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Biocontrol and ecophysiological characterization of bacterial and yeast BCA candidates isolated from two different environments to control postharvest diseases

Ana María Sánchez [a], Cristina Solsona [a], Jonàs Oliva [b], Neus Teixidó [a,*]

[a] IRTA, Postharvest, Fruitcentre, Lleida, Catalonia 25003, Spain
[b] Department of Crop and Forest Sciences, University of Lleida, Lleida, Catalonia 25198, Spain

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ABSTRACT

Biocontrol agents are a sustainable alternative to chemical fungicides in postharvest protection. Part of their success depends on using strains able to persistently colonize the host and maintain a broad activity from the field to the storage facilities. In this report, we evaluated 213 potential biocontrol agents (124 bacteria, 78 yeasts, and 11 filamentous fungi) isolated from two contrasting environments, the Pyrenees Mountains and the Ebro Valley (Catalonia, Spain). Their efficacy was tested against the three main postharvest pathogens of apples: *Penicillium expansum*, *Botrytis cinerea*, and *Rhizopus stolonifer*. The results showed that 4 bacteria and 25 yeast strains were effective against these pathogens, reducing their incidence by more than 75 %. From these results, two *Pantoea agglomerans* and two *Vishniacozyma carnescens* were further studied in terms of growth at different temperatures and water availability *in vitro* and their persistence on apple surfaces. The ecophysiological characterization showed that the valley *P. agglomerans* strain tolerated lower water activities better than the mountain strain, suggesting adaptation to the region's hot and dry climate. In contrast, the mountain *V. carnescens* performed better at low temperatures, while the valley strain showed superior growth at 30 ºC, consistent with their respective environmental origins. In conclusion, these results indicated the need to consider the environmental adaptability of potential biocontrol agents when searching for new BCA strains.

1. Introduction

Annually, plant diseases result in an estimated loss of $40 billion globally, either directly or indirectly, representing a significant economic problem for the agricultural industry (Wang et al., 2024). These losses become a global challenge (Kusstatscher et al., 2020), especially given that the demand for food production is expected to rise in the coming decades as the world's population grows (Syed Ab Rahman et al., 2018; Wang et al., 2024). It is estimated that pathogenic infections account for at least 20–40 % of crop yield losses (Syed Ab Rahman et al., 2018). Some of these occur during the storage of the fruits and are due to postharvest diseases caused by pathogenic filamentous fungi such as *Penicillium expansum*, *Botrytis cinerea*, and *Rhizopus stolonifer*, among others (FAO, 2011). These diseases are especially relevant in apples (Leng et al., 2023), the fourth most consumed fruit in the world (Fernandez-San Millan et al., 2023).

The application of synthetic fungicides during pre- and postharvest is an effective method for the control of diseases (Guijarro et al., 2008). However, an increasing social concern has led to a growing backlash against these practices. This concern has been enforced through the EU Directive 2009/128/CE, which aims to minimize the environmental and human health risks associated with these fungicides, and encourages the search for more environmentally sustainable alternatives (Di Canito et al., 2021; Guijarro et al., 2008; Kusstatscher et al., 2020). Therefore, there is a growing need for alternatives to synthetic pesticides that maintain the production of the crops and improve sustainability in agriculture (Palmieri et al., 2022). Antagonist epiphytic microorganisms from the fruit surface have been turned into commercial biocontrol agents (BCAs) to control postharvest fruit diseases (Carbó et al., 2018; Droby et al., 2016; Gotor-Vila et al., 2017a; Pereyra et al., 2021). These have been based on antagonistic bacteria, including various strains of *Bacillus*, such as Serenade® (Bayer AG, Leverkusen, Germany) or Amylo-X-WG® (Certis, Maryland, USA), and antagonistic yeasts, including different strains of *Candida*, such as Nexy® (Ennolys, Soustons, France)

* Corresponding author.
E-mail address: neus.teixido@irta.cat (N. Teixidó).

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or Candifruit® (IRTA, Lleida, Spain). Despite this, they have had limited success in the marketplace (Fernandez-San Millan et al., 2023), due to, among several reasons, their lower stability in field conditions when compared to synthetic pesticides (Sare et al., 2024), as well as the lengthy registration process required to commercialize these products in Europe (Usall et al., 2016).

Recovering native microorganisms from the surface of the fruit as potential BCAs is advantageous (Droby et al., 2016; Zhang et al., 2021). They are already adapted to the specific conditions of the fruit and its environment (Berg et al., 2020), improving their stability and therefore their efficacy. To harness this potential, a culturomics approach was used in a recent study (Sánchez et al., 2025a). Culturomics is a technique that uses plant-based media specifically designed to maximise the number of microbial isolates associated with the host plant, in contrast to classical isolation methods that employ meat-based conventional culture media (Elsawey et al., 2020; Sarhan et al., 2019). This cutting-edge methodology facilitates the recovery of a wide range of culturable plant microorganisms (Sarhan et al., 2019). In contrast to traditional methods that often focus on *in vitro* screening without first identification (Barbé et al., 2022; Pereyra et al., 2021; Settier-Ramírez et al., 2021), our strategy initially identifies the microbial community, both bacteria and fungi (Sánchez et al., 2025a). Subsequently, isolates belonging to well-known BCA species or genera were selected. This strategy also included recovering isolates from contrasting environments. As such, we recovered epiphytic and endophytic microorganisms from the apple carposphere in two different environments in Catalonia (Spain), the Ebro Valley and the Pyrenees Mountains (Sánchez et al., 2025a). The valley orchards are characterized by warmer and drier climates, while the mountain orchards are subjected to much colder and rainier conditions. These contrasting climatic conditions have previously been shown to shape microbial diversity and community composition in apples (Sánchez et al., 2025a, 2025b), which could lead to the finding of environment-specific biocontrol candidates.

Biocontrol agent candidates have to be resilient to environmental conditions where they have to be applied. When these microorganisms are applied onto the surface of the fruit, their efficacy can be reduced mainly because they are unable to cope with environmental fluctuations (Guijarro et al., 2008). Key environmental factors are water availability (a_w) and temperature (Carbó et al., 2018; Gotor-Vila et al., 2017a; Guijarro et al., 2008). As these factors vary between the studied environments, selecting microorganisms from contrasting habitats, such as the mountain and the valley, could help identify strains with complementary adaptations. We therefore hypothesized that mountain isolates may be adapted to cooler, more humid conditions, whereas valley strains may be better suited to warmer and drier climates.

The general objective of this study was to identify effective biocontrol candidates against postharvest diseases in apples that are capable of performing under a wide range of environmental conditions. To achieve that, the specific objectives were: i) To evaluate the *in vivo* biocontrol potential of a collection of microorganisms isolated from the apple microbiome in both the Ebro Valley and the Pyrenees Mountain environments, ii) to characterize the *in vitro* ecophysiological growth response of the most promising candidates under different temperatures and water activity conditions and iii) to assess the dynamics of selected candidates on apple fruits surface under varying humidity levels.

2. Material and methods

2.1. Isolation and selection of the candidates from our collection of microorganisms

The collection of microorganisms comprised 957 isolates, including 510 bacteria, 216 filamentous fungi, and 231 yeasts, obtained in our previous study using a culturomics approach (Sánchez et al., 2025a). The isolates were recovered from apple fruits from two varieties ('Mandy', with red skin, and 'Golden Reinders', with yellow skin) sampled in four different orchards, two from the Pyrenees Mountains and two from the Ebro Valley regions. The specific climatic conditions of each region are listed in Supplementary Figure 1. For the present work, a selection strategy was established focusing on biological control agents potential. Genera with antagonistic activity against any postharvest pathogens in fruit or other crops, as described in the literature, were identified using NCBI Literature resources (PubMed) (PubMed, 2023). From each of these genera, approximately 25 % of the isolates were selected using a random stratified approach. The selection was performed within each genus, ensuring representative isolates from both varieties ('Golden Reinders' and 'Mandy'), altitudinal environments (Pyrenees Mountains and Ebro Valley) and microbial niches (epiphytic and endophytic).

2.2. In vivo antagonists screening of the potential biocontrol isolates from the collection against major postharvest pathogens

2.2.1. Preparation of the potential biocontrol isolates from the collection

A total of 124 bacterial isolates, 78 yeast isolates, and 11 filamentous fungal isolates were tested against the main apple postharvest pathogen (*Penicillium expansum*). Bacteria were cultured on Luria Broth (LB) agar (Condalab, Madrid, Spain) at 25 ºC for 48 h and yeasts and filamentous fungi on Potato Dextrose agar (PDA) (Condalab, Madrid, Spain) at 25 ºC for 48 h and 7 days, respectively.

After growth, spores and conidial suspensions were prepared by scraping the surface of 48-hour-old and 7-day-old cultures with sterile water containing 0.01 % (w/v) Tween-80 using a sterile plastic loop. The concentration of each microorganism was determined using a spectrophotometer (Uvisco UV-1100, dDBioLab, Barcelona, Spain) based on transmittance measurements to obtain a final concentration of 10^8 CFU mL^{-1}. For the bacterial suspension, transmittance values of 30–40 % were used, measured at 700 nm for *Bacillus* isolates and at 420 nm for the remaining bacterial genera. For yeast suspension, transmittance values of 10–15 % were used, measured at 600 nm. The concentration of filamentous fungi was determined using a haemocytometer (Thoma BRAND™ Blaubrand™). All microbial suspensions were adjusted to 5 mL of potassium phosphate buffer (PB) solution. The PB solution was prepared with 70 mL of 0.2 mol L^{-1} KH_2PO_4, 30 mL of 0.2 mol L^{-1} K_2HPO_4, and diluted with deionised water to a final volume of 400 mL (pH 6.5) (Gotor-Vila et al., 2017a).

2.2.2. Preparation of the postharvest pathogens inoculum

The strains CMP-1 *Penicillium expansum* (CECT 20906), BC03 *Botrytis cinerea* (CECT 20973), and RSF *Rhizopus stolonifer* (CECT 21311) used in this study were obtained from the Postharvest Pathology Group Collection of the IRTA Centre (Lleida, Spain) and deposited at the Spanish Type Culture Collection (CECT) at the University of Valencia, Burjassot, Spain. These strains were isolated from infected apples in Lleida and maintained in 20 % glycerol (w/v) at −80 °C (Gotor-Vila et al., 2017a). CMP-1 and RSF were subcultured periodically in MEA and PDA medium, respectively, at 25 °C in the dark. BC03 was subcultured on PDA, with a daily 12 h photoperiod of near-ultraviolet light at 25 ºC and 12 h dark light at 18 ºC to induce sporulation.

After growth, conidial suspensions were prepared by scraping the surface of 7-day-old cultures with sterile water containing 0.01 % (w/v) Tween-80 using a sterile plastic loop. The concentration of each fungus was determined using a hemocytometer (Thoma BRAND™ Blaubrand™) and adjusted with sterile water containing 0.01 % (w/v) Tween-80 to obtain 10^5 conidia mL^{-1} of *P. expansum* and *B. cinerea* and 10^3 conidia mL^{-1} of *R. stolonifer* as described in Vilanova et al. (2014).

2.2.3. Inoculation of the potential biocontrol isolates and the postharvest pathogens

'Golden Reinders' apples were wounded twice on the equatorial part of each fruit using a sterile nail (1 mm wide and 2 mm deep). Each wound was first inoculated with 15 µL of the BCA aqueous suspension

and left to dry at room temperature. Once dried, the same wounds were subsequently inoculated with 15 µL of an aqueous suspension of the pathogens and allowed to dry between one and two hours at room temperature. A positive control was included in each assay, in which 15 µL of sterile water was applied instead of the BCA suspension (Vilanova et al., 2014).

Then, inoculated apples were incubated at 20 ºC and 85 % relative humidity. Rot lesion diameter (severity) and the number of infected wounds (incidence) were assessed after 5 days for *R. stolonifer* and 7 days for *P. expansum* and *B. cinerea*. Each potential BCA was tested in triplicate for each pathogen. Each replicate consisted of three apples, with each fruit containing two inoculated wounds. Those candidates that reduced the incidence and severity of the disease caused by *P. expansum* in more than 75 % were subsequently evaluated against *B. cinerea* and *R. stolonifer*.

2.2.4. Selection of the potential biocontrol agents

Of the 213 potential candidates, four isolates were further studied in terms of environmental tolerance. The criteria for the selection of these microorganisms were: i) they had to include one bacterial genus and one yeast genus that belong to the apple core microbiome (Sánchez et al., 2025b) controlling more than 50 % of incidence and severity reduction of the three postharvest pathogens and ii) from within these genera, there had to be one isolated from each area of study: Pyrenees Mountains and Ebro Valley).

2.3. In vitro ecophysiological characterization of the two best candidates from different environments (mountain and valley)

2.3.1. Preparation and inoculation of growth liquid media

The selected microorganisms were grown in LB or PDA plates and incubated at 25 ºC for 48 h. Fresh inoculum of each selected microorganism was prepared by transferring a 48-h-old culture to 5 mL of PB solution (Gotor-Vila et al., 2017a).

LB and PDB liquid media were prepared and adjusted to specific water activity (a_w) levels ranging from 0.94 to 0.998 (control) using glycerol as a non-ionic solute, following the methodology described by Dallyn and Fox (1980). The a_w levels (0.94, 0.96, and 0.98) were verified using an Aqualab 4TE a_w meter (Addium Inc., Pullman, Washington, USA) to an accuracy of ± 0.003. All media were sterilised for 15 min at 115 ºC. After sterilisation, the flasks were shaken and left at room temperature to cool. Each medium was inoculated with a fresh inoculum at a concentration of 10^5 CFU mL^{-1} of the corresponding microorganism. Then, 150 µL per well of the inoculated liquid LB or PDB medium, adjusted to the specified a_w condition, was dispensed into 96-well microplates. Three replicates were used for each a_w level and microorganism. An uninoculated LB or PDB medium (150 µL per well) for each a_w condition was used as a negative control. The microplates were incubated at six different temperatures: 0, 4, 10, 25, 30, and 36 ºC.

2.3.2. Growth monitoring at different temperatures and a_w

Microbial growth was monitored by measuring optical density at 600 nm (OD_{600}) using a spectrophotometer FLUOstar® Omega microplate reader (BMG Labtech, Ortenberg, Germany). Periodic readings were taken after 20 s of shaking throughout the incubation period. The OD_{600} was recorded until saturation depending on temperature: 72 h (for the higher temperatures) to 50 days (for the lower temperatures). Data was collected and processed by the software FLUOstar® Omega Data Analyst supplied by the microplate manufacturer. The resulting data were analyzed using add-in DMfit (ComBase, 2024) of Microsoft 365® Excel® (Microsoft Corporation, Redmond, Washington, USA) to model the microbial growth of the candidates by fitting the modified Gompertz model (Zwietering et al., 1990). The individual effect of each environmental factor (a_w and temperature) on the candidate's maximum growth rate (μ_{max}), lag phase duration (λ), and maximum growth capacity (yEnd) was obtained with the following equation:

$$y(t) = A\exp\{-\exp[\frac{\mu_{max}\ e}{A}\ (\lambda - t) + 1]\}$$

In this Equation, y indicates absorbance at time t, and A is the asymptotic value, μ_{max} signifies the maximum growth rate (h^{-1} or d^{-1}), t indicates the incubation duration (hours or days), and λ represents lag phase time (hours or days).

Before statistical analysis, OD_{600} values were corrected by taking out the mean absorbance of the three replicates of the uninoculated medium from the absorbance of each experiment.

2.4. Curve of growth in Erlenmeyer's under selected ecophysiological conditions

2.4.1. Growth in optimal conditions

To obtain the optimal growth curve of the selected candidates and determine their maximum growth capacity in CFU mL^{-1}, bacterial strains were incubated in flasks of 50 mL liquid LB medium, while yeast strains were incubated in 50 mL flasks of PDB medium at an initial concentration of 10^5 CFU mL^{-1}. The inoculum was prepared from 48-hour-old cultures grown on Petri plates. The liquid flasks were incubated at 25 °C in an orbital shaker set at 150 rpm to ensure suitable aeration and prevent oxygen limitation. Growth was monitored periodically at the following time points: 0, 2, 4, 6, 8, 16, 18, 20, 22, 24, 26, and 28 h for bacteria, and at 0, 2, 4, 6, 8, 16, 18, 20, 22, 24, 26, 28, 30, 40, and 48 h for yeasts, by determining the number of colony-forming units (CFU mL^{-1}). At each sampling time, three independent replicates were performed. Serial dilutions were prepared using a double dilution bank and were plated on LB agar for bacteria and PDA for yeasts. Plates were incubated at 25 °C for 48 h, and colony counts were recorded. Data were analyzed with the DMfit add-in (ComBase, 2024) to model the candidate's microbial growth, utilizing the modified Gompertz model (Zwietering et al., 1990).

2.4.2. Growth under different water activities (a_w)

To confirm the *in vitro* ecophysiological characterization, growth assays were performed in Erlenmeyer flasks with larger culture volumes and continuous agitation to minimize oxygen limitations. The same strains were incubated in 50 mL Erlenmeyer flasks at 25 °C in media without modification (0.998 a_w) and modified with glycerol (0.94, 0.96, and 0.98 a_w) following the methodology used in the previous ecophysiological assays. Bacteria were cultured in LB medium, and yeasts in PDB medium. The cultures were incubated at 150 rpm in an orbital shaker. Microbial growth was monitored at 0, 24, 48, 72, and 96 h. At each sampling time, three independent replicates were performed. Serial tenfold dilutions were prepared using a double dilution method and were plated on LB agar for bacteria and PDA for yeasts. Plates were incubated at 25 °C for 48 h, after which colony counts were recorded (CFU mL^{-1}).

2.5. Dynamics of the selected candidates under different relative humidities

The impact of two relative humidity (RH) levels (40 and 85 %) on the survival of candidate microbial populations was evaluated on 'Golden Reinders' apple surface previously treated with the selected microorganisms. Microbial suspensions from the liquid production in Erlenmeyer flasks of the bacteria and yeasts were prepared in water, and applied on the surface of apples at a concentration of 5×10^7 CFU mL^{-1} until runoff by using a 2 L manual sprayer, after which the fruit was air-dried for 2 h at room temperature.

For each selected microorganism, relative humidity level, and sampling time evaluated, 16 fruits were randomly selected without visible injuries or rots and homogeneous in maturity and size. The treated fruits were placed on packing trays following the protocol developed by Gotor-Vila et al. (2017b), covered with plastic chambers, and sealed before being stored in a climatic chamber set at 20 °C. The targeted RH

levels within the plastic chambers were maintained using a dehumidifier (FDC32S FRAL, Carmignano di Brenta, Padua, Italy) and monitored continuously with an external data logger (Testo 175H1, Testo Inc., Sparta Township, New Jersey, USA).

Each setup consisted of four replicates. For each replicate, four fruits were sampled, with analyzed per treatment and condition tested. From the surface of each fruit, 25 peel disks were superficially wounded at random using a cork borer (16 mm in diameter), resulting in a total of 100 peel disks per replicate. These peel disks were placed in sterile stomacher filter bags (BagPage 400 mL, Interscience BagSystem, St. Nom la Brèteche, France) containing 100 mL of PB solution. To extract microorganisms from the peel disks, the samples were homogenized using a Stomacher blender (IUL Masticator Basic 470, Barcelona, Spain) at 12 strokes sec^{-1} for 90 s (Gotor-Vila et al., 2017b). Serial ten-fold dilutions of the washings were prepared and plated onto LB agar medium for bacteria and PDA for yeasts. Plates were incubated at 25 ºC for 48 h, after which colonies were counted. Population dynamics of the selected candidates were recorded as CFU mL^{-1} and subsequently expressed as CFU cm^{-2} of the fruit surface. The population dynamics under the tested storage conditions were assessed at 0, 1, 2, 5, 7, 9, and 12 days.

2.6. Statistical analysis

The incidence and severity data were analysed and presented as incidence and severity reduction (%), which facilitated the integration of all assays and enabled comparisons across different treatments. The reductions in incidence (%) and severity (%) were calculated as the incidence and severity observed in the positive control (untreated, pathogen-inoculated samples) minus those from the antagonist-treated samples.

To evaluate the effect of water activity and temperature on the growth parameters of the selected microorganisms, a non-parametric Aligned Rank Transform (ART) ANOVA was used to test the main effects and interaction (microorganism * water activity) at each temperature. This approach was selected due to deviations from normality and heteroscedasticity observed in the data. Analysis was performed using RStudio in R version 4.2.3 (R Core Team, 2023) to assess their influence on the maximum growth rate (μ_{max}), lag phase duration (λ), and maximum growth capacity (yEnd). Statistical significance was set at p-value ≤ 0.05. When significant differences were detected, Tukey-adjusted ART contrasts were used for multiple comparisons to separate means.

The effect of the water activity and incubation time, as well as the relative humidity and storage time, on the population dynamics of the selected candidates was analysed using RStudio in R version 4.2.3 (R Core Team, 2023). The data were logarithmically transformed and analysed with an ANOVA to test the main effects and interaction (microorganism * factor). Statistical significance was determined with a p-value of ≤ 0.05. When differences were found to be significant, Tukey-adjusted contrasts were used for multiple mean comparisons.

3. Results

3.1. In vivo antagonists screening of the potential biocontrol isolates from the collection against major postharvest pathogens

A total of 124 bacterial, 78 yeast, and 11 filamentous fungal isolates were tested against *Penicillium expansum* CMP-1. The selected isolates included representatives from both altitudinal regions, as well as epiphytic and endophytic niches.

A total of 52 bacteria and 55 yeasts reduced rot incidence by more than 50 %, while 17 bacterial and 39 yeasts isolates successfully reduced the disease's incidence and severity by more than 75 % (Tables 1 and 2). Although the screening included isolates from contrasting altitudinal regions, no differences in biocontrol effectiveness were observed in relation to their origin (Supplementary Tables 1 and 2).

When grouped by taxonomy, the isolates tested against CMP-1 were comprised of 17 different bacterial genera, 8 yeast genera, and 3 filamentous fungi genera. The most abundant of which were *Bacillus* (28 isolates tested), *Rhodococcus* (18), *Streptomyces* (17), and *Pantoea* (16) (Table 1). In the case of the yeasts, *Aureobasidium* (24), *Vishniacozyma* (21), and *Meyerozyma* (15) were the most abundant (Table 2). The most effective isolates, those that reduced the disease by more than 75 % incidence and severity, belonged to 6 bacterial genera, *Microbacterium* (2 isolates tested), *Paenibacillus* (1), *Pantoea* (6), *Priestia* (1), *Rhodococcus* (4), and *Streptomyces*, and 7 yeast genera, *Aureobasidium* (2 isolates tested), *Metschnikowia* (2), *Candida* (1), *Meyerozyma* (13), *Pichia* (2),

Table 1
Bacterial isolates tested against the three postharvest pathogens after 7 days of infection for CMP-1, *Penicillium expansum*, and BC03, *Botrytis cinerea*, and 5 days of infection for RSF, *Rhizopus stolonifer*. The reduction of the disease was calculated as the reduction of the incidence of the disease in three different ranges (Control Rot < 50 %, 50–75 % and >75 %). Only isolates showing > 75 % control rot against CMP1 were subsequently tested against BC03 and RSF. The number of isolates tested per genus is therefore shown separately for each pathogen.

	CMP1 - 7d				BC03 - 7d				RSF - 5d			
	Isolates tested	Control Rot < 50 %	Control Rot 50–75 %	Control Rot > 75 %	Isolates tested	Control Rot < 50 %	Control Rot 50–75 %	Control Rot > 75 %	Isolates tested	Control Rot < 50 %	Control Rot 50–75 %	Control Rot > 75 %
Bacillus	28	27	1	0	0	0	0	0	0	0	0	0
Brevibacterium	1	0	1	0	0	0	0	0	0	0	0	0
Corynebacterium	1	1	0	0	0	0	0	0	0	0	0	0
Methylobacterium	2	0	2	0	0	0	0	0	0	0	0	0
Methylorubrum	2	1	1	0	0	0	0	0	0	0	0	0
Microbacterium	8	3	3	2	2	2	0	0	2	0	0	0
Micrococcus	2	2	0	0	0	0	0	0	0	0	0	0
Paenarthrobacter	5	3	2	0	0	0	0	0	0	0	0	0
Paenibacillus	3	0	2	1	1	1	0	0	1	0	0	1
Pantoea	16	1	9	6	6	2	2	2	6	0	0	6
Paracoccus	1	1	0	0	0	0	0	0	0	0	0	0
Peribacillus	2	2	0	0	0	0	0	0	0	0	0	0
Priestia	5	4	0	1	1	1	0	0	1	0	0	1
Pseudarthrobacter	9	8	1	0	0	0	0	0	0	0	0	0
Rhizobium	4	3	1	0	0	0	0	0	0	0	0	0
Rhodococcus	18	10	4	4	4	3	1	0	4	1	2	1
Streptomyces	17	6	8	3	3	0	1	2	3	0	0	3
TOTAL	**124**	**72 (58 %)**	**35 (28 %)**	**17 (14 %)**	**17**	**9 (52 %)**	**4 (24 %)**	**4 (24 %)**	**17**	**1 (6 %)**	**2 (12 %)**	**12 (72 %)**

Table 2
Yeast isolates tested against the three postharvest pathogens after 7 days of infection for CMP-1, *Penicillium expansum*, and BC03, *Botrytis cinerea*, and 5 days of infection for RSF, *Rhizopus stolonifer*. The reduction of the disease was calculated as the reduction of the incidence of the disease in three different ranges (Control Rot < 50 %, 50–75 % and >75 %). Only isolates showing > 75 % control rot against CMP1 were subsequently tested against BC03 and RSF. The number of isolates tested per genus is therefore shown separately for each pathogen.

	CMP1 - 7d				**BC03 - 7d**				**RSF - 5d**			
	Isolates tested	**Control < 50 %**	**Control Rot 50–75 %**	**Control Rot > 75 %**	**Isolates tested**	**Control < 50 %**	**Control Rot 50–75 %**	**Control Rot > 75 %**	**Isolates tested**	**Control < 50 %**	**Control Rot 50–75 %**	**Control Rot > 75 %**
Aureobasidium	**24**	15	7	2	**2**	0	0	2	**2**	0	0	2
Metschnikowia	**6**	2	2	2	**2**	0	0	2	**2**	0	0	2
Candida	**1**	0	0	1	**1**	0	0	1	**1**	0	0	1
Meyerozyma	**15**	1	1	13	**13**	0	1	12	**13**	0	0	13
Pichia	**4**	2	0	2	**2**	0	0	2	**2**	0	0	2
Rhodotorula	**6**	1	0	5	**5**	1	1	3	**5**	0	0	5
Tilletiopsis	**1**	1	0	0	**0**	0	0	0	**0**	0	0	0
Vishniacozyma	**21**	1	6	14	**14**	4	7	3	**14**	0	0	14
TOTAL	**78**	**23 (29 %)**	**16 (21 %)**	**39 (50 %)**	**39**	**5 (13 %)**	**9 (23 %)**	**25 (64 %)**	**39**	**0**	**0**	**39 (100 %)**

Rhodotorula (5) and *Vishniacozyma* (14) (Tables 1 and 2). None of the 11 filamentous fungi isolates tested against CMP-1, which belong to the genera *Acremonium* (8), *Epicoccum* (2), and *Trichoderma* (1), controlled the incidence and the severity of the disease by more than 50 %, so these isolates were excluded from later experiments (Supplementary Table 3).

The 17 bacterial and 39 yeast isolates that were more effective against PE01 were subsequently tested against *Botrytis cinerea* and *Rhizopus stolonifer*. Against BC03, 4 bacterial and 25 yeast isolates demonstrated a reduction of more than 75 %, meaning that 25 % of bacterial isolates and 65 % of yeast isolates effectively controlled the disease (Tables 1 and 2). In addition, when candidates were tested against RSF, 12 bacteria and 39 yeasts achieved a reduction of more than 75 % in disease incidence and severity, indicating that 70 % of the bacterial and 100 % of the selected yeast isolates were effective in controlling this pathogen (Tables 1 and 2).

The isolates that managed to control all three of the tested pathogens belonged to the genera *Pantoea*, *Rhodococcus*, and *Streptomyces* for bacteria, and *Aureobasidium*, *Metschnikowia*, *Candida*, *Meyerozyma*, *Pichia*, *Rhodotorula*, and *Vishniacozyma* for yeasts. With these results and based on our criteria of selection, the strains selected for further investigations were: two *Pantoea agglomerans* isolates, strain B109 from the mountain region (Bacterium mountain, BM) and strain B296 from the valley region (Bacterium valley, BV), as well as two *Vishniacozyma carnescens* isolates, strain L157 from the mountain region (Yeast mountain, YM) and strain L111 from the valley region (Yeast valley, YV). As can be seen in the Fig. 1, the four selected candidates reduced CMP-1, BC03 and RSF disease incidence and severity by more than 75 % except for YM, which achieved a 55 % reduction in incidence against BC03.

3.2. *In vitro ecophysiological characterization of the four selected candidates from different environments (mountain and valley)*

The absorbance growth curves fitted by the Gompertz model of the selected candidates' in vitro characterization are illustrated in Fig. 2 (bacteria) and Fig. 3 (yeasts).

Regarding the bacterial candidates, the most limiting growth factor was water activity. At all temperatures, isolates of *Pantoea agglomerans*

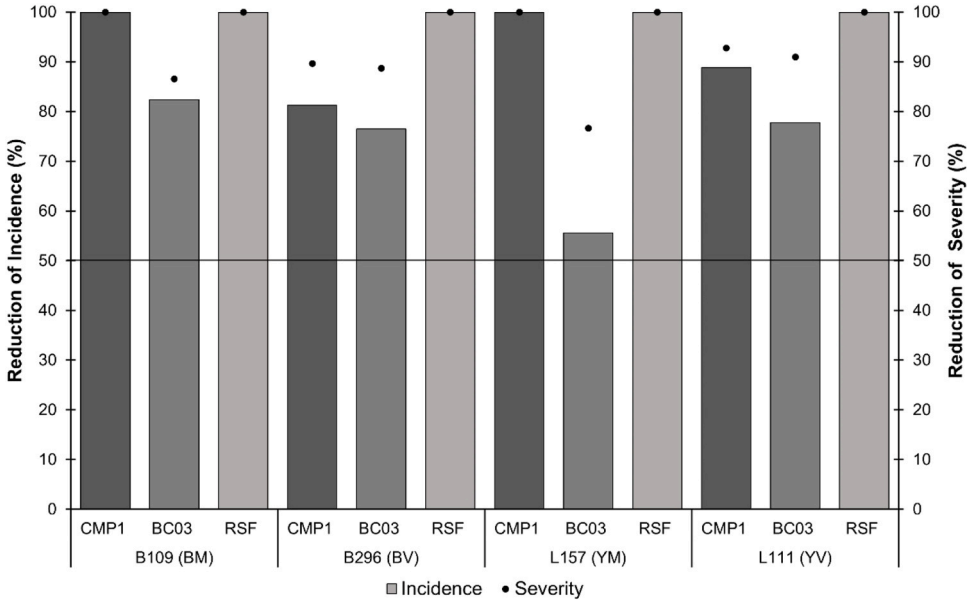

Fig. 1. Reduction of Incidence (%) and Severity (%) of the four selected microorganisms (BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296; YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111) against the three pathogens tested (CMP-1, *Penicillium expansum*; BC03, *Botrytis cinerea*; and RSF, *Rhizopus stolonifer*) on artificially inoculated wounded apples after 7 days for CMP-1 and BC03 and 5 days for RSF. The line that crossed the graph was the minimum acceptable reduction (50 %) that our criteria established for considering isolates as candidates.

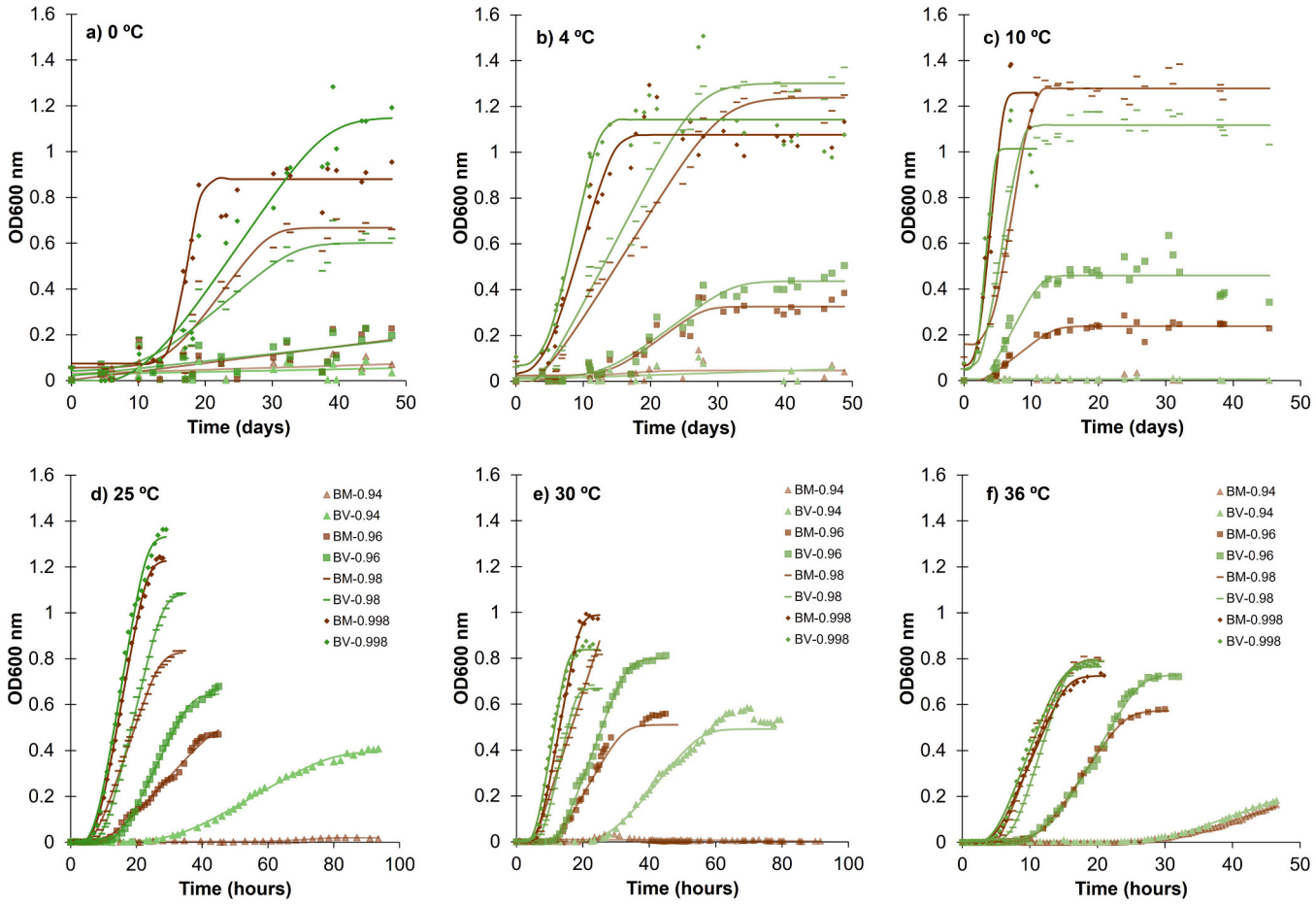

Fig. 2. Absorbance growth curves (600 nm) of the bacteria candidates (BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296) at different water activity modified with glycerol, 0.94, 0.96, 0.98 and 0.998, and different temperatures a) 0 ºC, b) 4 ºC, c) 10 ºC, d) 25 ºC, e) 30 ºC and f) 36 ºC. Symbols refer to experimental data, and solid lines refer to curves fitted by the Gompertz model. The temperatures a) 0 ºC, b) 4 ºC and c) 10 ºC were represented in days and the rest of the temperatures d) 25 ºC, e) 30 ºC and f) 36 ºC in hours.

could grow in the media without water restrictions (0.98 and 0.998 a_w). However, at minimum water activity (0.94 a_w), the mountain strain was unable to grow at any tested temperatures. In contrast, the valley strain could grow at this aw at most temperatures, except for the lower range temperatures (0–10 ºC) (Fig. 2 and Table 3).

At higher water availabilities (0.98 and 0.998 a_w) and lower temperatures (0–10 ºC), BM showed a higher or similar growth rate and maximum growth than BV, indicating better adaptation to colder environments (Figs. 2a, 2b and 2c; Tables 4 and 5). At 25 ºC, which represents the optimum temperature for the growth of both bacterial strains, BV grew quicker than BM at every water activity (Fig. 2d and Table 3). This higher growth trend for BV continued at higher temperatures of 30 ºC and 36 ºC (Figs. 2e and 2f).

Regarding the yeast candidates, water activity was not restrictive at low temperatures, but it became a limiting factor when interacting with high temperatures above 30 °C. At the two lowest temperatures (0 ºC and 4 ºC), YM exhibited a shorter or similar lag phase compared to YV under all water activities. The biggest difference between them being at 0.98 a_w, where YM showed a 6.23 days lag phase, compared to YV a 12.02 days lag phase (Figs. 3a, 3b and Table 6). Also at 0 ºC, YM was able to grow more significantly than YV in every water activity treatment, particularly at lower availabilities of water in the medium (0.94 and 0.96 a_w) (Fig. 3a and Table 8).

At higher temperatures, a shift in the growth of the yeast was observed. Although at 10 ºC YM still grew faster, YV achieved a higher maximum growth (Fig. 3c, and Tables 7 and 8). It is at 25 ºC and 30 ºC, however, where YV exhibited a shorter lag phase, along with a higher growth rate and increased absorbance at all water activities (Figs. 3d, 3e and Tables 7 and 8). Finally, at 36 ºC, none of the yeasts could grow (Fig. 3f).

3.3. Curve of growth in Erlenmeyer's under selected ecophysiological conditions

3.3.1. Growth in optimal conditions

For the bacteria, maximum growth at optimal conditions of temperature and aeration occurred after 18 h of growth in both candidates, achieving 1.55×10^9 CFU mL^{-1} for BV and 2.17×10^9 CFU mL^{-1} for BM. As shown in Fig. 4, the two bacteria exhibited very similar growth trends, starting their exponential growth phase at 2 h. For yeasts, however, maximum growth was achieved at 48 h, reaching 6.28×10^8 CFU mL^{-1} for YV and 6.73×10^8 CFU mL^{-1} for YM, with the exponential growth phase starting at approximately 8 h (Fig. 4).

3.3.2. Growth under different water activity (a_w) levels

The mountain *Pantoea* strain could not grow at 0.94 a_w, whereas the valley bacterial strain started to grow after 24 h and reached its maximum growth at 72 h of incubation at a concentration of 1.44×10^8 CFU mL^{-1}. At 0.96 a_w, BM grew significantly slowly than BV, although final concentrations were comparable after 48 h, around 2×10^8 CFU mL^{-1}. At 0.98 and 0.998 a_w, both strains achieved maximum growth of 2×10^9 CFU mL^{-1} after 24 h (Fig. 5a).

For *Vishniacozyma* strains, maximum growth occurred at 48 h in the media with all water activities tested (0.96, 0.98, and 0.998 a_w),

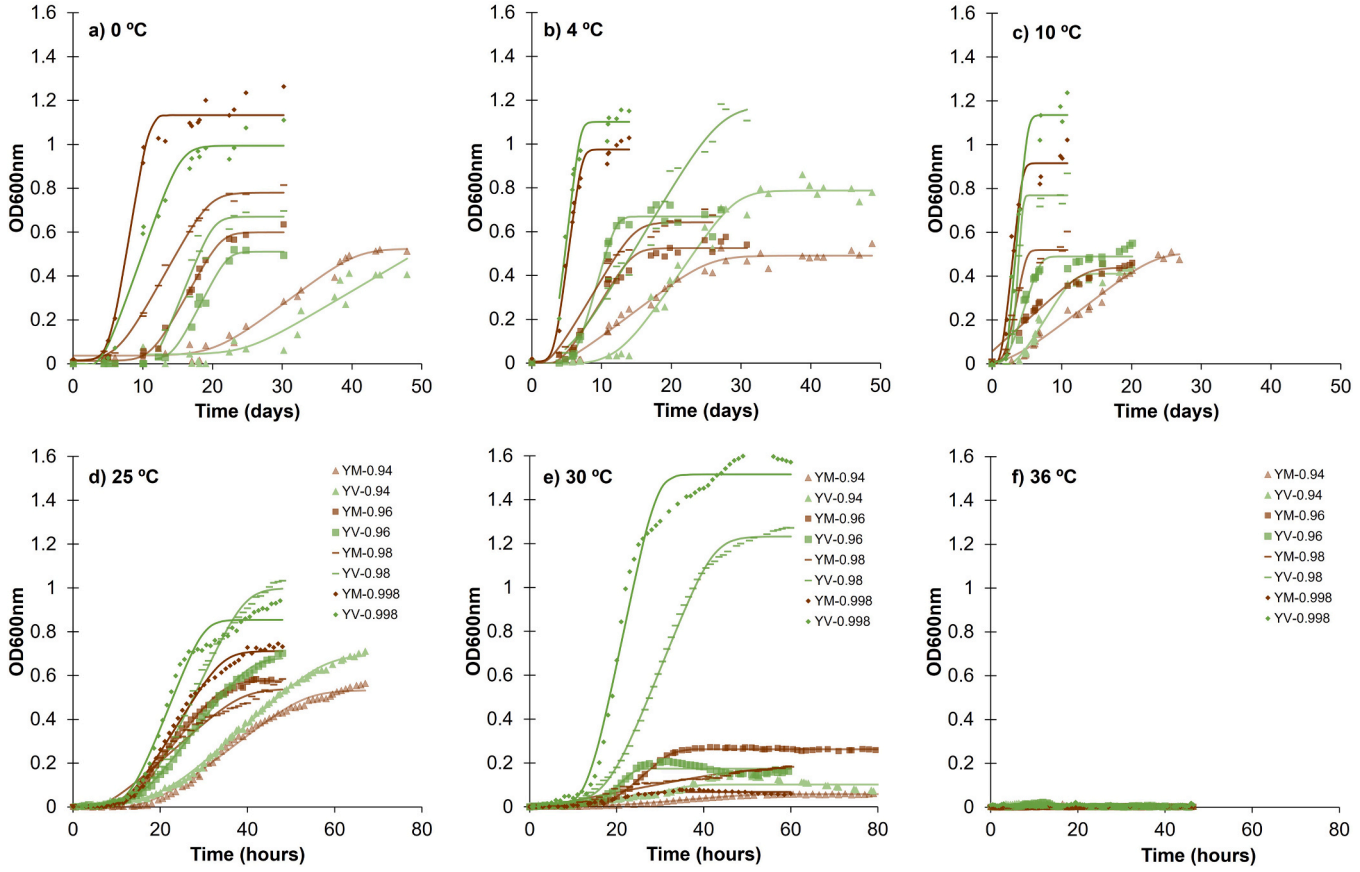

Fig. 3. Absorbance growth curves (600 nm) of the yeasts candidates (YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111) at different water activity modified with glycerol, 0.94, 0.96, 0.98 and 0.998, and different temperatures a) 0 ºC, b) 4 ºC, c) 10 ºC, d) 25 ºC, e) 30 ºC and f) 36 ºC. Symbols refer to experimental data, and solid lines refer to curves fitted by the Gompertz model. The temperatures a) 0 ºC, b) 4 ºC and c) 10 ºC were represented in days and the rest of the temperatures d) 25 ºC, e) 30 ºC and f) 36 ºC in hours.

Table 3
Duration of the lag phase (h or d) for selected bacteria (BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296) at varying temperatures and water activity, as estimated by the Gompertz model. Statistical analysis was performed with ART-ANOVA, followed by Tukey-adjusted ART contrasts. Asterisks (*) indicate significant differences between isolates in each water activity and temperature ($p \leq 0.05$). (–) signifies no growth.

α_w	Temperature (ºC)											
	0 ºC		4 ºC		10 ºC		25 ºC		30 ºC		36 ºC	
	BM	BV	BM	BV	BM	BV	BM	BV	BM	BV	BM	BV
0.94	–	–	–	–	–	–	–	**33.00***	–	**27.98***	30.97	30.78
0.96	–	–	14.13	12.90	3.32	2.98	11.85	14.97	10.89	12.47	11.27	12.53
0.98	14.30	11.49	2.75	2.38	3.04	2.69	**5.98***	**9.87***	**4.02***	**7.68***	4.74	7.96
0.998	13.09	11.42	4.17	4.02	2.02	1.84	7.72	7.19	6.57	5.24	5.06	4.67

Table 4
Maximum growth rate (h^{-1} or d^{-1}) for selected bacteria (BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296) at varying temperatures and water activity, as estimated by the Gompertz model. Statistical analysis was performed with ART-ANOVA, followed by Tukey-adjusted ART contrasts. Asterisks (*) indicate significant differences between isolates in each water activity and temperature ($p \leq 0.05$). (–) signifies no growth.

α_w	Temperature (ºC)											
	0 ºC		4 ºC		10 ºC		25 ºC		30 ºC		36 ºC	
	BM	BV	BM	BV	BM	BV	BM	BV	BM	BV	BM	BV
0.94	–	–	–	–	–	–	–	**0.01***	–	**0.02***	0.01	0.01
0.96	–	–	0.02	0.02	0.04	0.08	**0.02***	**0.03***	0.03	0.04	0.05	0.05
0.98	**0.04***	**0.03***	0.05	0.06	0.16	0.18	0.04	0.06	0.05	0.07	0.08	0.11
0.998	**0.14***	**0.04***	0.10	0.13	0.31	0.35	0.08	0.09	0.08	0.08	0.08	0.08

Table 5
Maximum absorbance growth capacity ($OD_{600\ nm}$) for selected bacteria (BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296) at varying temperatures and water activity, as estimated by the Gompertz model. Statistical analysis was performed with ART-ANOVA, followed by Tukey-adjusted ART contrasts. Asterisks (*) indicate significant differences between isolates in each water activity and temperature ($p \leq 0.05$). (–) signifies no growth.

α_w	Temperature (°C)											
	0 °C		4 °C		10 °C		25 °C		30 °C		36 °C	
	BM	BV	BM	BV	BM	BV	BM	BV	BM	BV	BM	BV
0.94	–	–	–	–	–	–	–	**0.40***	–	**0.49***	0.00	0.00
0.96	–	–	0.33	0.48	0.24	0.46	0.53	0.66	0.54	0.80	0.58	0.78
0.98	**0.67***	**0.60***	1.24	1.31	**1.28***	**1.12***	0.83	1.08	0.87	0.69	0.80	0.79
0.998	**0.89***	**1.16***	1.08	1.14	**1.26***	**1.01***	1.23	1.34	**1.01***	**0.86***	0.73	0.76

Table 6
Duration of the lag phase (h or d) for selected yeasts (YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111) at varying temperatures and water activity, as estimated by the Gompertz model. Statistical analysis was performed with ART-ANOVA, followed by Tukey-adjusted ART contrasts. Asterisks (*) indicate significant differences between isolates in each water activity and temperature ($p \leq 0.05$). (–) signifies no growth.

α_w	Temperature (°C)											
	0 °C		4 °C		10 °C		25 °C		30 °C		36 °C	
	YM	YV	YM	YV	YM	YV	YM	YV	YM	YV	YM	YV
0.94	19.68	25.02	6.29	12.61	4.23	4.51	17.89	19.66	–	–	–	–
0.96	**11.15***	**14.90***	3.77	6.09	2.3	2.33	10.91	14.94	**18.36***	**15.75***	–	–
0.98	**6.23***	**12.02***	2.92	3.78	**1.79***	**3.04***	**7.89***	**16.01***	**20.15***	**18.71***	–	–
0.998	4.79	3.22	3.27	3.27	**1.36***	**2.30***	12.76	13.84	**20.15***	**13.22***	–	–

Table 7
Maximum growth rate (h^{-1} or d^{-1}) for selected yeasts (YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111) at varying temperatures and water activity, as estimated by the Gompertz model. Statistical analysis was performed with ART-ANOVA, followed by Tukey-adjusted ART contrasts. Asterisks (*) indicate significant differences between isolates in each water activity and temperature ($p \leq 0.05$). (–) signifies no growth.

α_w	Temperature (°C)											
	0 °C		4 °C		10 °C		25 °C		30 °C		36 °C	
	YM	YV	YM	YV	YM	YV	YM	YV	YM	YV	YM	YV
0.94	0.02	0.02	0.03	0.05	**0.02***	**0.09***	0.02	0.02	–	–	–	–
0.96	0.06	0.08	0.05	0.11	**0.03***	**0.01***	0.03	0.03	0.02	0.02	–	–
0.98	0.06	0.07	0.06	0.05	**0.16***	**0.50***	**0.02***	**0.04***	**0.01***	**0.05***	–	–
0.998	0.19	0.09	0.24	0.27	0.30	0.39	0.04	0.06	**0.01***	**0.10***	–	–

achieving around 1×10^8 CFU mL^{-1} except for 0.94, in which 72 h are needed. There were no differences between the two strains for each water activity at any sampled time (Fig. 5b).

3.4. Dynamics of the four candidates under different relative humidities

The candidates were also characterised *in vivo* on the surface of apples at a low relative humidity of around 40 % and at a high relative humidity of 85 % usual in fruit storage cold chambers (Fig. 6). Yeasts survived better on the surface of apples at both relative humidity levels than bacteria. After 12 days, yeasts exhibited lower population reductions, with values of 0.59 and 0.41 log reductions for YV and YM at 40 % relative humidity, and 1.18 and 0.71 log reductions at 85 % relative humidity. In contrast, bacterial isolates showed greater population declines, with 1.79 and 2.51 log reductions for BV and BM at 40 % relative humidity, and 2.68 and 2.56 log reductions for BV and BM at 85 % relative humidity. In general, the studied strains performed better at 40 % than at 85 % RH. Both bacterial populations suffered a significant decline of 1 log CFU cm^{-2} 24 h post-application. Then, these strains decreased more gradually over time (Fig. 6a). At 85 % RH, a short decline occurred after 7 days, followed by recovery at 9 days. After 12 days, BCA concentrations were around 10^2 CFU cm^{-2} under both relative humidities.

Yeasts, however, showed a different population dynamic as these strains were more stable over the days on the surface of the fruit, not lowering their population below 1.79×10^3 CFU cm^{-2} (Fig. 6b). YM was

Table 8
Maximum absorbance growth capacity ($OD_{600\ nm}$) for selected yeasts (YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111) at varying temperatures and water activity, as estimated by the Gompertz model. Statistical analysis was performed with ART-ANOVA, followed by Tukey-adjusted ART contrasts. Asterisks (*) indicate significant differences between isolates in each water activity and temperature ($p \leq 0.05$). (–) signifies no growth.

α_w	Temperature (°C)											
	0 °C		4 °C		10 °C		25 °C		30 °C		36 °C	
	YM	YV	YM	YV	YM	YV	YM	YV	YM	YV	YM	YV
0.94	**0.53***	**0.50***	**0.40***	**0.84***	**0.61***	**0.45***	0.53	0.70	–	–	–	–
0.96	**0.60***	**0.51***	**0.52***	**0.67***	0.44	0.49	0.58	0.68	0.26	0.17	–	–
0.98	0.78	0.67	**0.61***	**1.19***	**0.51***	**0.76***	**0.54***	**1.00***	**0.14***	**1.25***	–	–
0.998	1.13	1.00	0.97	1.1	0.90	1.12	0.74	0.81	**0.07***	**1.52***	–	–

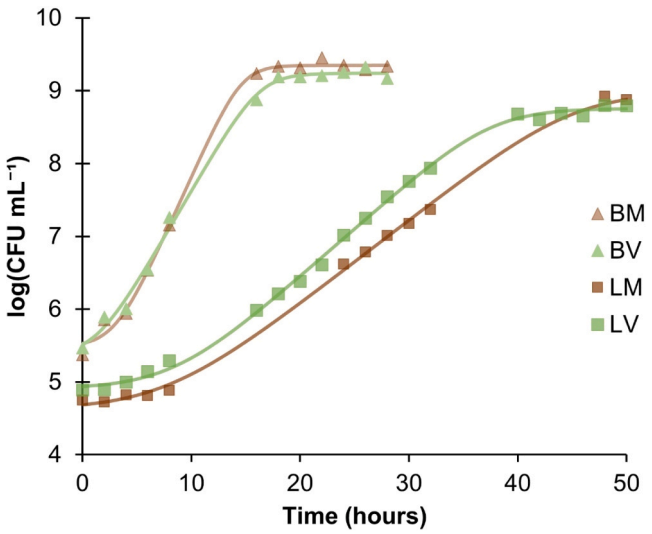

Fig. 4. Growth curves (log (CFU mL^{-1})) of the four candidates after 28 h of incubation at 25 ºC in LB liquid media for the bacteria, BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296, and 48 h of incubation in PDB liquid media for the yeasts, YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111. Symbols denote experimental data, while solid lines indicate curves fitted by the Gompertz model.

more stable than YV at almost all times at both relative humidities, stabilising around 1×10^4 CFU cm^{-2}.

4. Discussion

This study aimed to evaluate the biocontrol potential of several microorganisms against apple postharvest diseases. The microorganisms were isolated using a culturomics approach from the apple carposphere. They were obtained from two contrasting environments, the Pyrenees Mountains and the Ebro Valley, and two different niches, epiphytic and endophytic communities. The aim of this study is to explore whether the ecological origin provides adaptability traits to the most promising strains that may enhance resilience and biocontrol performance.

In this work, we evaluated the biocontrol potential of 124 bacterial isolates, 78 yeast isolates, and 11 filamentous fungal isolates against key postharvest pathogens, *Penicillium expansum*, *Botrytis cinerea* and *Rhizopus stolonifer* in artificially inoculated wounded apples. The result was that 4 bacterial isolates and 25 yeast isolates controlled both diseases, reducing the rot incidence by more than 75 % with respect to the control. These results, as other authors discussed, highlight the importance of recovering microorganisms from the surface of apples, as this technique gave us a broad starting range of candidates that share the same ecological niche as postharvest fungal pathogens (Droby and Wisniewski, 2018; Fernandez-San Millan et al., 2021; Sare et al., 2024). However, further validations through experiments under field conditions are needed, as these assays were conducted on artificially wounded apples under controlled conditions.

Our screening results reflect an improvement in the methodology for the search for biocontrol agents with the implementation of a culturomics approach. Whereas previous trials tested all recovered microorganisms without prior identification, in this study, we first identified the isolates and selected genera with reported biocontrol potential in postharvest. This resulted in an increase in the biocontrol efficacy of the isolates tested. For example, Viñas et al. (1998) recovered 933 epiphytic bacteria and yeast from apples, pears and apple leaves, and obtained 10 % of the isolates capable of reducing the disease against *P. expansum* by more than 50 %. Also, Nunes et al. (2001) tested 247 microorganisms against *P. expansum,* and only 2 % of them reduced the incidence of infected wounds by more than 50 %. In contrast, we increased the percentage of candidates capable of reducing the incidence by more than 50 % of *P. expansum* up to 50 %.

Previous studies have already shown that some genera are more suitable than others to work as viable BCAs (Bonaterra et al., 2022; Villavicencio-Vásquez et al., 2025). This is consistent with the current situation in the European market, where only a few microbial products are approved, partly due to the long and demanding registration procedures required for market approval (Romanazzi et al., 2016; Soto-Muñoz et al., 2020; Wenneker and Thomma, 2020). The biocontrol products approved for postharvest diseases in apples are: *Aureobasidium pullulans* (BoniProtect®/ BOTECTOR®, SAN Agrow Holding, Austria), *Bacillus amyloliquefaciens* (Amylo-X-WG®, Certis, USA), *Bacillus subtilis* (Serenade®, Bayer AG, Germany), *Candida oleophila* (Nexy®, Ennolys, France), or *Metschnikowia fructicola* (Shemer®, Bayer AG, Germany). In our study, we found partial similarities with these genera, particularly with *Aureobasidium*, *Metschnikowia* and *Candida*. We also identified *Pantoea* as one of the most successful bacterial genera, which correlates with results previously obtained by our group that led to the development of the formulated product Pantovital® (IRTA, Spain) (Soto-Muñoz et al., 2020). Other promising candidates in our study not currently represented in commercial formulations include *Rhodococcus* and *Streptomyces* for bacteria, and *Meyerozyma*, *Pichia*, *Rhodotorula*, and *Vishniacozyma* for yeasts. The identification of these additional genera for postharvest diseases highlights opportunities to complement existing commercial BCAs rather than replace them. For example, targeting

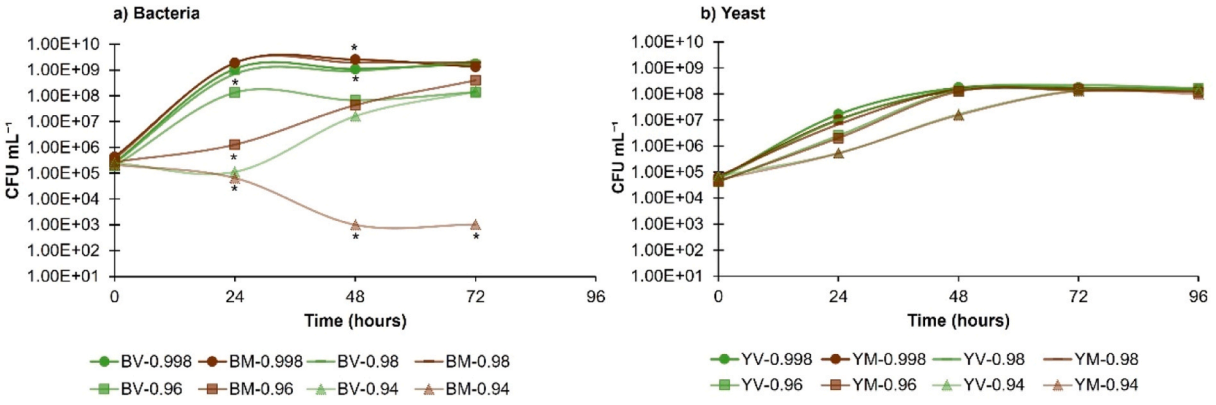

Fig. 5. Growth curves (CFU mL^{-1}) of the four candidates after 72 h of incubation in LB liquid media at different water activity modified with glycerol, 0.94, 0.96, 0.98 and 0.998 for the a) bacteria, BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296, and 96 h of incubation in PDB liquid media at different water activity modified with glycerol, 0.94, 0.96, 0.98 and 0.998 for the b) yeasts, YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111. The values represent the mean of three replicates. Asterisks denote significant differences between conditions according to Tukey's post-hoc test ($p \leq 0.05$).

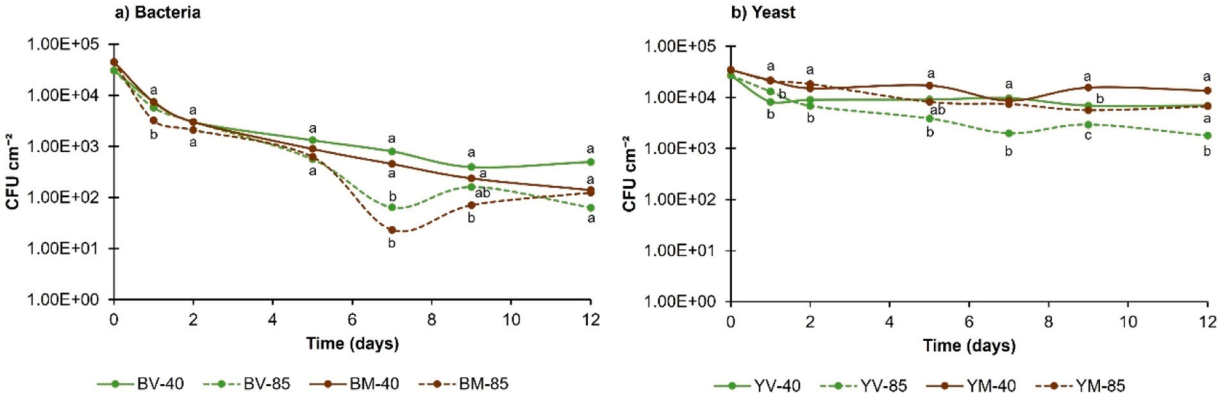

Fig. 6. Dynamics (CFU cm^{-2}) on apples' surface of the selected candidates: a) Bacteria (BM, *Pantoea agglomerans* strain B109; BV, *Pantoea agglomerans* strain B296) and b) Yeast (YM, *Vishniacozyma carnescens* strain L157; and YV, *Vishniacozyma carnescens* strain L111), were observed under two different relative humidity conditions (40 % and 85 %) over a period of 12 days at room temperature. The values represent the mean of four replicates. Letters denote significant differences between conditions according to Tukey's post-hoc test ($p \leq 0.05$).

specific pathogens or environmental conditions, combining different modes of action, or creating a synthetic microbial community that combines all of the previous ones together (Ferraz et al., 2016; Fenta et al., 2023; Droby et al., 2025; Pereyra et al., 2024).

From the collection of potential BCA isolates, two *P. agglomerans* and two *V. carnescens* strains were selected. The following *in vitro* characterization results showed that the bacterial candidate, *P. agglomerans* from the valley, was more adapted to the dry and hot climatic conditions typical of the Ebro Valley region. Meanwhile, the yeast valley strain was also more suited to hot and dry climates, whereas the mountain isolate is adapted to colder conditions. These results were further confirmed by the growth of these microorganisms in a larger volume of culture medium. At 25 °C, BM was unable to grow (0.94 a_w) or grew slower (0.96 a_w) compared to BV under more limiting water activity conditions. These patterns are consistent with the contrasting climatic conditions of the two regions. The Ebro Valley orchards (low altitude) are characterized by lower relative humidity, lower solar radiation levels, and higher temperatures, from maximum to minimum. In contrast, Pyrenees Mountains orchards (high altitude) exhibited cooler conditions, where the temperature rarely exceeds 30 ºC (Supplementary Figure S1A, S1B, S1C, and S1D). We know that environmental conditions of these locations shape microbial diversity and community composition in apples (Sánchez et al., 2025a, 2025b) and impact the quality and physiology of the apple fruit (Fernández-Cancelo et al., 2021, 2022; Vall-llaura et al., 2025).

While in some studies it has been shown that the preprocessing of the BCAs is useful to improve their stress tolerance, adaptability and the final product's effectiveness in field conditions (Cañamás et al., 2011; Droby et al., 2016; Sui et al., 2015; Teixidó et al., 2006, 2011). In the present approach, the selection of microorganisms already adapted to the environment may remove the need for this preprocessing step. This is important because withstanding biotic stresses is one of the key characteristics that a BCA needs to combat plant disease (Costa et al., 2002; Fernandez-San Millan et al., 2021). In this context, microorganisms isolated from distinct ecological niches offer valuable insights into their adaptability and survival under changing environmental conditions (Droby et al., 2016; Palmieri et al., 2022). Some examples are Palmieri et al. (2022) who reviewed studies showing that the cold-adapted yeasts *Leucosporidium scottii* recovered from Antarctic soil and *Cryptococcus laurentii* from Tibet which exhibited stronger biocontrol activity against *Botrytis cinerea* in apples and tomatoes stored at low temperatures (Hu et al., 2017; Vero et al., 2013).

Both genera form part of the core microbiome of the apple fruit (Sánchez et al., 2025b), microbial taxa consistently found in apples in various geographical areas (Abdelfattah et al., 2021). Suggesting a natural ecological adaptation to the apple environment result of a possible coevolution between the microorganisms populating the fruit and their host, that could have been maintained throughout the process of domestication (Abdelfattah et al., 2021). This ecological perspective is increasingly recognised as a potential area in microbiome-based biocontrol research (Droby et al., 2025).

Although the mode of action of the isolates selected in our study was not evaluated, previous reports provide insights into potential mechanisms of the selected genera. For instance, several *P. agglomerans* strains have been shown to act through physical contact, competition for nutrients and space, as well as, to a lesser extent by producing antimicrobial metabolites against postharvest pathogens (Bonaterra et al., 2022; Dutkiewicz et al., 2016; Moreno González, 2006; Poppe et al., 2003). Similarly, *V. victoriae* has been reported to control the pathogen through nutrient competition, utilizing the host's organic acid and energy to colonize the wound (Nian et al., 2023). This is why studying the optimal growth conditions of these BCAs is essential to understand the best conditions for them to thrive and, in turn, be effective when competing against pathogens.

We evaluated the production of these candidates at laboratory scale, reaching high cell concentrations in a laboratory liquid medium, 1.50×10^9 CFU mL^{-1} for the bacterial strains after 18 h and 6×10^8 CFU mL^{-1} for the yeast strains after 48 h. These results are comparable with those of other *P. agglomerans* strain CPA-2, where concentrations between 3.2×10^9 CFU mL^{-1} to 5.50×10^9 CFU mL^{-1} were achieved (Costa et al., 2001). Also, *V. victoriae* NPCC 1263 obtained concentrations in a bioreactor between 5.78×10^8 CFU mL^{-1} to 7.46×10^8 CFU mL^{-1} (Gorordo et al., 2022). Although our results were performed in small-volume Erlenmeyer flasks, it provides a promising starting point for future optimization and scale-up in bioreactor systems (Teixidó et al., 2022). Further development will require addressing formulation challenges, including the selection of appropriate carriers and adjuvants to ensure viability and efficacy during storage and application (Teixidó et al., 2022).

Finally, a characterization of the dynamics of the strains was carried out *in vivo* on apple fruits. It was done under varying relative humidity conditions to evaluate the microorganisms' persistence under a range of environments. While bacterial populations declined quickly, the yeast candidates, on the other hand, showed notable survival across varying hypothetical relative humidity conditions, highlighting their resilience as biocontrol agents. This behaviour is consistent with previous studies showing that *P. agglomerans* CPA-2 populations decrease rapidly when applied on unwounded oranges but remain stable in wounds, the natural entry point of pathogens (Teixidó et al., 2001). A similar result was found for *Candida sake* CPA-1 on unwounded apples (Usall et al., 2001). Such behaviour could be beneficial because the biocontrol agent could persist in the microenvironments where disease prevention is required,

while decreasing to very low or undetectable levels on the fruit surface (Teixidó et al., 2001). Although the observed differences in survival at 40 % compared to 85 % relative humidity were not statistically significant, the trend suggests that these microorganisms may survive in drier conditions without major population losses. This resilience under reduced water availability is consistent with previous reports highlighting the importance of abiotic stress tolerance for the persistence of biocontrol agents in field applications (Carbó et al., 2019; Gotor-Vila et al., 2017b).

Moreover, Teixidó et al. (2011) emphasized that the crucial aspect of preharvest biocontrol application lies in the agent's capability to effectively colonize and persist on fruit surfaces from field conditions through subsequent storage, thus continuously preventing decay. Also, previous studies underline that insufficient survival in field conditions has traditionally limited the success of biological control agents (Fernandez-San Millan et al., 2021; Varo et al., 2016).

Overall, these findings improved our existing BCA screening methodology and provided new insights into the environmental adaptability of potential biocontrol agents. Selecting strains with specific environmental adaptations could optimize their efficacy in diverse postharvest conditions. In this context and as future research, the combination of the BV and YM in a microbial consortium would represent a promising approach, as these strains exhibit complementary environmental adaptations. Such consortia could contribute to the development of more resilient and stable biocontrol formulations. Specifically, improving performance under field conditions where environmental variability is common.

Data availability

All data are available under NCBI BioProject PRJNA1265908, and the BioSample accession numbers for each candidate tested in this study are provided in Supplementary Tables 1, 2 and 3.

CRediT authorship contribution statement

Neus Teixidó: Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization. **Jonàs Oliva:** Writing – review & editing, Validation, Supervision. **Cristina Solsona:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Ana María Sánchez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Grammarly to ensure clarity, cohesiveness, and comprehensiveness. After using this tool, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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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.2026.114174.

Data availability

All data are available under NCBI BioProject PRJNA1265908, and the BioSample accession numbers for each candidate tested in this study are provided in Supplementary Tables 1, 2 and 3.

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