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Molecular characterization of *Aureobasidium* spp. strains isolated during the cold season. A preliminary efficacy evaluation as novel potential biocontrol agents against postharvest pathogens

R. Cignola · A. Boato · A. Sadallah · G. Firrao · A. Di Francesco

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Abstract Several *Aureobasidium* spp. strains isolated from wild environments during winter 2022 were characterized by sequence analysis of the internal transcribed spacer (ITS), the translation elongation factor EF-1α gene (EF1), and part of the elongase gene (ELO). The variability in the EF1 and ELO *loci* are higher than in the ITS. All strains but one (UC14), were identified as *A. pullulans*. To assess the effectiveness of the characterized strains as biocontrol agents (BCAs) of diseases occurring during postharvest storage, a selection of the strains was evaluated by *in vitro* and *in vivo* assays. On average, the reduction of *Monilinia* spp. colony growth was more marked for non-volatile metabolites than for volatile (VOCs). Strain UC14 provided the strongest mycelial growth reduction of *Monilinia fructicola* by VOCs (66%). According to the *in vivo* results, all strains were effective in controlling brown rot during cold storage and remarkably in restricting the growth of *Monilinia polystroma*. In particular, VB23 was the most effective in controlling brown rot incidence, by 80%, 60%, 100%, and severity, by 79.5%, 72.7% and 100%, for *Monilinia laxa, M. fructicola,* and *M. polystroma,* respectively.

Keywords Yeast · Antagonists · Stone fruits · Biological control · Brown rot

Introduction

The increasing limitation to the use of pesticides, the allowable limit of residues on fruits, and the risk of development of resistant strains together with the request of the market for a more sustainable fruit production, is leading plant defence science into a change (Casals et al., 2022). The new European plant health regulation (EU 2016/2031) jointly with the "European Green Deal" aims to accelerate the transition to a more sustainable food system recommending a reduction by 50% of pesticides and an increment of the land used for organic by 25% farming by 2030. A significant number of pesticides are presently used to prevent brown rot on stone fruits, caused by *Monilinia laxa* (Aderh. & Ruhland) Honey, *Monilinia fructicola* (Winter) Honey, and *Monilinia polystroma* Kohn. These species are notorious pathogens of rosaceous plants all around the world (Petróczy et al., 2012). Fruits that has been infected in the field can lead to losses during the postharvest phase in packing houses (Larena et al., 2005a). In Europe, *M. laxa* is the prevalent species (Larena et al., 2005b) but since the detection

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R. Cignola · A. Boato · A. Sadallah · G. Firrao · A. Di Francesco (✉)
Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy
e-mail: alessandra.difrancesco@uniud.it

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of *M. fructicola* in France (EPPO, 2002), Spain (de Cal et al., 2009), Switzerland (Bosshard et al., 2006), Hungary (Petróczy & Palkovics, 2009), Italy (Pellegrino et al., 2009) and *M. polystroma* (Côté & Meldrum, 2004;Petróczy & Palkovics, 2009; EPPO, 2011; Martini et al., 2014) on peaches, it is now apparent that these species can co-exist (Villarino et al., 2013).

A sustainable solution to limit brown rot diseases during storage is biological control by exploiting microorganisms (Droby et al., 2009) referred to as biocontrol agents (BCAs). Recent studies proved the efficacy of *Aureobasidium pullulans* (de Bary) G. Arnaud in controlling some of the most detrimental pathogens of fruits in the postharvest phase (Di Francesco et al., 2018, 2020a, 2020b; Ippolito et al., 2000; Mari et al., 2012,). *Aureobasidium pullulans* is a polyextremotolerant black yeast, epiphyte or endophyte, globally distributed, found in tropical, temperate, and polar areas (Di Francesco et al., 2023; Gostinčar et al., 2014). Other interesting species within the *Aureobasidium* genus are *A. melanogenum*, *A. subglaciale,* and *A. namibiae* (Gostinčar et al., 2014), although *A. melanogenum* could be associated to human infections, being able to grow at 37 °C (Di Francesco et al., 2020b).

Unfortunately, not much data is available regarding the biocontrol ability of *A. namibiae*, a species isolated from extreme environments and able to tolerate up to 10% of NaCl and to grow between 10 °C and 30 °C (Zalar et al., 2008; Gostincar et al., 2014). Such features make it a promising source for new antimicrobial metabolites and a candidate for applications in agriculture. Recently, new species such as *Aureobasidium vineae, Aureobasidium mustum, Aureobasidium uvarum* (Onetto et al., 2020)*, Aureobasidium castaneae* (Jiang et al., 2021), *Aureobasidium aerum* (Cheng et al., 2022) and *Aureobadidium acericola* (Lee et al., 2021) were described on the ground of phylogenetic and morphological analyses, but their potential as BCAs has not been assessed yet (Di Francesco et al., 2023).In general, the most studied environment for isolation of antagonistic yeasts to be applied during the postharvest phase, is the fruit surface (Mari et al., 2012; Parafati et al., 2015). In general, the isolation of wild yeasts from fruit surface holds a great promise for disease control in postharvest, although the biocontrol ability of the isolated strains may vary greatly, and hence needs careful assessment.

In order to improve the knowledge on the potential of wild yeasts, we undertook the present work with the aim to molecularly characterize the *Aureobasidium* spp. population from wild environments sampled during the cold season and evaluate the biocontrol ability of selected strains through *in vitro* (co-culturing, non-volatile and volatile metabolites assays) and *in vivo* assays on apricots against *Monilinia* spp.

Material and methods

Microorganisms' isolation and morphological identification

Thirty-seven wild black yeasts were isolated during winter 2022 from wild plants located in Friuli Venezia Giulia, Italy. Olive, laurel, plum, and viburnum healthy leaves were sampled. Plants material (2 g) was washed with 20 mL of sterile distilled water (SDW) amended with Tween 20 (0.05% v/v, Sigma-Aldrich, USA), collected in sterile flasks subsequently placed at 20 °C for 1 h under continuous stirring on a rotary shaker (250 rpm). Samples were centrifuged for 30 min at 3500 rpm and the supernatant was discarded. The pellet was resuspended with 1 mL of SDW and then 100 µL of each solution wase spread on Petri dishes containing NYDA medium (non-selective general-purpose medium for the cultivation of microorganisms) (8 g L^{-1} Nutrient broth, 5 g L^{-1} Yeast extract, 10 g L^{-1} Dextrose, and 25 g L^{-1} of technical Agar) (Oxoid, UK). Plates were incubated for 48 h at 20 °C. Only single colonies displaying *A. pullulans* morphological characteristics like smooth, slimy, pale pink or cream color, as reported by Barnett and Barry (1998), were individually isolated on new plates and then purified. Pure cultures were observed with a microscope (Zeiss AXIO Observer. Z1). Only the strains with common *Aureobasidium* morphological characteristics such as hyaline, thin-walled and septate hyphae, and ellipsoidal, variable in shape and dimension, hyaline conidia were retained, according to Zalar et al. (2008).

Fifteen *Monilinia* spp. strains were isolated from symptomatic apricots collected in the experimental orchards of the University of Udine. Purified fungal isolates were grown on PDA (Potato Dextrose Agar, Oxoid, UK) plates for 5 d at 20 °C. Yeast isolates were processed for DNA extraction and molecular

identification. For the subsequent experiments, yeast cells and fungal conidia were collected and suspended in SDW containing 0.05% (v/v) Tween 20 and the suspensions were adjusted to 1×10^8 cells mL^{-1} and 1×10^5 conidia mL^{-1}, respectively, by using a hemocytometer. Before proceeding to the *in vivo* assay, fungal strain suspensions (1×10^5 conidia mL^{-1}) were wound-inoculated on apricots to test their pathogenicity.

Fruits

Apricots (*Prunus armeniaca* L.) cv "Bella d'Imola" (12°Brix) were harvested in an experimental orchard of the University of Udine (Italy) (46°01′56.4"N 13°13′23.6"E) and immediately processed. Fruits homogeneous in size and injuries-free were selected.

Molecular characterization of Aureobasidium spp. and Monilinia spp

Yeast isolates were grown on flasks containing NYDB (NYDA without agar). Cellular cultures were centrifuged at 3500 rpm for 30 min and the supernatant was discarded. Cells were frozen with liquid nitrogen and then stored at – 80 °C. The genomic DNA extraction was conducted by using 150 mg of each sample collected in 2 mL microcentrifuge tubes (Eppendorf AG, Hamburg, Germany) and by following the protocol described by Carlucci et al. (2013).

For molecular characterization of *Aureobasidium* spp., a *multi-locus* approach was used. The nucleotide sequences of Internal Transcribed Spacer (ITS), translation elongation factor EF-1α gene (EF1) and part of the elongase gene (ELO) were determined as described by Zalar et al. (2008). The ITS region was PCR amplified by using the primers ITS1 (5'-TCC GTAGGTGAACCTGCGG-3') and ITS4 (5'-TCC TCCGCTTATTATTGATATGC-3') described by White et al. (1990). Primers ELO2-F(5'-CACTCT TGACCGTCCCTTCGG-3') and ELO2-R (5'-GCG GTGATGTACTTCTTCCACCAG-3') (Zalar et al., 2008) were used for the PCR amplification of the elongase gene. Finally, for the amplification of the elongation factor 1α gene, EF1-728F (5'-CATCGA GAAGTTCGAAGG-3') and EF1-986R (5'-TACTTG AAGGAACCTTTACC-3') (Carbone & Kohn, 1999) were used. PCR amplifications were carried out separately on a MiniAmp Plus thermal cycler (Thermo Fisher Scientific, USA). For the ITS region, an initial denaturation of 94 °C for 2 min was followed by 40 cycles of 94 °C for 40 s, 55 °C for 40 s, and 72 °C for 1 min, with a final extension of 5 min at 72 °C. For ELO and EF1, the parameters were slightly different: an initial denaturation of 94 °C for 5 min, 35 cycles of 94 °C for 15 s, 56 °C for 40 s and 40 s at 72 °C, with a final extension of 8 min at 72 °C. The PCR products were purified and sequenced by BMR Genomics (Padova, Italy).

For *Monilinia* spp. isolates identification, DNA was extracted as above,after collecting 150 mg of mycelium scraped off the PDA plates after 7 days of growth. DNA was amplified by using primers UniMon_Rev (5'-GAGCAAGGTGTCAAAACT TCCAT-3') and UniMon_Forw (5'-ATCGGCTTG GGAGCGG-3') (Petróczy et al., 2012), based on the different lengths of the ITS. The amplified region is 397 bp for *M. laxa,* 417 bp for *M. fructigena*, 433 bp for *M. polystroma*, and 594 bp for *M. fructicola* (Petróczy et al., 2012).

RAPD analysis

To corroborate the findings concerning the strain diversity highlighted among the isolates by gene sequencing, DNA of 8 strains, selected as the most representative of each strain cluster, was subjected to Random Amplification of Polymorphic DNA (RAPD) analysis. The protocol described by Di Francesco et al. (2018) was used, and the purified genomic DNA was subjected to PCR for RAPD analysis by using a MiniAmp Plus thermal cycler (Thermo Fisher Scientific, USA). Four random primers were used (Supplemental material S1) and PCR products were separated and visualized on 1% agarose gel.

In vitro assays

To evaluate the effectiveness of the selected strains as BCAs, three different *in vitro* experiments were conducted as reported by Di Francesco et al. (2017). The interaction between the yeast strains and fungal pathogens belonging to three different species (*M. laxa* AML1, *M. fructicola* AMF1, *M. polystroma* AMP1) was evaluated by co-culturing assays. On PDA plates (90 mm diameter), a mycelial plug of each pathogen (6 mm diameter, from 7-d old colony) was placed 25 mm from the edge of the plate, and a streak of

each yeast strain (from a 48-h old culture) was placed 25 mm from the other side of the edge of the plate. The non-volatile organic compounds (non-VOCs) assays were carried out by using sterile cellophane layers (BIORAD, USA), positioned on PDA plates, on which 100 µL of each yeast suspension (1×10^8 cells mL^{-1}) were spread. After 48 h, the cellophane layer was removed and a mycelial plug from 7 d old colony of each pathogen species (6 mm diameter) was placed in the centre of the plate (Di Francesco et al., 2020a). For the evaluation of BCAs VOCs antifungal activity, a double Petri dish assay was performed according to the method of Rouissi et al. (2013) as modified by Di Francesco et al. (2020b).

The plates were incubated for 5 days at 25 °C for *M. laxa* and *M. fructicola* and for 14 d at 20 °C in the case of *M. polystroma* (Di Francesco et al., 2017). The sample unit of each assay consisted in 6 plates for each interaction. The experiments were conducted twice. The inhibition rate of mycelial growth was calculated using the equation (Dennis & Webster, 1971):

$$\%inhibition = \frac{d1 - d2}{d1} * 100$$

where d1 and d2 are the control colony and the treated colony diameters, respectively.

In vivo assay

Apricots were disinfected by immersion for 1 min in sodium hypochlorite solution (1%) and washed twice in sterile water. Fruits were wounded (2×2×2 mm) on the equatorial area with a sterile needle and inoculated with 20 µL of each selected yeast suspension (UC14, VB23, UC11, UOR18, UOR112, SE0, AL25, UC12) (1×10^8 cells mL^{-1}). After the complete adsorption (1 h), 20 µL of conidial suspension (1×10^5 conidia mL^{-1}) of each of the three pathogens (AML1, AMF1, AMP1) were pipetted into the wounds. After air drying, fruits were stored at 0 °C and 90% R.H. for 30 d in plastic boxes. The percentage of rotten fruits was detected after shelf-life of 7 d at 20 °C. Fruits inoculated with SDW (20 µL) and Scholar® (active substance fludioxonil, 2 mL/L) (Syngenta, Switzerland) were controls. Each treatment was represented by 20 fruits, and there were three replicates per treatment. The experiment was conducted twice.

Data analysis

All the obtained data from *in vitro* and *in vivo* experiments were subjected to a one-way analysis of variance (ANOVA). The statistical comparisons of the means were established by using Tukey's HSD test ($\alpha=0.01$). All the analyses were conducted by using Minitab 17® statistical software (Minitab, USA). DNA sequences were aligned by using Clustal Omega (Sievers et al., 2011) and the phylogenetic trees were built from pairwise distances using Seaview 5.0.5 (Gouy et al., 2010). The Neighbor-Net graph was built by using Split Trees Version 4 (Huson & Bryan, 2006). The same software tool was used to build and display a tree based on DNA fragment presence/absence data from RADP patterns as detected on agarose gels.

Results

Characterization and identification of the strains

From a first screening of the microorganisms grown on NYDA plates, thirty-seven colonies morphologically referable to *Aureobasidium* by microscope identification were selected, isolated on new agar plates, and purified. All the pure cultures were molecularly analysed.

The amplification of the target genes produced sequences of 508, 691, and 230 bp respectively for ITS, ELO, and EF-1alpha. According to the analysis, thirty-six strains were identified as *A. pullulans*, while one strain (UC14) was found to belong to the *Aureobasidium* genus but could not be unambiguously ascribed to a species, as reported in Table 1. The analysis carried out on the ITS region showed no differences among the strains (Fig. 1A), with the exception of the sequence of strain UC14 that displayed seven different nucleotides different from the others. Conversely, the variability at the ELO and EF1 loci was larger and more discriminant than that of the ITS, in particular for strain UC14 (on average, 65 and 44 nts difference for ELO and EF1 genes, respectively, vs. the remaining strains). Overall, the analysis of the sequences of the ELO gene revealed 8 distinct clusters of strains (Fig. 1B). The groups arising from the ELO gene sequence analysis did not match with those based on EF1 gene (Fig. 1C). This

Table 1 List of identified isolates based on ITS region

Sample ID	Identification	Origin	Accession number	Identity (%)
AL14	*A. pullulans*	Laurel, leaf	KY552634.1	99.82
AL18	*A. pullulans*	Laurel, leaf	KY552634.1	99.63
AL23	*A. pullulans*	Laurel, leaf	KY552634.1	99.63
AL25	*A. pullulans*	Laurel, leaf	KY552634.1	99.81
SE0	*A. pullulans*	Plum, endophyte	MT573468.1	99.08
SE2	*A. pullulans*	Plum, endophyte	KT223358.1	99.26
SE3	*A. pullulans*	Plum, endophyte	MT573468.1	98.90
UC11	*A. pullulans*	Olive, leaf	ON712557.1	99.63
UC13	*A. pullulans*	Olive, leaf	MT035961.1	99.63
UC14	Unknown	Olive, leaf	MT325792.1	99.44
UC21	*A. pullulans*	Olive, leaf	KY552634.1	99.82
UC22	*A. pullulans*	Olive, leaf	ON712557.1	99.62
UC23	*A. pullulans*	Olive, leaf	ON712557.1	99.01
UOR11	*A. pullulans*	Olive, leaf	KT223358.1	99.81
UOR110	*A. pullulans*	Olive, leaf	KY552634	99.44
UOR111	*A. pullulans*	Olive, leaf	KY552634	99.62
UOR112	*A. pullulans*	Olive, leaf	KT223358.1	99.62
UOR12	*A. pullulans*	Olive, leaf	KY552634.1	99.81
UOR13	*A. pullulans*	Olive, leaf	KY552634.1	99.63
UOR15	*A. pullulans*	Olive, leaf	ON248013.1	99.81
UOR17	*A. pullulans*	Olive, leaf	MT573468.1	99.81
UOR18	*A. pullulans*	Olive, leaf	ON248013.1	99.81
UOR19	*A. pullulans*	Olive, leaf	KY552634.1	99.63
UOR32	*A. pullulans*	Olive, leaf	ON248013.1	99.63
UOR33	*A. pullulans*	Olive, leaf	ON248013.1	99.62
UOR34	*A. pullulans*	Olive, leaf	KY552634.1	99.44
VB11	*A. pullulans*	Viburnum, leaf	KT223358.1	99.44
VB12	*A. pullulans*	Viburnum, leaf	KT223358.1	99.44
VB13	*A. pullulans*	Viburnum, leaf	ON712557.1	99.63
VB15	*A. pullulans*	Viburnum, leaf	KY552634.1	99.44
VB17	*A. pullulans*	Viburnum, leaf	ON712557.1	99.81
VB18	*A. pullulans*	Viburnum, leaf	ON712557.1	99.62
VB19	*A. pullulans*	Viburnum, leaf	KY552634.1	99.44
VB21	*A. pullulans*	Viburnum, leaf	KT223358.1	99.25
VB22	*A. pullulans*	Viburnum, leaf	MT107050.1	99.27
VB23	*A. pullulans*	Viburnum, leaf	KT223358.1	99.44
VB24	*A. pullulans*	Viburnum, leaf	MT573468.1	99.81

issue could probably be explained by the presence of a recombinant population (Fig. 1D).

One strain from each group (AL25, SE0, UC11, UC14, UC22, UOR112, UOR18, VB23), based on the ELO gene, was selected and the relative diversity was assessed by RAPD (A9) with electrophoretic separation (Fig. 2). The analysis of the RAPD patterns supported the notion of a diversified sample of strains, with strains UC14 and UC11 that differ the most from the others (Fig. 3).

Regarding the identification of *Monilinia* spp. isolates, the most represented species was *M. laxa* (13 isolates). The remaining strains were identified as either *M. fructicola* (1 isolate) and *M. polystroma* (1 isolate) (data not shown).

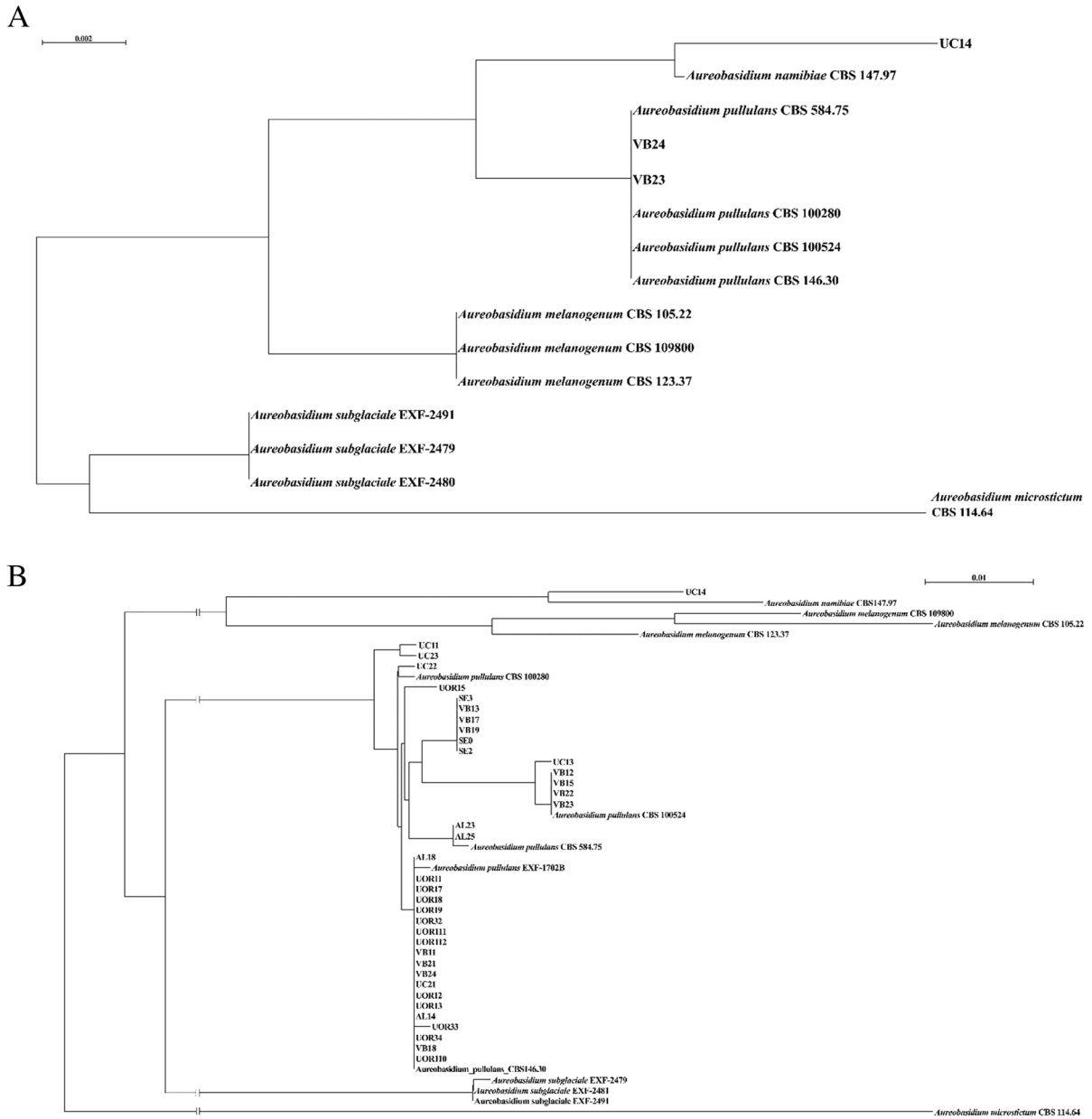

Fig. 1 Phylogenetic analysis of the isolates. Trees built using BIONJ distance methods (Gascuel, 1997) based on different regions: ITS (**A**), ELO gene (**B**) and EF1 (partial; **C**). **D**) Graph built using Neighbor-Net method (Bryant & Moulton, 2004) based on the concatenated ELO + EF1 genes

In vitro assays

Strains isolated in this work were evaluated through different assays. Regarding the co-culturing assay, all the selected strains provided on average a growth inhibition of AML1 and AMF1 by 23% and 25%, respectively, with no significant difference between the tested strains. *Monilinia polystroma* AMP1 strain was the tested fungal pathogen inhibited to the highest degree (on average 40%), in particular by strains SEO, AL25, UC22 with an inhibition of

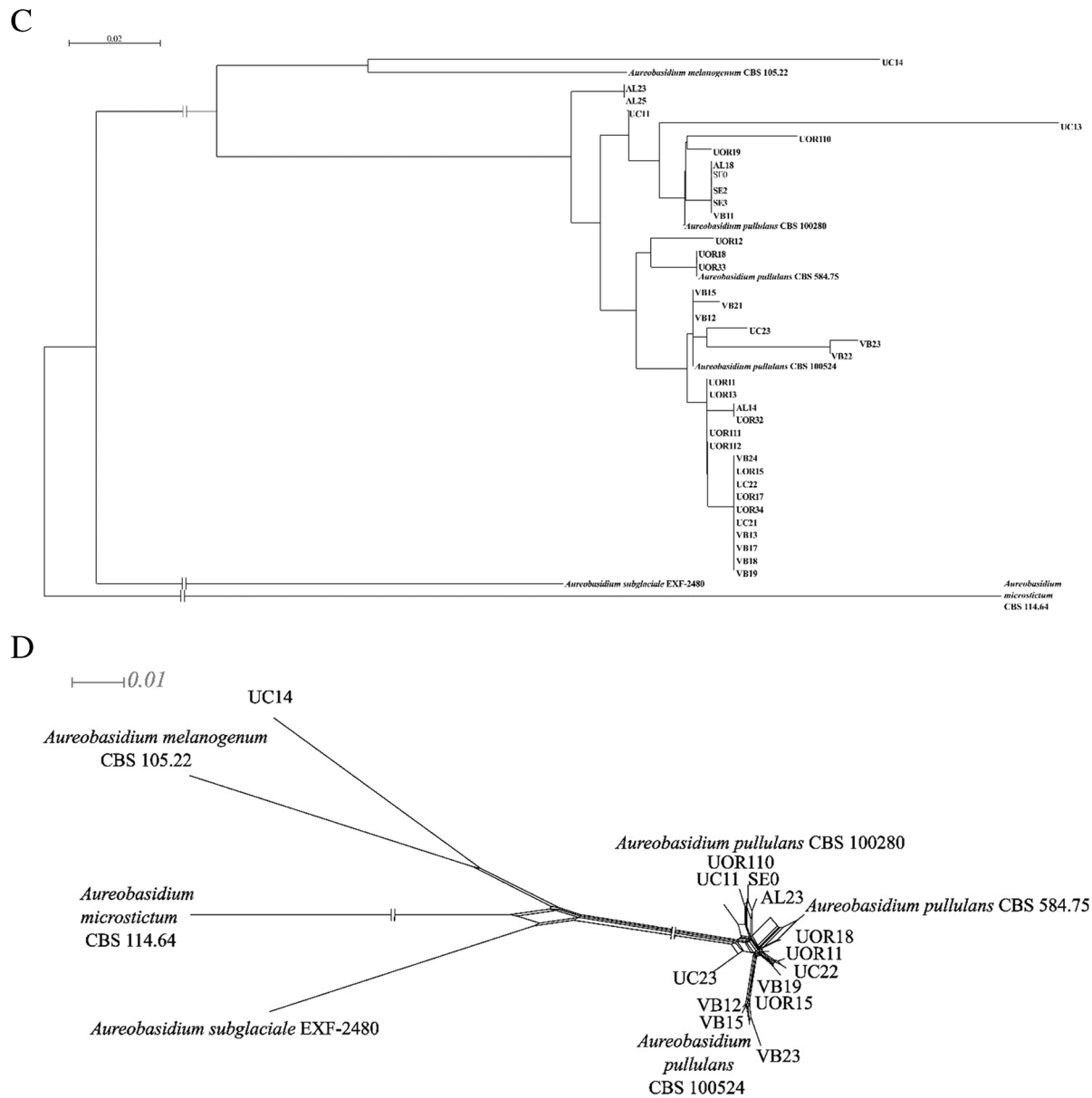

Fig. 1 (continued)

49.1%, 46.3% and 50%, respectively, as compared to the control (Fig. 4).

In the case of non-volatile assay, strains UC14, UOR18, AL25, and UC22 completely inhibited *M. polystroma* AMP1 mycelial growth (Fig. 5). The mycelial growth of both *M. laxa* AML1 and *M. fructicola* AMF1 was reduced by 70%, on average, by the tested strains. Noticeably, VB23 inhibited by 76% AML1 and UC11 by 81% AMF1.

The efficacy of VOCs produced by the tested strains was strictly dependent on the targeted pathogen species. As shown in Fig. 6, AML1 compared to AMF1, while AMP was less affected by the VOCs produced by the wild yeast strains. In fact, on average, the percentage of reduction detected for AML1, AMF1, and AMP1 was 28.6%, 32.8%, and 40.3%, respectively. Globally, isolates UC14 and

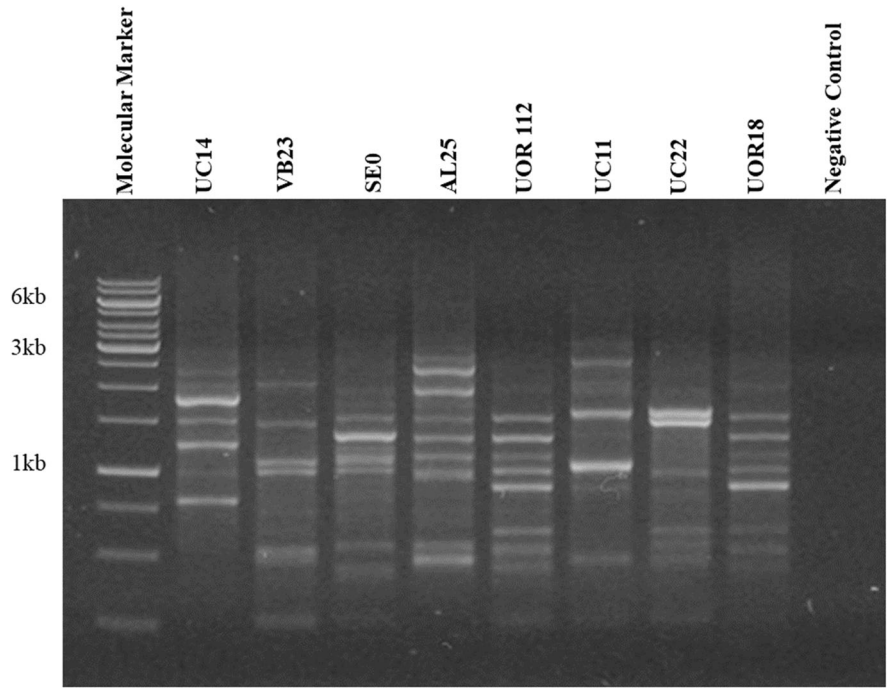

Fig. 2 RAPD patterns of the isolates, one for each group based on the ELO gene, obtained using primer A9

AL25 resulted the most effective strains according to bio-active VOCs production results.

In vivo assay

A relevant inhibitory activity of the tested strains was detected by *in vivo* assays (Fig. 7). It was evidenced that isolate VB23 was the most effective strain in controlling brown rot (Supplemental material S2) reducing by 80%, 60%, 100% the incidence (data not reported) and by 79.5%, 72.7% and 100% the severity of brown rot caused by AML1, AMF1, AMP1, respectively, as compared to the control.

Strain UC14 showed the same efficacy as strain VB23 in reducing AML1 severity symptoms. Strains AML1 (average lesion size: 44 mm) and AMF1 (55 mm) resulted more aggressive than AMP1 (19 mm). The synthetic product (Scholar®) was confirmed as the most effective treatment, although the effectiveness of strain VB23 was comparable to that of the chemical treatment against AMP1 strain.

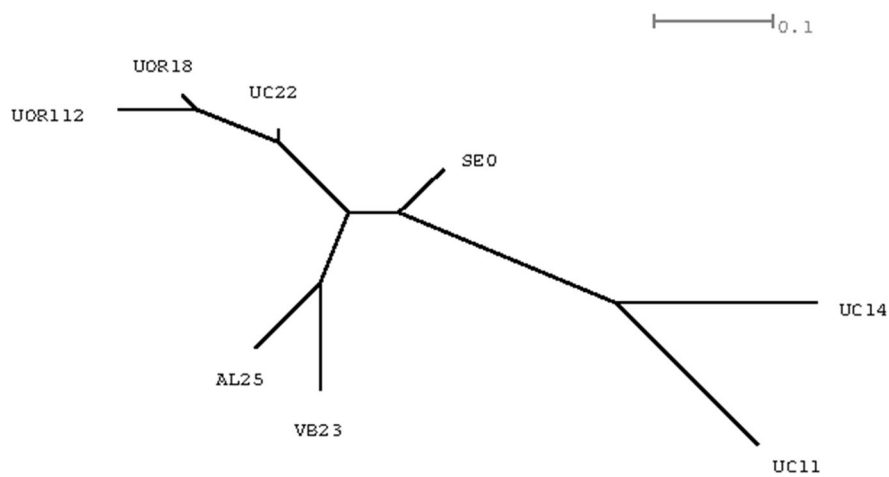

Fig. 3 RAPD patterns analysis

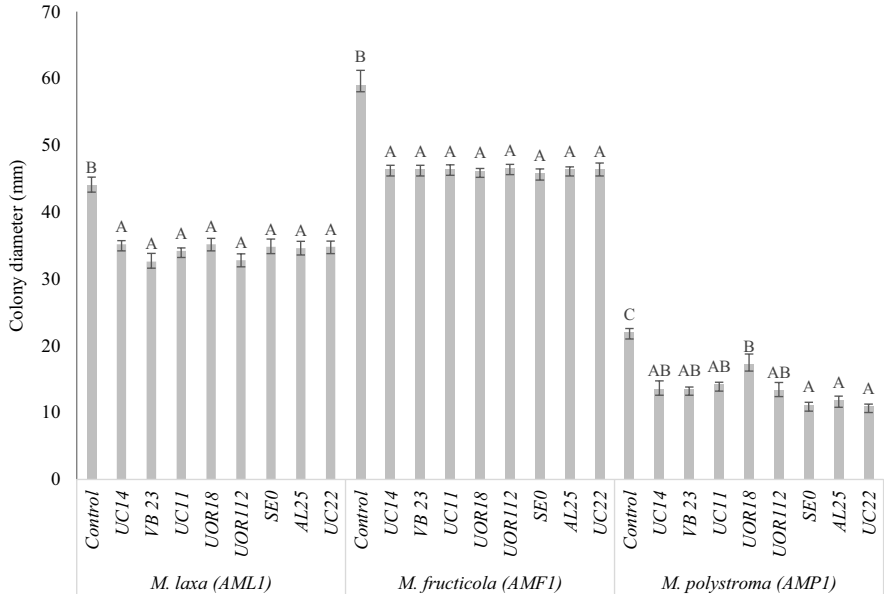

Fig. 4 Co-culturing assay between the selected *Aureobasidium* spp. strains and *Monilinia* spp. Colony diameters (mm) were measured after 5 days at 25 °C for *M. laxa* and *M. fructicola,* and after 14 days at 20 °C for *M. polystroma*. Each value is the mean of 6 plates (replicates) ± standard error. Different letters represent significant differences among the strains according to Tukey's Test (α = 0.01)

Discussion

It is well known that the phenotypic plasticity *Aureobasidium* spp. promotes its tolerance and adaptability to different environmental conditions (Pinto et al., 2018; Di Francesco et al., 2023). The best-studied species of *Aureobasidium,* namely *A. pullulans*, has been predominantly isolated from the fruit carposphere (Bozoudi & Tsaltas, 2018). Indeed, microorganisms naturally present on the surface of fruits are the most common source of BCAs being used against postharvest fungal fruit diseases (Lima et al., 1997; Janisiewicz & Korsten, 2002; Talibi et al., 2014). In fact, strains belonging to extreme-tolerant species are potential candidates for use during cold storage.

The present study reports the identification of strain UC14, an isolate molecularly distinct from the others and likely related to the species *A. namibiae,* according to the results of the analysis of ITS and ELO gene sequences. Unfortunately, no EF1 gene sequences were available for *A. namibie.* Therefore, further investigations are needed to better characterize this strain.

Although the isolation was carried out during the winter period, no strain with similarity to *A. subglaciale* was isolated. With the exception of isolate

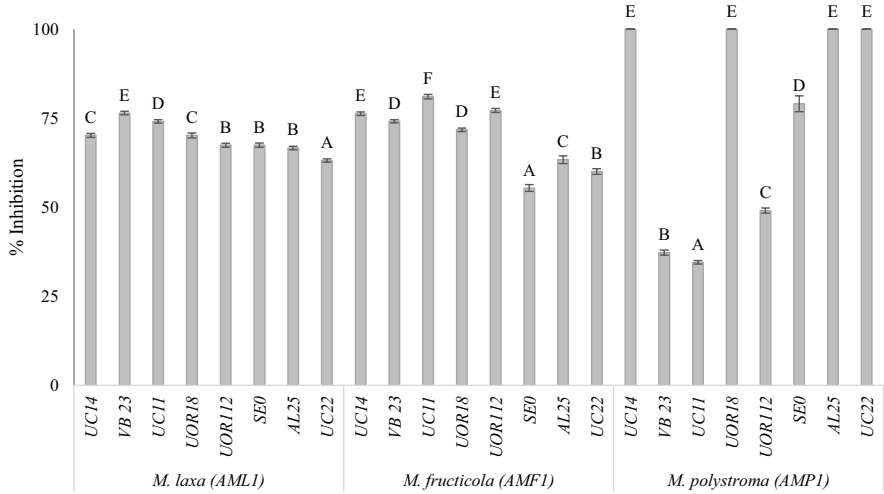

Fig. 5 Effect of non-volatile metabolites produced by selected isolates on the mycelial growth (mm) of *Monilinia* spp. Colony diameter (mm) was measured after 5 days at 25 °C for *M. laxa* and *M. fructicola,* and after 14 days at 20 °C for *M. polystroma*. Each value is the mean of 6 plates (replicates) ± standard error. Different letters indicate significant differences according to Tukey's Test (α = 0.01)

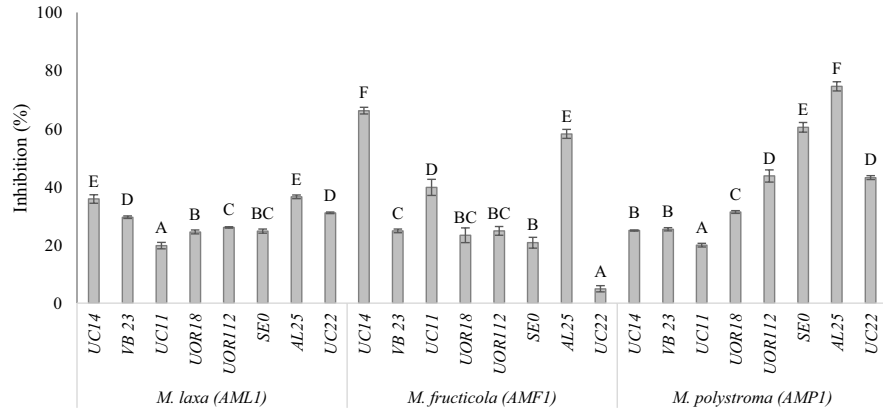

Fig. 6 Effect of volatile compounds produced by selected *Aureobasidium* spp. strains on the mycelial growth (mm) of *Monilinia* spp. Colony diameter (mm) was measured after 5 days at 25 °C for *M. laxa* and *M. fructicola,* and after 14 days at 20 °C for *M. polystroma*. Each value is the mean of 6 plates (replicates) ± standard error. Different letters indicate significant differences according to Tukey's Test (α = 0.01)

UC14, there was no evidence that the population sampled in this work may significantly differ from those sampled during spring and on fruit surfaces in more conventional environments.

Cold can induce biochemical, biophysical, and physiological changes to cells (Garcia-Rios et al., 2016). Most microorganisms resist to a variety of changing conditions in their environment in order to survive. Nevertheless, the well-equipped for cold temperature growth, psychrophilic and psychrotrophic microorganisms have to make numerous adjustments when facing temperatures lower than *optimum* (Berry & Foegeding, 1997). Moreover, the cold shock response has not been fully delineated but seems to be an adaptive trait of *A. pullulans,* characterized by a high physiological adaptability.

The lack of population structure (Fig. 1A, B, C), despite the large observed diversity within all the yeast isolates, might be explained by a high recombination among *A. pullulans* strains (Gostincar et al., 2019).

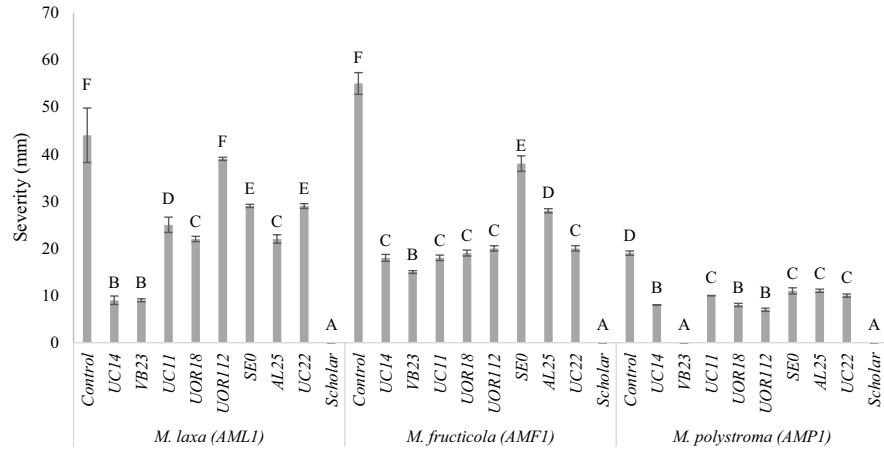

Fig. 7 Antagonistic effect by *Aureobasidium* spp. strains against *Monilinia* spp. in apricot. Fruits were artificially inoculated with antagonist cells and conidial suspensions (20 µL) (1×10^8 cells mL^{-1} and 1×10^5 conidia mL^{-1}, respectively). Apricots were incubated at 0 °C and 90% RH for 30 days. Severity of rotten fruits was detected after 7 d at 20 °C. Fruit inoculated with water and Scholar® were used as negative and positive control treatments, respectively. According to Tukey's HSD Test, different letters represent significant differences within the treatments among the fungal pathogens (α = 0.01)

In this study, the mechanism of action of the antibiosis was deeply investigated by *in vitro* assays in view of future exploitation for the control of *Monilinia* spp. Hence, the results obtained provided preliminary information on the effectiveness of the strains and on their most active modes of action. The results showed a higher reduction of the growth of the pathogens by non-volatile metabolites with respect to volatile metabolites, particularly in the case of *M. polystroma* (AMP1). These results are in agreement with other studies reporting limited efficacy of VOCs in the control of *Monilinia* spp. mycelial growth (Di Francesco et al., 2020a), rather than conidial germination.

Non-volatile organic compounds produced by BCAs have also been reported to inhibit other postharvest pathogens such as *Alternaria alternata* (Pérez-Corral et al., 2020; Wang et al., 2022). According to the literature, *Aureobasidium* spp. secondary metabolites involved in the antagonism against pathogens comprehend extracellular hydrolytic enzymes, such as chitinase and beta-1,3-glucanase, and various antimicrobial compounds such as aureobasidin A, exophilin A, and siderophores (Castoria et al., 2001; Zhang et al., 2010; Gostinčar et al., 2014a).

Strains UC14 and AL25 showed the best inhibition results by producing VOCs against *M. fructicola* mycelial growth compared to the rest of the tested yeast population. However, compared to the synthetic fungicide (Scholar®), which completely controlled the insurgency of the disease caused by all three pathogens, the BCAs exhibited lower effectiveness, and only AMP was totally controlled by the BCA, strain VB23. Other studies reported similar results with BCA effectiveness significantly lower than the synthetic fungicides (Lahlali et al., 2020; Palmieri et al., 2022). Since *Aureobasidium* spp. are considered highly tolerant microorganisms, a desired trait in the biocontrol application (Zajc et al., 2019), we hypothesize that the efficacy of yeasts as BCAs will be greater when applied in a stressful situation, such as the low temperature condition during fruit storage.

Based on these preliminary results, future studies will be carried out to test the effectiveness of the target yeasts in extreme storage conditions (e.g., temperature, relative humidity—R.H.) and further investigate on the possible physiological differences that exist between species isolated during cold or warm seasons.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval This research did not involve any animal and/or human participants.

Competing interests The authors declare that they have no conflict of interests.

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