10 days at 90% RH. Values of each trial, followed by the same letter, are not significantly different by Tukey HSD test ($p < 0.05$).

| A) Epiphytic yeasts trial | | B) Endophytic yeasts trial | |
|---|---|---|---|
| Treatment | Disease incidence (%) | Treatment | Disease incidence (%) |
| PL5 | 19.0 ± 3.9 a | FR4A | 14.8 ± 8.0 a |
| MS | 19.5 ± 9.7 a | FR9B | 20.7 ± 6.0 a |
| *M. fructicola* NRRL Y-27328 (Noli) | 25.2 ± 3.7 a | FR9A | 23.1 ± 7.9 ab |
| AP47 | 26.6 ± 5.9 ab | MEL1A | 24.2 ± 6.1 ab |
| AP2 | 31.3 ± 2.0 b | AL1B | 24.8 ± 7.6 ab |
| AP1 | 32.4 ± 9.3 bc | *M. fructicola* NRRL Y-27328 (Noli) | 26.5 ± 2.9 ab |
| CAR1 | 35.4 ± 2.1 bc | ROS2D | 35.6 ± 9.4 b |
| Untreated control | 40.1 ± 6.1c | ROS2C | 36.0 ± 8.4 b |
| | | FR9C | 36.4 ± 2.7 b |
| | | Untreated control | 42.4 ± 5.8c |

Considering the endophytic yeasts, five strains (FR4A, FR9B, FR9A, MEL1A and AL1B) significantly reduced the percentage of infected fruit compared to the untreated control (42.4%), with values ranging from 14.8% to 26.5%. Strain FR4A was the most effective (disease incidence 14.8%), followed by FR9B (20.7%), with both values comparable to those reported for fruit treated with the commercial *M. fructicola* (26.5%). Based on these findings, strains PL5, MS, FR4A and FR9B were selected for further analyses.

The strain PL5 had previously been identified as *Aureobasidium pullulans* (Zhang et al., 2010a), while the identity of strains MS, FR4A and FR9B was determined in this study. Strains FR4A and FR9B showed 100% sequence identity with the *A. pullulans* type strain (CBS 584.75), while strain MS shared 99% identity with the *Metschnikowia pulcherrima* reference strain NRRL Y-7111, based on BLAST analysis of the D1/D2 domains of the LSU rRNA gene. The obtained sequences were deposited in GenBank under the accession numbers PX602228 (FR4A), PX602229 (FR9B) and PX602230 (MS).

### 3.2. Biocontrol efficacy trial

An efficacy trial was performed with the strains selected from the preliminary screening. The efficacy of the yeasts in reducing postharvest decay on strawberries was assessed after 4, 7 and 10 days of storage at 1 ± 1 °C (Supplementary Figure 1), as well as after 2 days of shelf-life at 20 ± 1 °C. Decay was due to gray mold, confirmed by isolation of *Botrytis cinerea* from infected fruit.

Considering disease incidence (Fig. 1A), all strains significantly reduced the percentage of infected fruit compared to the untreated control at all time points, with the exception of *A. pullulans* FR9B at 4 days of storage. Although disease incidence increased slightly over time in treated fruit, values remained consistently low throughout the storage period and ranged from 9% to 12% at 4 days, 10–16% at 7 days and 13–19% at 10 days. The untreated control exhibited a more pronounced increase, with gray mold incidence values of 19%, 30% and 32% at 4, 7 and 10 days, respectively. During shelf-life, disease incidence further increased in all samples but remained significantly lower in treated fruit (44–52%) compared to the control (70%).

All the tested strains performed comparably to the commercial biocontrol agent *M. fructicola* NRRL Y-27328, which showed disease incidence values of 9%, 10%, 13% and 52% at 4, 7 and 10 days of storage and after 2 days of shelf-life, respectively. Among the tested strains, *A. pullulans* FR4A consistently showed the lowest values.

Weighted disease severity, expressed with the McKinney index, was significantly lower in treated fruit than in the control at all time points (Fig. 1B), reflecting the trend observed for disease incidence. Although

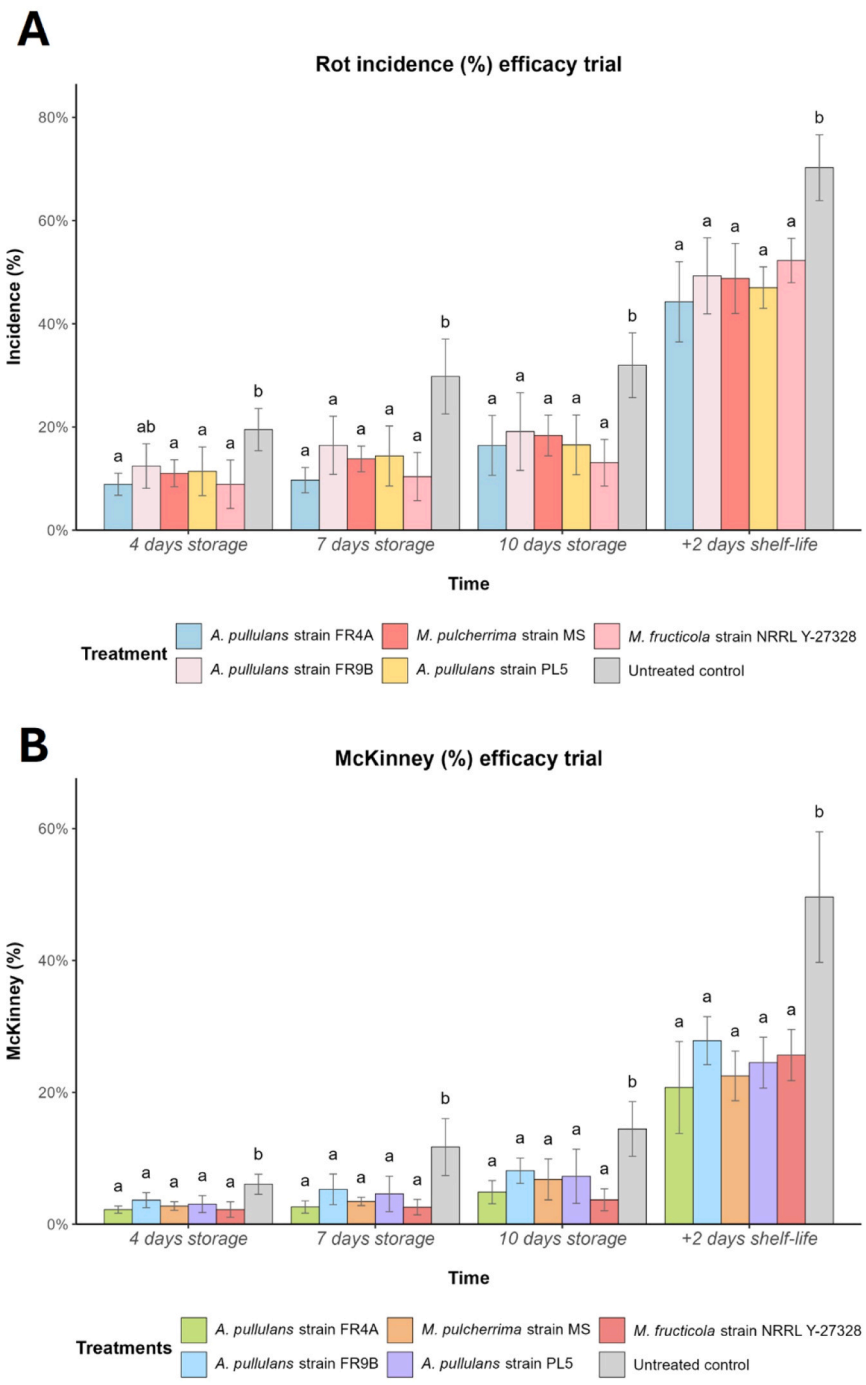


**Fig. 1.** Disease incidence (A) and McKinney index (B) of strawberries treated with antagonistic yeasts after 4, 7 and 10 days of storage at 1 ± 1°C at 90% RH and after 2 days of shelf-life at 20 ± 1°C. Values at the same time point, followed by the same letter, are not significantly different by Tukey HSD test ($p < 0.05$).

differences were detectable after 4 days of storage, they became more pronounced at 7 and 10 days, with treated fruit showing McKinney index values ranging from 2.6% to 5.3% and 3.7–8.1%, respectively, compared to 11.7% and 14.4% in the untreated control. During shelf-life, severity increased in all samples, but remained considerably lower in treated fruit (20.7%-27.8%) than in the control (49.6%). Among the treatments, *A. pullulans* strain FR4A exhibited the lowest values, followed by *M. pulcherrima* strain MS.

### 3.3. Fruit quality analyses

Firmness, total soluble solids (TSS) and titratable acidity (TA) were measured at harvest, after 10 days of cold storage and following a subsequent 2-day shelf-life at 20°C (Table 3) to evaluate the effects of the treatments on fruit quality parameters. Detected changes were consistent with the normal ripening process of strawberries over time, with a general decrease in firmness and TA and an increase in TSS. Regarding the effects of the treatments, no significant differences were observed between treated strawberries and the untreated control for all the three parameters both after storage and after shelf-life ($p > 0.05$).

**Table 3**
Firmness, total soluble solids (TSSs), and titratable acidity (TA) of strawberries treated with antagonistic yeasts. Values are expressed as the mean of n = 3 replicates of 10 fruit ± standard deviation (SD). For each analysis, values with the same letter are not statistically different by Tukey HSD test ($p < 0.05$).

| Time point | Treatment | Firmness (N/cm$^2$) ± SD | TSS (° Brix) ± SD | TA (%) ± SD |
|---|---|---|---|---|
| Harvest | - | 1.93 ± 0.37 | 8.30 ± 0.10 | 0.88 ± 0.09 |
| 10 days storage (1 ± 1 °C) | *M. pulcherrima* MS | 1.69 ± 0.28 a | 8.68 ± 0.60 a | 0.87 ± 0.05 a |
| | *A. pullulans* FR4A | 1.88 ± 0.46 a | 8.98 ± 0.45 a | 0.88 ± 0.10 a |
| | *A. pullulans* FR9B | 1.88 ± 0.57 a | 8.98 ± 0.32 a | 0.78 ± 0.04 a |
| | *A. pullulans* PL5 | 1.58 ± 0.11 a | 9.15 ± 0.33 a | 0.87 ± 0.18 a |
| | *M. fructicola* NRRL Y-27328 (Noli) | 1.83 ± 0.03 a | 8.45 ± 1.03 a | 0.86 ± 0.08 a |
| | Untreated control | 1.52 ± 0.32 a | 8.37 ± 0.52 a | 0.75 ± 0.08 a |
| 2 days shelf-life (20 ± 1 °C) | *M. pulcherrima* MS | 1.40 ± 0.04 a | 8.92 ± 0.46 a | 0.78 ± 0.08 a |
| | *A. pullulans* FR4A | 1.48 ± 0.21 a | 9.02 ± 0.80 a | 0.75 ± 0.10 a |
| | *A. pullulans* FR9B | 1.31 ± 0.24 a | 9.18 ± 0.28 a | 0.77 ± 0.04 a |
| | *A. pullulans* PL5 | 1.52 ± 0.28 a | 9.22 ± 0.32 a | 0.81 ± 0.08 a |
| | *M. fructicola* NRRL Y-27328 (Noli) | 1.50 ± 0.41 a | 8.78 ± 0.10 a | 0.76 ± 0.01 a |
| | Untreated control | 1.35 ± 0.13 a | 8.73 ± 0.62 a | 0.71 ± 0.05 a |

### 3.4. Fungal microbiome

#### 3.4.1. Fungal diversity

Alpha diversity analysis of the fungal community (Fig. 2) revealed that overall richness, as measured by the Shannon index, was statistically comparable at the end of storage between the yeast treatments and the untreated control (1.88 ± 0.65). Among treatments, *A. pullulans* strain FR9B exhibited a significantly higher Shannon index (3.83 ± 0.15) than both *A. pullulans* strains PL5 (0.81 ± 0.26) and FR4A (1.36 ± 0.78).

Considering the number of observed features, no significant differences were found among the treatments and the control at the end of the storage (32.33 ± 4.72) or the harvest samples (33.67 ± 12.50). However, *A. pullulans* FR9B-treated strawberries showed significantly higher values than those treated with *M. fructicola* NRRL Y-27328 (26.75 ± 8.26) or *A. pullulans* PL5 (22.0 ± 4.55).

Similarly, Pielou evenness values did not differ significantly between treatments and the control after storage (0.37 ± 0.12), or samples at harvest (0.60 ± 0.06). Nonetheless, *A. pullulans* FR9B-treated samples showed significantly higher evenness (0.67 ± 0.03) compared to those treated with *A. pullulans* strains PL5 (0.18 ± 0.06) and FR4A (0.26 ± 0.14).

Beta diversity analysis based on Adonis revealed that both treatments and sampling timepoints significantly influenced the fungal community composition (Table 4). However, treatments explained a much larger proportion of the total variance ($R^2 = 0.88$, $q = 0.001$) compared to the sampling timepoints ($R^2 = 0.03$, $q = 0.016$).

Pairwise PERMANOVA (Supplementary Table 1) confirmed that treatments significantly affected the fungal community structure. Strawberries treated with *M. fructicola* NRRL Y-27328 harboured fungal communities that were statistically distinct from those observed in all other groups. A clear separation was also observed for samples treated with *A. pullulans* FR4A, whose fungal profile differed significantly from all the other treatments except for *A. pullulans* PL5. In contrast, no significant differences were detected between the communities of untreated fruit, both at harvest and after storage, and those treated with *M. pulcherrima* MS. Additionally, fungal communities of *M. pulcherrima* MS-treated samples did not significantly differ from those treated with *A. pullulans* FR9B.

PERMDISP analysis (Supplementary Table 1) revealed that differences in dispersion among groups were generally limited. Significant variation in within-group dispersion was observed only between samples treated with *A. pullulans* FR4A and *M. fructicola* NRRL Y-27328, and between *A. pullulans* FR9B and both *M. fructicola*-treated and untreated samples at the end of storage.

These patterns were reflected in the PCoA plot (Fig. 3), which revealed a clear clustering of samples according to the treatment. Strawberries treated with *M. fructicola* NRRL Y-27328 formed a distinct group, separated from all other treatments. This separation was strongly correlated with an increased abundance of ASVs assigned to the genus *Metschnikowia*. Untreated samples at harvest and at the end of storage generally clustered in a similar region of the ordination space, suggesting limited changes in the microbial community over time in the absence of treatment. Samples treated with *M. pulcherrima* MS and *A. pullulans* FR9B formed another group, associated with higher relative abundances of ASVs from the genera *Cladosporium* and *Vishniacozyma*. Lastly, samples treated with *A. pullulans* FR4A and PL5 clustered together and were characterized by ASVs assigned to the genera *Aureobasidium*, *Cystofilobasidium*, and *Pichia*.

#### 3.4.2. Compositional analysis of fungal populations

The composition of the fungal microbiota varied across sampling time and treatments (Fig. 4). At harvest, *Cladosporium* was the dominant genus (46.83%), followed by *Vishniacozyma* (13.75%) and *Metschnikowia* (12.39%). Apart from the pathogenic genus *Botrytis* (8.74%), all the other taxa with a relative abundance greater than 1% were yeasts: *Rhodotorula* (6.82%), *Candida* (2.58%), *Hanseniaspora* (2.15%), *Aureobasidium* (1.52%) and *Sporobolomyces* (1.28%).

At the end of storage, the community composition shifted considerably. In the untreated control, *Botrytis* increased significantly (62.0%), while *Cladosporium* abundance declined to 2.9%. Among the genera with a relative abundance above 1% at harvest, only *Metschnikowia* (24.53%) persisted at high levels, with smaller proportions of *Vishniacozyma* (1.68%), *Aureobasidium* (1.49%) and *Rhodotorula* (1.20%).

Strawberries treated with *A. pullulans* strains FR4A and PL5 showed a pronounced dominance of *Aureobasidium* (76.3% and 87.8%, respectively), confirming an effective colonization of the biocontrol agent. In the PL5 treated-fruit, other genera exceeding 1% relative abundance were *Botrytis* (5.83%), *Vishniacozyma* (1.43%) and *Cladosporium* (1.40%). Conversely, FR4A-treated samples showed almost no *Botrytis* and a more varied composition, including *Cystofilobasidium* (11.13%), *Curvibasidium* (4.00%), *Vishniacozyma* (3.05%), *Cladosporium* (1.90%) and *Rhodosporidiobolus* (1.06%).

Similarly, treatments with *M. pulcherrima* MS and *M. fructicola* NRRL Y-27328 resulted in a high abundance of the genus *Metschnikowia* (58.30% and 95.24%, respectively), again indicative of successful fruit colonization. *M. fructicola* treated fruit showed a highly unbalanced community composition, dominated almost exclusively by *Metschnikowia*, with only *Vishniacozyma* above 1% (1.60%). In contrast, the *M. pulcherrima* treatment maintained a more heterogeneous population, with several genera exceeding 1% relative abundance, including *Cladosporium* (10.60%), *Botrytis* (9.93%), *Vishniacozyma* (5.05%), *Aureobasidium* (4.09%), *Rhodotorula* (3.91%), *Hanseniaspora* (2.12%), *Rhodosporidiobolus* (1.51%) and *Curvibasidium* (1.11%).

In strawberries treated with *A. pullulans* FR9B, colonization by the biocontrol agent was lower than the other treatments (5.72%), although higher than in the untreated control. The most abundant genus was *Cladosporium* (22.46%), followed by *Vishniacozyma* (15.59%), *Botrytis* (13.81%) and *Rhodotorula* (12.16%). Other detected genera included *Hanseniaspora* (5.29%), *Gnomoniopsis* (4.60%), *Metschnikowia* (3.70%), *Curvibasidium* (2.81%), *Filobasidium* (2.19%), *Sporobolomyces* (1.91%)

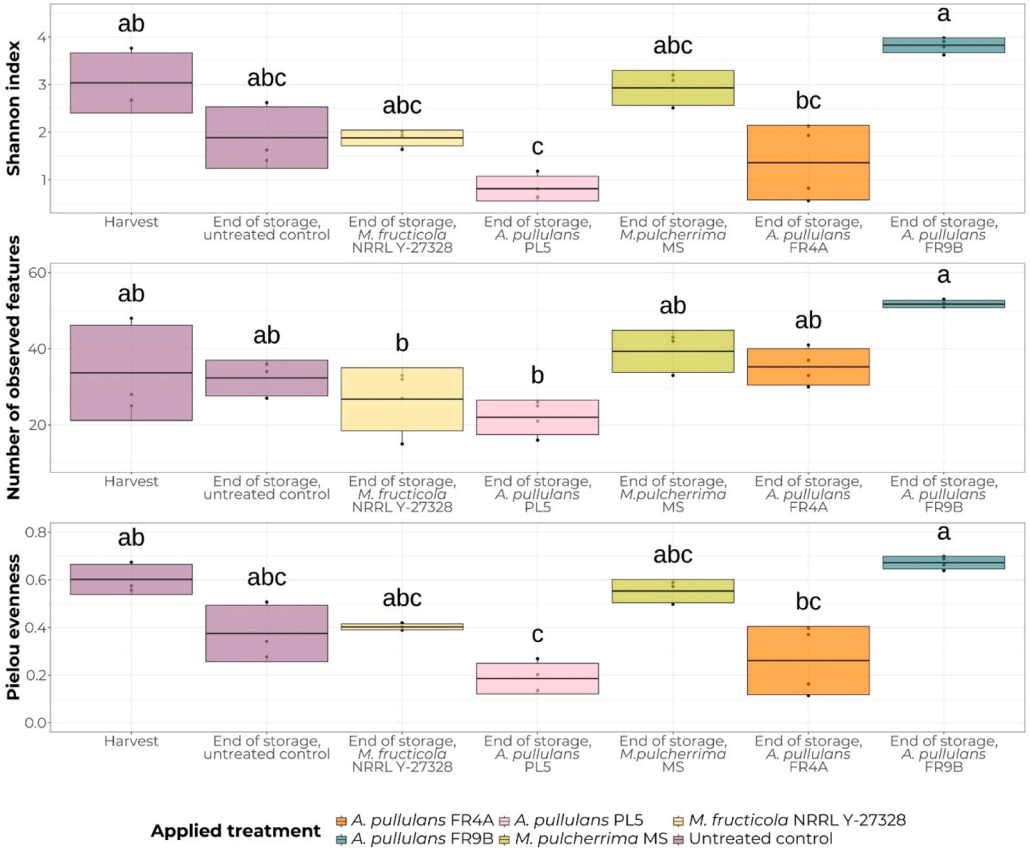


**Fig. 2.** Alpha diversity of fungal communities associated with strawberries across sampling timepoints and treatments. Panels display the Shannon index (top), number of observed features (middle) and Pielou evenness (bottom). In each boxplot, the central line represents the mean, box limits indicate one standard deviation above and below the mean, and whiskers extend to the minimum and maximum values. Statistical differences among groups were evaluated using a Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. Significance groups were assigned using a q-value (FDR-adjusted p-value) threshold of 0.05.

**Table 4**
Results of the Adonis analysis for fungal community composition. Df indicates the degrees of freedom for each variable; F.Model is the pseudo-F test statistic; $R^2$ represents the proportion of total variance explained by the corresponding variable and q-value is the false discovery rate (FDR)-adjusted p-value. The significance threshold was set at 0.05.

| Variable | Df | SumsOfSqs | MeanSqs | F.Model | $R^2$ | q-value |
|---|---|---|---|---|---|---|
| Treatment | 5 | 24.44 | 4.89 | 34.9 | 0.88 | **0.001** |
| Timepoint | 1 | 0.85 | 0.85 | 6.07 | 0.03 | **0.016** |
| Residuals | 18 | 2.52 | 0.14 | NA | 0.09 | NA |
| Total | 24 | 27.81 | NA | NA | 1 | NA |

and *Udeniomyces* (1.28%).

### 3.4.3. Differential abundance analysis of fungal populations

The differential abundance analysis (ANCOM-BC) was performed to identify taxa showing statistically significant differences in relative abundance between each treatment and the untreated control at the end of storage. The analysis highlighted significant shifts induced by the biocontrol agents (Fig. 5). The *A. pullulans* FR4A treatment showed higher abundances of the genera *Aureobasidium* (log fold-change, LFC, 4.32 ± 0.50), *Cystofilobasidium* (LFC 3.06 ± 1.09), *Cryptococcus* (LFC 1.63 ± 0.23) and *Rhodosporidiobolus* (LFC 1.53 ± 0.18) compared to the control, alongside a lower presence of *Botrytis* (LFC −7.02 ± 0.54), *Udeniomyces* (LFC −4.90 ± 1.42), *Metschnikowia* (LFC −4.75 ± 0.77) and *Sporobolomyces* (LFC −0.77 ± 0.23).

In the *A. pullulans* FR9B treated-fruit, higher levels of *Rhodotorula* (LFC 1.60 ± 0.47) and *Sporobolomyces* (LFC 0.63 ± 0.24) were observed, while the genera *Botrytis* (LFC −3.75 ± 0.58) and *Metschnikowia* (LFC −4.00 ± 0.74) showed significant decreases. The *A. pullulans* PL5 treatment resulted in higher abundances of the genera *Aureobasidium* (LFC 5.70 ± 0.40), *Candida* (LFC 2.36 ± 0.58), *Itersonilia* (LFC 2.31 ± 0.85), *Rhodotorula* (LFC 1.81 ± 0.60), *Filobasidium* (LFC 1.42 ± 0.34) and *Sporobolomyces* (LFC 1.19 ± 0.35), together with significantly lower levels of *Botrytis* (LFC −2.44 ± 0.96) and *Metschnikowia* (LFC −3.98 ± 0.88).

The *M. pulcherrima* MS treatment induced higher abundances of genera *Itersonilia* (LFC 2.23 ± 0.79), *Rhodotorula* (LFC 1.77 ± 0.66) and *Aureobasidium* (LFC 1.01 ± 0.20), along with a reduction in *Botrytis* (LFC −2.66 ± 0.60).

Finally, the *M. fructicola* treatment resulted in significantly higher abundances of *Itersonilia* (LFC 3.33 ± 0.71), *Metschnikowia* (LFC 2.53 ± 0.75), *Cryptococcus* (LFC 2.19 ± 0.33), *Rhodosporidiobolus* (LFC 2.11 ± 0.39), *Filobasidium* (LFC 1.77 ± 0.27), *Sporobolomyces* (LFC 1.55 ± 0.41) and *Rhodotorula* (LFC 1.45 ± 0.49), along with a marked reduction of the genus *Botrytis* (LFC −5.09 ± 0.78).

## 3.5. Bacterial microbiome

### 3.5.1. Bacterial diversity

Alpha diversity analysis of the bacterial community (Fig. 6) showed that Shannon index values for all treatments were comparable to those of the untreated control at the end of storage (2.27 ± 0.25) and the samples at harvest (1.46 ± 0.92). The only exception was observed in fruit treated with *A. pullulans* FR9B, which exhibited a significantly lower Shannon index (0.33 ± 0.32) compared to both the untreated control and the commercial strain *M. fructicola* NRRL Y-27328 (2.07 ± 0.56). However, FR9B value did not differ significantly from those of

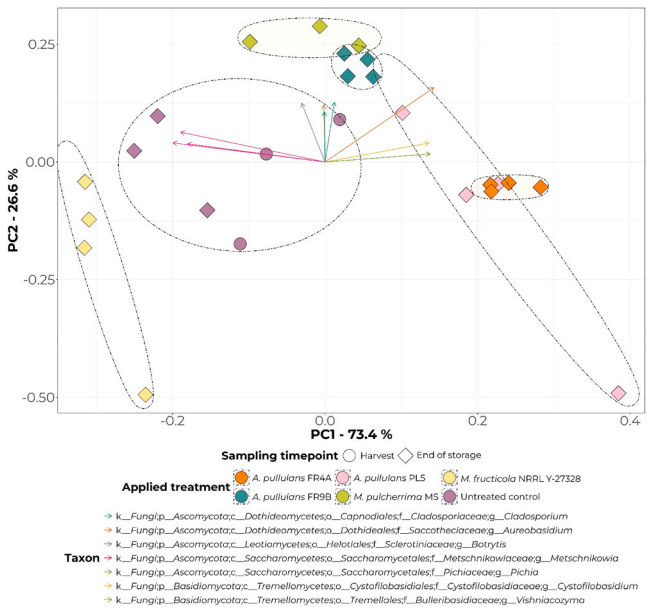


**Fig. 3.** Principal Coordinates Analysis (PCoA) plot of fungal communities associated with strawberries.
Each point represents an individual sample, with colours and shapes indicating treatments and sampling timepoints, respectively. Arrows represent Amplicon Sequence Variants (ASVs) most strongly correlated with the ordination axes: the direction of each arrow reflects the correlation with the principal components, its length is proportional to the strength of the correlation and its colour denotes the corresponding taxonomic assignment. The percentage of variance explained by each principal component is indicated on the respective axis.

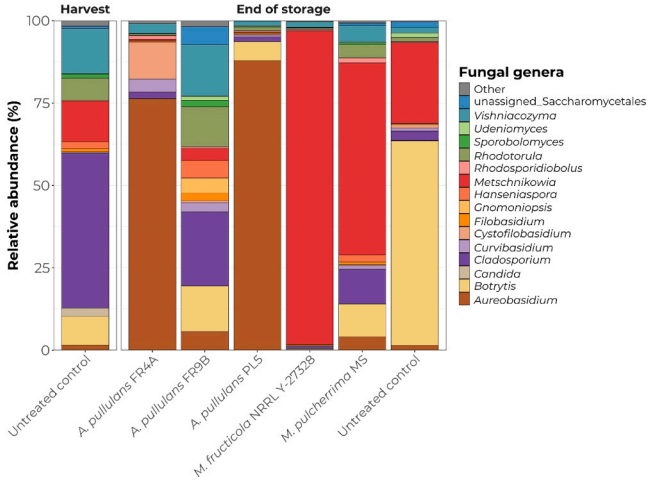


**Fig. 4.** Relative abundance of fungal genera associated with strawberry fruit. The "Other" category includes taxa with less than 1% relative abundance across all groups.

the other treatments.

Regarding the number of observed features, significantly lower values were detected in samples treated with *A. pullulans* strains FR9B (4.25 ± 0.96) and FR4A (4.75 ± 0.96) compared to both the untreated control (11.67 ± 0.58) at the end of storage and to the *M. fructicola* NRRL Y-27328-treated strawberries (13.33 ± 0.58), but comparable with the other treatments. Pielou evenness values were similar across all treatments and timepoints, with no significant differences detected.

For beta diversity, Adonis analysis (Table 5) showed that bacterial community composition was significantly affected by the treatments ($R^2 = 0.71$, $q = 0.001$), whereas the sampling timepoint had no significant effect ($R^2 = 0.01$, $q = 0.616$).

Pairwise PERMANOVA comparisons (Supplementary Table 2) revealed significant differences in bacterial community composition among treatments. Specifically, communities associated with untreated fruit (both at harvest and at the end of storage) and those treated with *M. fructicola* NRRL Y-27328 differed significantly from those treated with the *A. pullulans* strains. No significant differences were found between the three *A. pullulans* treatments, nor between *A. pullulans* FR4A and fruit treated with *M. pulcherrima* MS. PERMDISP analysis indicated that dispersion differences were limited. Specifically, significant differences in dispersion were detected only between *A. pullulans* FR4A and FR9B and the untreated control after storage, and between *A. pullulans* FR9B and PL5.

Sample distribution in the PCoA plot generally reflected these patterns (Fig. 7). Samples treated with *M. fructicola* NRRL Y-27328 and the controls at harvest and after storage clustered together. Samples treated with *A. pullulans* FR4A, FR9B and PL5 grouped together, forming a distinct cluster, whereas FR4A-treated samples partially overlapped with those treated with *M. pulcherrima* MS. Feature vector fitting further supported these trends: the separation of *A. pullulans*-treated samples from *M. fructicola* and control samples was associated with higher relative abundance of ASVs assigned to *Achromobacter*, and to a lesser extent, *Pseudomonas* and *Frateuria*. A lower abundance of *Tatumella*, *Gluconobacter* and unidentified members of the *Enterobacteriaceae* family also contributed to this separation. Additionally, two *Tatumella* ASVs and one ASV assigned to the *Erwiniaceae* family were associated with the distinction between harvest and both post-storage control and *M. fructicola*-treated samples.

### 3.5.2. Compositional analysis of bacterial population

Compositional analysis of the bacterial microbiome revealed distinct community structures among treatments (Fig. 8). At harvest, *Tatumella* was the dominant genus (62.09%), followed by *Achromobacter* (22.14%) and *Pseudomonas* (6.94%). Other genera detected included *Gluconobacter* (2.73%), *Sphingomonas* (2.54%), *Pantoea* (2.36%), *Frigobacterium* (0.68%) and *Methylobacterium–Methylorubrum* (0.35%).

By the end of storage, *Tatumella* remained highly abundant in both *M. fructicola* NRRL Y-27328-treated samples (70.59%) and the untreated control (66.38%). Similarly, *Sphingomonas* increased in both groups, reaching 4.41% and 4.02%, respectively. However, a different trend was observed considering the other bacterial genera. In *M. fructicola*-treated strawberries, *Pseudomonas* increased (8.00%), as did *Methylobacterium–Methylorubrum* (0.68%) and *Erwinia* (0.65%), which was not detected at harvest. The genera *Achromobacter* (12.78%) and *Pantoea* (1.31%) decreased, *Frigobacterium* remained stable (0.61%) and *Gluconobacter* was no longer detected. Conversely, in the untreated control a decrease in *Pseudomonas* (4.82%) and *Achromobacter* (5.02%) was detected, while *Methylobacterium–Methylorubrum* was no longer present. The relative abundance of *Frigobacterium* (2.33%), *Pantoea* (3.53%) and particularly *Gluconobacter* (13.12%) increased compared with harvest levels.

Strawberries treated with the strains FR4A, FR9B and PL5 of *A. pullulans* and with *M. pulcherrima* MS showed a marked increase of the genus *Achromobacter*, with relative abundances of 87.36%, 94.55%, 82.85%, and 75.28%, respectively. However, two distinct patterns emerged within this group. In FR4A- and FR9B-treated fruit, *Pseudomonas* levels were very low (0.28% and 0.11%, respectively), as were *Sphingomonas* (0.28% and 0.02%). By contrast, PL5- and MS-treated fruit showed higher abundances of these genera: *Pseudomonas* increased at 9.33% and 5.96%, while *Sphingomonas* at 4.38% and 1.89%, respectively.

Considering the other taxa, FR9B-treated fruit showed low levels of *Tatumella* (5.26%) and *Gluconobacter* (0.04%), while in FR4A-treated strawberries *Tatumella* (11.18%) and small amounts of *Pantoea* (0.31%) and *Frigobacterium* (0.11%) were detected. In MS-treated samples, *Tatumella* persisted (14.45%) along with small proportions of

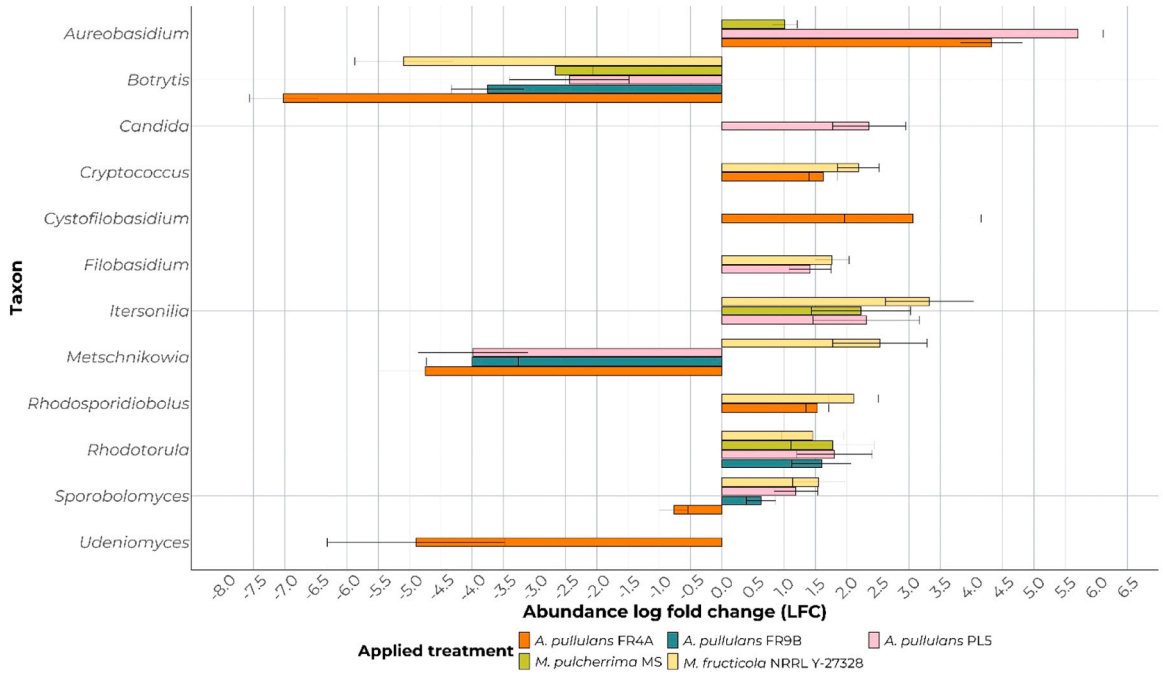


**Fig. 5.** Bar plot of the Analysis of Composition of Microbiomes with Bias Correction (ANCOM-BC) results for fungal genera, comparing each treatment with the untreated control at the end of storage. Only genera showing significant changes in relative abundance (adjusted $p < 0.05$) are displayed. Bar length and direction indicate the log fold change (LFC), while bar colour represents the treatment. Error bars denote the LFC ± one standard error.

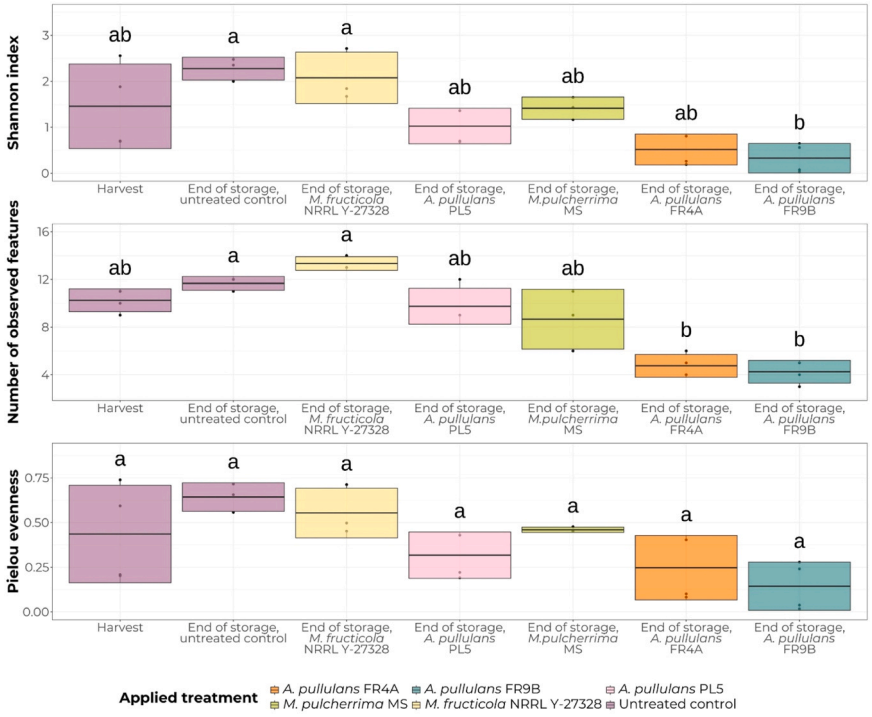


**Fig. 6.** Alpha diversity of bacterial communities associated with strawberries across sampling timepoints and treatments. Panels display the Shannon index (top), number of observed features (middle) and Pielou evenness (bottom). In each boxplot, the central line represents the mean, box limits indicate one standard deviation above and below the mean, and whiskers extend to the minimum and maximum values. Statistical differences among groups were evaluated using a Kruskal-Wallis test, followed by Dunn's post-hoc comparisons. Significance groups were assigned using a q-value (FDR-adjusted p-value) threshold of 0.05.

*Pantoea* (0.69%), *Erwinia* (0.21%) and *Gluconobacter* (0.63%). Strawberries treated with PL5 contained only minor amounts of other genera, including *Tatumella* (0.63%), *Frigobacterium* (0.83%), *Pantoea* (0.43%), *Methylobacterium-Methylorubrum* (0.15%) and *Erwinia* (0.05%).

### 3.5.3. Differential abundance analysis of bacterial population

The differential abundance analysis revealed that only a few bacterial genera showed statistically significant changes in response to the treatments compared to the control (Fig. 9). Strawberries treated with *M. pulcherrima* MS and *A. pullulans* FR9B, FR4A and PL5 showed a

**Table 5**
Adonis analysis results for bacterial community samples. *Df* are the degrees of freedom for each variable; *F.Model* is the pseudo-F test statistic; $R^2$ represents the proportion of total variance explained by the corresponding variable and *q*-value is the false discovery rate (FDR)-adjusted p-value. The significance threshold was set at 0.05.

| Variable | Df | SumsOfSqs | MeanSqs | F.Model | R2 | q-value |
|---|---|---|---|---|---|---|
| Treatment | 5 | 18.76 | 3.75 | 8.93 | 0.71 | **0.001** |
| Timepoint | 1 | 0.13 | 0.13 | 0.31 | 0.01 | 0.616 |
| Residuals | 18 | 7.56 | 0.42 | NA | 0.28 | NA |
| Total | 24 | 26.46 | NA | NA | 1 | NA |

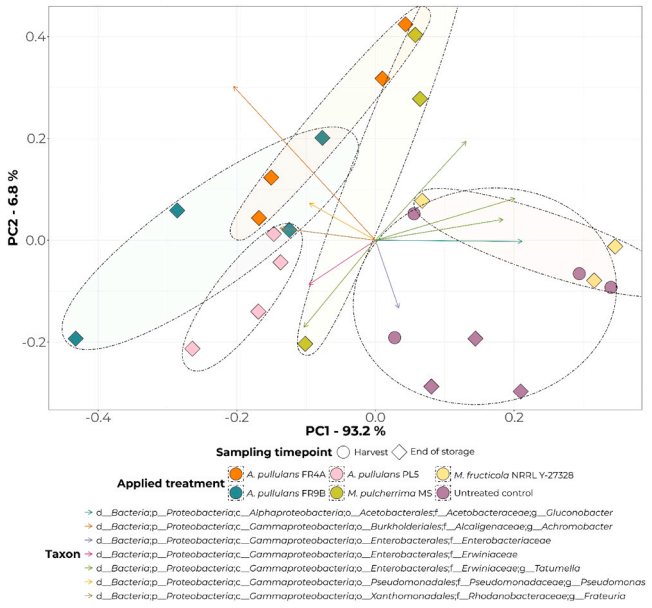


**Fig. 7.** PCoA plot of bacterial communities associated with strawberries. Each point represents an individual sample, with colours and shapes indicating treatments and sampling timepoints, respectively. Arrows represent ASVs most strongly correlated with the ordination axes: the direction of each arrow reflects the correlation with the principal components, its length is proportional to the strength of the correlation, and its colour denotes the corresponding taxonomic assignment. The percentage of variance explained by each principal component is indicated on the respective axis.

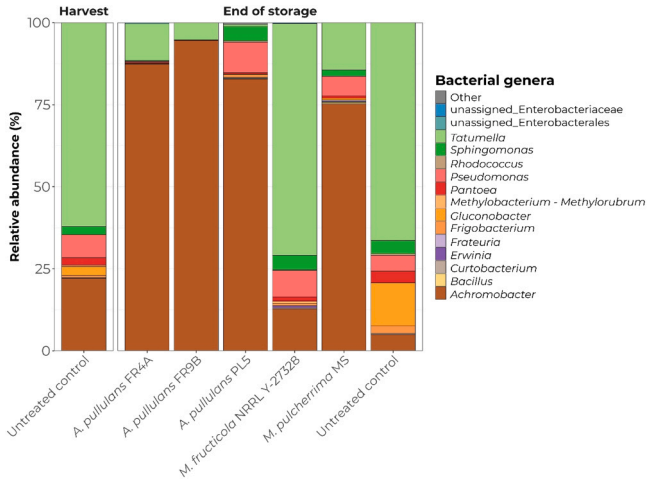


**Fig. 8.** Relative abundance of bacterial genera associated with strawberry fruit. The "Other" category includes taxa with less than 0.05% relative abundance across all groups.

significant increase in *Achromobacter* (LFC = 4.41 ± 0.52, 7.39 ± 0.48, 6.65 ± 0.46, and 2.88 ± 0.34, respectively). Additionally, treatment with *A. pullulans* PL5 resulted in a significant decrease in *Tatumella* (LFC = –4.96 ± 0.58). No significant changes were detected in *M. fructicola* NRRL Y-27328-treated samples.

## 4. Discussion

Effective postharvest management of fruit decay is a critical challenge for strawberry production (Feliziani and Romanazzi, 2016). The use of BCAs has gained increasing interest over time to control pre- and postharvest fruit diseases. However, their impact on the fruit-associated microbial communities, besides the effect on the quality parameters, has not been widely investigated. This study explored the use of antagonistic yeasts as a sustainable strategy to reduce postharvest decay of strawberries, focusing on screening and selecting the most effective strains and evaluating their effects on both fruit quality and microbiome composition.

Several strains, including epiphytes isolated from several fruit surfaces and endophytes obtained from strawberry tissues, were screened for their antagonistic activity through *in vivo* assays under controlled conditions. *In vivo* screening was preferred over *in vitro* testing, as it provides more reliable results that better reflect real postharvest conditions. This preliminary evaluation permitted to identify four yeast strains, both epiphytes and endophytes, that significantly reduced gray mold incidence compared to the untreated control after 10 days of cold storage and were selected for further investigation. While the use of epiphytes is well established for managing postharvest fruit diseases (Droby et al., 2019), the application of endophytes, particularly on strawberries, has only recently gained attention. Their potential lies in efficient colonization of fruit tissues, high adaptability to the host environment and ability to produce bioactive compounds, that may contribute to the biocontrol activity. Moreover, endophytes can potentially be efficient in competing against latent pathogens, such as *Botrytis cinerea* on strawberries, whose infections often occur in the field and can remain quiescent until storage (Kumar et al., 2021; Romanazzi et al., 2019). Previous studies have reported the successful use of a few endophytic bacteria on strawberries, such as *Bacillus* spp. and *Pantoea* spp. (Moura et al., 2021), as well as *Lactobacillus plantarum* (Chen et al., 2020), to control gray mold. However, information about the application of endophytic yeasts on strawberries to control postharvest decay is currently lacking.

The most effective yeasts, identified as *Aureobasidium pullulans* and *Metschnikowia pulcherrima*, were subsequently tested on a larger number of fruit to confirm their antagonistic activity. All four strains significantly reduced both disease incidence and severity compared to the untreated control, both during cold storage and subsequent shelf-life. Although a gradual increase in decay was observed over time and strawberries were generally highly deteriorated by the end of the shelf-life period, the treated fruit consistently showed significantly lower disease incidence than the untreated control. Notably, *A. pullulans* strain FR4A was the most effective, followed by *M. pulcherrima* strain MS.

*Aureobasidium pullulans* has been widely studied for its biocontrol activity against several pathogens. Specifically, its efficacy to control gray mold has been demonstrated on various fruit, including apples (Mari et al., 2012), kiwifruit (Di Francesco et al., 2018) and table grapes (Parafati et al., 2015). On strawberries, successful control of gray mold has been achieved through preharvest applications in the field over two years, where *A. pullulans* reduced postharvest gray mold severity and extended the shelf-life of the harvested fruit (Iqbal et al., 2021). Lima et al. (1997) also reported effective control of *B. cinerea* on strawberries grown under plastic tunnels when a strain of *A. pullulans* was applied during flowering or at fruit maturity. It should be emphasized that the efficacy of *A. pullulans* is strain-specific. Zajc et al. (2020) analysed several *A. pullulans* strains and reported differences in traits such as enzyme production, salt tolerance and biofilm formation, which may

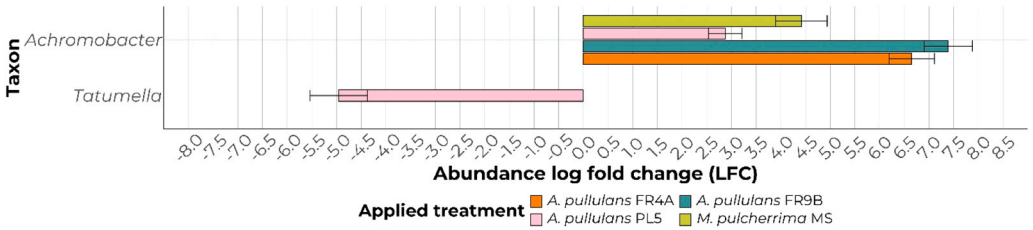


**Fig. 9.** Barplot of the ANCOM-BC results for bacterial genera, comparing each treatment with the untreated control at the end of storage. Only genera showing significant changes in relative abundance (adjusted $p < 0.05$) are displayed. Bar length and direction indicate the log fold change (LFC), while bar colour represents the treatment. Error bars denote the LFC ± one standard error.

explain their variable efficacy. In this study, a strain-specific biocontrol activity was observed, highlighting the importance of strain selection for biocontrol applications.

Similarly, species of the genus *Metschnikowia* have been shown to effectively control gray mold. *M. pulcherrima* has shown promising antagonistic activity on apples (Spadaro et al., 2002) and grapes (Liu et al., 2025). On strawberries, the closely related species *M. fructicola* has been successfully tested as a preharvest treatment in greenhouses, open-field culture and plastic tunnels, reducing fruit rot incidence in both pre- and postharvest conditions (Karabulut et al., 2004; Zhimo et al., 2021). To further validate our findings, we compared the selected strains with a commercial biofungicide based on *M. fructicola* NRRL Y-27328 (Kurtzman and Droby, 2001). Results confirmed the efficacy of this commercial strain in controlling postharvest decay, and notably, all the tested strains demonstrated comparable efficacy in reducing gray mold incidence and severity, highlighting their potential as biocontrol agents for managing strawberry postharvest rots.

The mechanisms of action of the selected yeasts were not characterized in this study. However, the biocontrol activity of *A. pullulans* and *M. pulcherrima* has been associated with several mechanisms in previous works, including rapid colonization of host surfaces, competition for nutrients and space (Bencheqroun et al., 2007; Di Francesco et al., 2017; Zhang et al., 2010b), siderophore (Saravanakumar et al., 2008; Sipiczki, 2006; Zajc et al. 2020), biofilm formation (Di Francesco et al., 2021; Parafati et al., 2015) and secretion of hydrolytic enzymes such as β-1, 3-glucanase, chitinase, and protease (Banani et al., 2014, 2015; Saravanakumar et al., 2009; Zajc et al. 2020; Zhang et al., 2012). Both yeasts also produce volatile organic compounds (VOCs) with antifungal activity, which have been shown to inhibit *B. cinerea* both *in vitro* and on strawberries (Oro et al., 2018; Tulukoglu-Kunt et al., 2024).

Quality analyses revealed that all selected strains had no significant impact on fruit firmness, total soluble solids (TSS) and titratable acidity (TA), with all measured parameters comparable to those of the untreated control, after both storage and shelf-life exposure. Maintaining fruit quality is a critical factor for commercial acceptability, and in this study, the selected yeasts preserved fruit quality without any negative alterations, supporting their potential application. Similar findings were reported on strawberries treated with *Debaryomyces hansenii*, which did not affect soluble solids content, firmness or titratable acidity compared to the untreated fruit (Zhao et al., 2023), and with *Cryptococcus laurentii*, where preharvest treatment also had no negative effects on key quality parameters (Wei et al., 2014).

The effect of the applied yeast treatments on the strawberry microbiome during storage was assessed. In order to avoid significant variability, disease surveys and microbiome analyses were performed on fruit from the same batch. Analyses highlighted the colonization of biocontrol agents on the treated fruit, along with a clear impact on *Botrytis* abundance and marked alterations in both fungal and bacterial community.

Compositional analysis of the fungal microbiome revealed that the pathogenic genus *Botrytis* was already present at harvest, as a result of latent infections established during flowering or early fruit development. During storage, its relative abundance dramatically increased in the untreated control. This trend confirms the data obtained in the phytopathological survey and aligns with previous studies on the strawberry microbiome (Zhimo et al., 2021). Conversely, *Botrytis* abundance was significantly reduced in all the yeast-treated samples, as also supported by ANCOM-BC analysis, providing evidence of the biocontrol efficacy of the applied strains.

*Botrytis* reduction generally corresponded to a noticeable colonization of the applied yeasts within the fruit microbiome. The genus *Aureobasidium* predominated in strawberries treated with *A. pullulans* PL5 and FR4A, whereas *Metschnikowia* was enriched in fruit treated with *M. fructicola* NRRL Y-27328 and *M. pulcherrima* MS, indicating successful colonization of the biocontrol agents. These findings are consistent with previous studies on fruit microbiomes following treatments with antagonistic yeasts, which reported a high persistence of the applied strains during storage. Strong colonization of *Aureobasidium* was observed in tomatoes (Shi et al., 2022) and apples (Remolif et al., 2024b; Zhimo et al., 2025). Notably, Remolif et al. (2024b) reported that strain PL5 can efficiently colonize apples, and the results of this study confirm that it can also successfully establish on strawberries. Similarly, a good colonization of *Metschnikowia* was previously observed after the application of *M. fructicola* on strawberries (Zhimo et al., 2021) and apples (Biasi et al., 2021). In our study, *M. fructicola* exhibited colonization levels exceeding 95%, consistent with its observed efficacy in reducing postharvest decay.

In contrast, strawberries treated with *A. pullulans* FR9B showed lower *Aureobasidium* abundance compared to fruit treated with the other *A. pullulans* strains, although still higher than in the untreated control. Despite this, its application significantly reduced *Botrytis* abundance, as confirmed by both disease incidence data and ANCOM-BC analysis, and induced notable shifts in the bacterial community. These findings suggest that its biocontrol activity may rely more on indirect mechanisms, such as modulation of the resident fruit microbiome or production of bioactive metabolites, rather than on direct colonization. A similar pattern has been reported for *Debaryomyces hansenii* on strawberries, which, despite being detected at low levels in the microbiome, effectively reduced decay and influenced fungal community compared to untreated fruit (Zhao et al., 2023).

Interestingly, yeast application also modulated the abundance of other fungal genera. Differential abundance analysis highlighted that the treatments promoted the presence of genera with reported biocontrol activity on fruit, such as *Rhodotorula* and *Sporobolomyces* (Sanzani et al., 2021; Zhang et al., 2007). This effect was particularly pronounced for *A. pullulans* FR4A. Among the tested strains, FR4A had the strongest impact on decay reduction, resulting in the lowest *Botrytis* abundance. Beyond its effective establishment in the fruit microbiome, FR4A treatment also significantly increased the abundance of *Cystofilobasidium*, which became the second most abundant genus, a pattern further supported by the differential abundance analysis. This genus was previously reported as an effective biocontrol agent against postharvest diseases on apples and citrus (Garat et al., 2010), suggesting that its enrichment may contribute to pathogen suppression.

The application of antagonistic yeasts also influenced the bacterial community composition. At the end of storage, yeast-treated

strawberries showed a marked increase of *Achromobacter*, reaching relative abundances above 75%. Species of this genus have previously been reported as biocontrol agents on various crops, including tomato (Moretti et al., 2008) and melon (Dhaouadi et al., 2019). Beyond its direct antagonistic role, *Achromobacter* has been shown to interact with fungal species, modulating their biocontrol activity. For instance, *Fusarium oxysporum* MSA35, an antagonistic strain used to control Fusarium wilt of lettuce (Gilardi et al., 2007), harbours *Achromobacter* ectosymbionts, whose removal restored the pathogenic phenotype (Minerdi et al., 2008). In our study, *Achromobacter* was not physically associated with the applied yeasts (data not shown). However, yeast treatments appeared to promote the abundance of resident *Achromobacter*, initially present at lower abundance at harvest, potentially contributing to the observed biocontrol activity.

Two distinct patterns emerged for other bacterial genera depending on the type of yeast applied. Treatments with epiphytic strains (*M. pulcherrima* MS and *A. pullulans* PL5) were associated with higher relative abundances of *Pseudomonas* and *Sphingomonas*, whereas treatments with endophytes (*A. pullulans* FR4A and FR9B) showed reduced levels of these genera and lower overall diversity, probably as a consequence of the stronger dominance of *Achromobacter* These differences likely reflect the distinct colonization strategies of epiphytic and endophytic yeasts, with endophytic strains potentially exerting stronger selective pressure on the resident bacterial community.

The genus *Tatumella*, already present at harvest, remained the dominant bacterial taxon at the end of storage both in the untreated control and in fruit treated with the commercial formulation of *M. fructicola*. However, a clear distinction emerged: unlike the untreated control, *M. fructicola* treatment markedly reduced the relative abundance of *Gluconobacter* This pattern partially aligns with previous findings on the same strain applied preharvest on strawberries, where *Tatumella* increased in treated fruit at harvest and, after storage, was still present in both treated and untreated samples, albeit at lower levels than in our study (Zhimo et al., 2021). Importantly, a reduction of *Gluconobacter*. at the end of storage following *M. fructicola* application was detected, consistent with our observations. Since this acetic acid-producing bacterium was associated with fruit rot of apples and pears (Van Keer et al., 1981) and is implicated in sour rot development in grapes (Brischetto et al., 2024; Hall et al., 2018), its reduction suggests that yeast-based treatments may contribute to limiting bacteria potentially associated with fruit decay.

The shifts observed in both fungal and bacterial communities indicate that the application of yeast biocontrol agents not only reduced pathogen abundance but also reshaped the resident microbiome. Particularly, the enrichment of potentially beneficial genera, both fungal and bacterial, suggests that part of the antagonistic effect may derive from interactions with the native microbiota. These findings further support previous observations that the fruit-associated microbial communities are an integral component of postharvest disease dynamics and should be considered in biocontrol strategies (Zhimo et al., 2025). Thus, the activity of a single antagonist on the fruit surface may not be limited to direct pathogen suppression but can involve the establishment of a beneficial microbiome that may jointly contribute to preventing disease development.

To further explore the impact of yeast treatments on the strawberry microbiome, overall community diversity was assessed. Beta diversity analyses highlighted the significant impact of the treatments on both fungal and bacterial community composition. PCoA revealed distinct clustering of fungal communities based on the applied yeast: untreated fruit at harvest and after storage formed a single cluster, while *M. fructicola*-treated fruit were separated due to the high abundance of *Metschnikowia*. Treatments with *A. pullulans* strains FR4A and FR9B clustered together and were dominated by *Aureobasidium*, while *M. pulcherrima* MS grouped separately, likely due to a more balanced fungal composition. Similarly, bacterial communities showed treatment-dependent clustering. Fruit treated with *M. fructicola* and untreated controls grouped together, largely due to the dominance of *Tatumella*, whereas the other treatments formed a distinct cluster characterized by higher *Achromobacter* abundance.

Finally, alpha diversity revealed that both richness and evenness of fungal and bacterial communities were generally maintained in treated fruit compared to controls. While previous studies have often reported reductions in microbial richness following BCAs applications, our results indicate that it was not significantly affected, despite the high abundance of some taxa introduced or promoted by the treatments, such as *Aureobasidium*, *Metschnikowia* and *Achromobacter*. These findings suggest that yeast-based treatments can modulate the microbiome composition without negatively impacting microbial diversity, potentially preserving a more resilient community.

## 5. Conclusions

This study highlighted the potential of selected antagonistic yeasts, including both epiphytic and endophytic strains, to effectively control postharvest decay of strawberries without affecting key fruit quality parameters. Beyond direct pathogen inhibition, yeast treatments induced shifts in both fungal and bacterial communities. Specifically, the applied strains showed good colonization ability, reduced the pathogen abundance and promoted the enrichment of potentially beneficial taxa.

These findings suggest that the activity of a single antagonist may involve interactions with the native microbial communities and may promote the establishment of a healthier fruit-associated microbiome that can contribute to disease suppression. Overall, this work underscores the importance of considering the fruit-associated communities as an integral component of postharvest disease dynamics.

Future research will focus on understanding the mechanisms of action of the selected antagonistic yeasts, optimizing application strategies by considering preharvest treatments and exploring synergistic effects with other management practices and storage technologies. Moreover, further studies are needed to investigate the complex microbial interactions occurring during shifts in the structure of bacterial and fungal communities. Such insights will be crucial to improve the efficacy of yeast-based biocontrol strategies and promote the establishment of a beneficial microbiome to prevent disease development.

## CRediT authorship contribution statement

**Gianfranco Romanazzi:** Writing – review & editing. **Davide Spadaro:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Vladimiro Guarnaccia:** Writing – review & editing. **Samir Droby:** Writing – review & editing. **Giulia Remolif:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simona Prencipe:** Validation, Methodology. **Ilario Ferrocino:** Validation, Methodology. **Marco Garello:** Validation, Software, Methodology, Formal analysis, Data curation.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Davide Spadaro reports financial support was provided by European Commission, PRIMA programme. Davide Spadaro reports financial support was provided by Euroepan Commission, NextGeneration EU. Davide Spadaro reports travel was provided by European Cooperation in Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2026.114414.

## Data availability

Data will be made available on request.

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