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Biopreservation of tomatoes using fermented media by lactic acid bacteria

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ABSTRACT

Post-harvest spoilage fungi in tomatoes represent an economic loss for industry and consumers. There is currently an increasing demand for novel applications of bio-preservatives as replacers of chemical additives and pesticides in food. In this study, nine lactic acid bacteria strains isolated from tomato and sourdough were screened for antifungal activity *in vitro* against 33 fungal strains and used as bio-preservatives of tomato inoculated with *Penicillium expansum* and *Aspergillus flavus*. The identification of the compounds potentially responsible for the antifungal activity, such as organic acids, phenolic acids and volatile organic compounds (VOCs), were identified and quantified. Several cell-free supernatants (CFS) showed *in vitro* antifungal activity against toxigenic fungi. The highest antifungal activity was observed in the CFS of *Lactobacillus plantarum* TR7 and *L. plantarum* TR71, these showed a range of MFC values of 6.3–100 g/L. Antifungal compounds were identified in CFS as organic acids, phenolic acids and VOCs. Lactic acid, acetic acid, phenyllactic acid and pyrazine derivatives can be related for the antifungal activity. Bio-preservation of tomato with the CFS fermented by *L. plantarum* TR7 and *L. plantarum* TR71 decreased the microbial count by 1.98–3.89 $\log_{10}$ spores/g in comparison to the treatment with medium non-fermented.

1. Introduction

Fungi are currently a problem for the food industry due to their ability to degrade food and produce mycotoxins (Saladino, Luz, Manyes, Fernández-Franzón, & Meca, 2016). Fungal contamination can occur at various stages during cultivation, collection, transport, storage and processing (Yang et al., 2014), resulting in economic losses for the agricultural sector, and potential health problems in livestock and humans (Rodríguez-Carrasco, Moltó, Mañes, & Berrada, 2014; Zain, 2011). It is estimated that between 5% and 10% of world food production is lost due to the action of fungi growth (Varsha & Nampoothiri, 2016). Tomato spoilage by fungi during storage contributes to post-harvest losses of around 10–30% of the total (Fisher et al., 2012). The genera of fungi mainly involved in spoilage are *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* (Stankovic, Levic, Petrovic, Logrieco, & Moretti, 2007).

To date, the use of synthetic antifungals has been the most common way to combat this type of food alteration, but its use has certain drawbacks (Vandghanooni et al., 2013). The repeated use of synthetic antifungals has generated resistance to this type of pesticide (Taylor, 2009). In addition, the use of these agrochemicals is related to environmental problems, due to their high stability, and their toxicity causes human health issues in numerous organs, besides teratogenic, carcinogenic and irritant effects (Jabłońska-Trypuć, Wołejko, Wydro, & Butarewicz, 2017; Leuschner et al., 2010). These two problems have added to the consumer demand to reduce the use of synthetic pesticides, motivating the search for new methods of food spoilage control that can ensure food safety (Calvo, Marco, Blanco, Oria, & Venturini, 2017).

Bio-preservation is the use of microorganisms, as well as their metabolic products, to prevent fungal growth and improve the food shelf-life. Lactic acid bacteria (LAB) are microorganisms commonly used in numerous industrial fermentation processes. They are considered by the Food and Drug Administration (FDA) and European Union as a microorganism Generally-regarded-as-safe (GRAS) and with Qualified Presumption of Safety (QPS) status (Martinez, Balciunas, Converti, Cotter, & Oliveira, 2013). LAB represent a promising strategy to prevent spoilage of fruits and vegetables (Ribes, Fuentes, Talens, & Barat, 2017). The antifungal compounds produced by LAB have been extensively studied, but knowledge about the mechanism of action and synergies of the biocomplex produced during fermentation is still lacking (Salas et al., 2017).

This study investigated the antifungal activity of isolated LAB from tomato and sourdough against several toxigenic fungi. It identified and

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quantified the organic acids, phenolic acids and VOCs in fermented media, which was subsequently used as a novel application for improving the post-harvest shelf-life of tomato.

2. Materials and methods

2.1. Chemicals

The phenolic compounds gallic acid, chlorogenic acid, caffeic acid, syringic acid, vanillin, *p*-coumaric, hydroxybenzoic acid, vanillic acid, hydroxycinnamic acid, sinapic acid, benzoic acid, DL-3-phenyllactic acid, 1,2-dihydroxybenzene, 3,4-dihydroxyhydrocinnamic acid and DL-*p*-hydroxyphenyllactic acid were provided from Sigma–Aldrich (Dublin, Ireland). Phenyllactic acid (PLA) was obtained from BaChem (Weil am Rhein, Germany). Ferulic acid was purchased from MP Biomedicals, and protocatechuic acid came from HWI Pharma Services (Ruelzheim, Germany). All analytes had a purity of 95%.

Liquid chromatography grade solvents, including acetonitrile (ACN), methanol, ethyl acetate and formic acid (99%) were obtained from VWR Chemicals (Radnor, USA). Magnesium sulphate ($MgSO_4$), C18, ammonium formate and sodium chloride (NaCl) were obtained from Sigma–Aldrich. Potato dextrose broth (PDB), potato dextrose agar (PDA), de Man–Rogosa–Sharpe (MRS) broth and MRS agar were obtained from Liofilchem (Teramo, Italy). Deionised water (< 18 MΩ/cm) was obtained from a Milli-Q purification system (Millipore Corp., Bedford, MA, USA).

2.2. Fungi and bacteria isolation

Nine *Penicillium* strains (*P. camemberti* CECT 2267, *P. expansum* CECT 2278, *P. roqueforti* CECT 2905, *P. digitatum* CECT 2954, *P. brevicompactum* CECT 2316, *P. nordicum* CECT 2320, *P. pinophilum* CECT 2912, *P. commune* CECT 20767, *P. solitum* CECT 20818) and eight *Aspergillus* strains (*A. parasiticus* CECT 2681, *A. niger* CECT 2088, *A. steynii* CECT 20510, *A. brasilensis* CECT 2574, *A. sclerotioniger* CECT 20583, *A. tubingensis* CECT 20543, *A. tubingensis* CECT 20544, *A. lacticoffeatus* CECT 20581) were obtained from the Spanish Type Culture Collection (Spain). *Penicillium verrucosum* VTT D-01847 was obtained from the VTT Culture Collection (Finland). *Aspergillus flavus* ITEM 8111, *Aspergillus carbonarius* ITEM 5010, ten *Fusarium* strains (*F. graminearum* ITEM 126, *F. graminearum* ITEM 6352, *F. graminearum* ITEM 6415, *F. proliferatum* ITEM 12072, *F. verticillioides* ITEM 12052, *F. verticillioides* ITEM 12043, *F. verticillioides* ITEM 12044, *F. sporotrichioides* ITEM 12168, *F. langsethiae* ITEM 11031, *F. poae* ITEM 9151), and three *Alternaria* strains (*A. alternata* ITEM 8121, *A. alternata* ITEM 8122, *A. alternata* ITEM 8123) were obtained from the Agro-Food Microbial Culture Collection (Italy). These fungi were cryopreserved in sterile 30% glycerol at −80 °C. Before antifungal studies, were defrosted and cultured in PDB at 25 °C for 48 h and inoculated on PDA plates to obtain spores.

A total of nine LAB isolates from sourdough and tomato were screened for antifungal activity against 33 toxigenic fungi belonging to the genera *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria*. These LAB were isolated by dilution of sourdough and tomato at 1:10 (w/w) with 0.1% sterile peptone water and spread plating on MRS agar. The plates were incubated under an anaerobic condition using the Anaerocult® system in anaerobic jars at 37 °C for 72 h. Isolated colonies with different morphologies were sub-cultured in MRS agar plates to obtain a pure culture. Gram-staining, morphology and catalase reaction were carried out to discard Gram-negative, yeast and catalase-positive microorganisms.

The LAB were preserved in sterile 30% glycerol and stored at −80 °C before use. Prior to the fermentation experiments, the LAB were recovered in culture medium (MRS broth) at 37 °C for 48 h under anaerobic conditions.

2.3. 16S rRNA gene sequencing for bacterial identification

Identification of isolates was performed using the method described by Chenoll et al. (2019) with some modifications. DNA culture was extracted using the High Pure PCR Template Preparation Kit (Roche). 16S rRNA sequence was amplified and sequenced using an Applied Biosystems ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Foster City, USA). DNA templates were amplified by PCR using the universal primers amplifying a 1000-bp region of the 16S rRNA gene; 616V: 5'-AGAGTTTGATYMTGGCTCAG-3' and 699R: 5'-RGGGTTGCGCTCGTT-3'. 616V and 699R primers, *Taq* DNA polymerase and dNTP mix were obtained from Thermo Fisher Scientific (Waltham, USA). The DNA templates were amplified by initial denaturation at 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The integrity of the PCR products was assayed by the development of single bands following electrophoresis for 1 h at 100 V in 2% (w/v) agarose gels in Tris–borate EDTA buffer. Amplicons were purified using the commercial Metabion GmbH mi-PCR Purification Kit (Planegg, Germany), followed by sequencing reactions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), premixed format. The resulting sequences were automatically aligned and inspected visually, and then compared with the on-line tool BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The strain was identified on the basis of the highest scores.

2.4. Growth of strains for preparation of cell-free supernatant

After their defrost and recovery, the LAB were cultivated in MRS at 37 °C until the exponential phase growth (12 h). Then, LAB were inoculated at a concentration of 10^7 CFU/mL in 50 mL of MRS broth and incubated at 37 °C for 72 h. After fermentation, LAB were separated by centrifugation at 3200g for 10 min. Cell-free supernatants (CFS) were stored at −80 °C for 24 h before lyophilisation (FreeZone 2.5 L, Labconco, Kansas City, MO, USA) and then stored at −19 °C (Nazareth et al., 2020).

2.5. Qualitative assay of antifungal activity in solid medium

The effect of the LAB on different fungal strains growth was evaluated using two qualitative methods. On the one hand, the diffusion agar method was used to study the effect of fermented CFS against fungi. PDA plates were inoculated with fungal spores using sterile cotton swabs. Then, the wells were made using sterile pipette tips, and each well was loaded with 50 μL of lyophilised CFS and suspended in PDB to a concentration of 100 g/L. A well with lyophilised MRS broth was included as the negative control. Afterward, the plates were incubated at 25 °C for 72 h. Finally, the inhibition halo diameter was measured. Halos larger than 8 mm were considered positive for antifungal activity (Varsha, 2014).

On the other hand, the culture overlay assay was undertaken to study the LAB strains with potential antifungal activity. LAB cultures of 24 h at 37 °C were inoculated as 2-cm-long lines in MRS A with a sterile handle and incubated at 37 °C for 72 h. Spores of fungi were next suspended in sterile 0.1% Tween–water and counted using a Neubauer chamber. The incubated plates were then covered with 15 mL of PDA at 50 °C containing 10^4 fungal spores/mL and incubated at 25 °C for 72 h. Inhibition of fungal growth was quantified after incubation (Guimarães, Santiago, Teixeira, Venâncio, & Abrunhosa, 2018).

2.6. Quantitative assay of antifungal activity in a microplate

The assay was performed as described by Luz, Izzo, Ritieni, Mañes, and Meca (2019). A volume of 100 μL of fermented CFS at final concentrations from 0.1 to 100 g/L was added to 96-well sterile microplates. Next, the microwells were inoculated with 100 μL of a 5×10^4

spores/mL suspension in PDB of the toxigenic fungi. The positive control consisted of inoculated PDB medium with non-fermented CFS (100 g/L), and the negative control was a non-inoculated PDB medium without any treatment. Inoculated microplates were incubated at 25 °C for 72 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the fermented CFS at which the fungi did not show any visible growth. Four replicates of each assay were realised.

After determining the MIC, the concentration corresponding to the MIC and higher concentrations were sub-cultured on PDA plates for the determination of the minimum fungicidal concentration (MFC). After incubation of the plates at 25 °C for 72 h, the MFC results were defined as the lowest extract concentration in which a visible growth of the subculture was prevented. The *in vitro* antifungal activity data were used to select LAB with relatively greater antifungal potential.

2.7. Identification of organic and phenolic acids in CFS

For the analysis of organic acids, lyophilised CFS was diluted in water and injected into the high-performance liquid chromatography (HPLC) system (Agilent 1100 Series HPLC System, Agilent Technologies, Palo Alto, CA, USA), equipped with a quaternary pump and a diode array detector, using a 20 μL sample injection loop (Khosravi, Rastakhiz, Iranmanesh, & Olia, 2015). The analytical separation was achieved with a Spherisorb S5 ODS2 (4.6 mm × 250 mm, 5 μm) reverse-phase column (Waters Corp., Milford, MA, USA) using an isocratic mobile phase of acidified water (pH 2.1) at a flow rate of 0.6 mL/min for 25 min. The chromatogram was monitored at 210 nm. Data were acquired by the HP-CORE ChemStation system (Agilent Technologies, Santa Clara, CA, USA). Results were expressed as g/Kg.

For the identification of phenolic acids, the CFS was purified using the QuEChERS method to remove possible interferents before the chromatographic analysis (Brosnan, Coffey, Arendt, & Furey, 2014). Ten millilitres of fermented CFS was extracted with 10 mL ethyl acetate, 1% formic acid, 4 g $MgSO_4$ and 1 g NaCl, then vortexed for 1 min. The extract was centrifuged. The supernatant was combined with 150 mg C18 and 900 mg $MgSO_4$ and vortexed for 1 min. The extract was centrifuged again, and the supernatant was evaporated under a nitrogen flow. Immediately before chromatographic analysis, the purified extract was resuspended in 1 mL of H_2O:ACN (90:10 v/v).

The HPLC system used for the chromatographic determination was an Agilent 1200 (Agilent Technologies, Santa Clara) equipped with a vacuum degasser, autosampler and binary pump. The column was a Gemini C18 (50 mm × 2 mm, 100 Å, 3-μm particle size; Phenomenex).

The mobile phases consisted of water as solvent A, ACN as solvent B, both acidified (0.1% formic acid), with gradient elution, as follows: 0 min, 5% B; 30 min 95% B; 35 min, 5% B. The column was equilibrated for 3 min before every analysis. The flow rate was 0.3 mL/min, and 20 μL of sample was injected.

Mass spectrometry (MS) analysis was conducted using a Q-TOF-MS (6540 Agilent Ultra High Definition Accurate Mass), equipped with an Agilent Dual Jet Stream electrospray ionisation (Dual AJS ESI) interface in negative ionisation mode under the following conditions: drying gas flow (N_2), 8.0 L/min; nebuliser pressure, 30 psig; gas drying temperature, 350 °C; capillary voltage, 3.5 kV; fragmentor voltage, 175 V; scan range, *m/z* 20–380. Targeted MS/MS experiments were carried out using collision energy values of 10, 20 and 40 eV. Integration and data elaboration were managed using MassHunter Qualitative Analysis software B.08.00 (Denardi-Souza, Luz, Mañes, Badiale-Furlong, & Meca, 2018). Results were expressed as g/L.

2.8. Analysis of VOCs of CFS

Lyophilised CFS (200 mg) was mixed with 2 mL of water and placed in a 10-mL glass vial. VOCs were identified by gas chromatography with a single quadrupole mass spectrometer detector (GC/MS) analysis. Prior to analysis, samples were incubated in a water bath at 55 °C for 45 min, while being gently stirred with a rod. VOCs were extracted from the vial headspace by solid-phase microextraction (SPME). An SPME holder (Supelco, Bellafonte, PA, USA) containing a fused-silica fibre coated with a 50/30 μm layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was used to trap VOCs in the vial headspace. The fibre was introduced into the split-less inlet of an Agilent 6890N GC system (Agilent Technologies, Palo Alto), and thermal desorption of the analytes was performed at 250 °C for 5 min. The GC system was equipped with an HP-5MS (30 m × 0.25 mm, 0.25 μm 5% diphenyl/95% dimethylpolysiloxane) capillary column (J&W Scientific, Folsom, CA, USA). The oven was programmed to start at 40 °C (held for 2 min) and to ramp up to 160 °C at 6 °C/min, then increase to 260 °C at 10 °C/min (held for 4 min). Helium (99.999%) was used as the carrier gas, and the flow rate was 1 mL/min. The flow was transferred from the column into an Agilent 5973 MS detector (Agilent Technologies, Palo Alto). The ion source temperature was set at 230 °C, the ionising electron energy was 70 eV, and the mass range was 40–450 Da in full scan acquisition mode. Compounds were identified using the NIST Atomic Spectra Database version 1.6 (Gaithersburg, MD, USA), considering spectra with 95% similarity. Results were expressed as a percentage of the VOC by dividing the area of each peak by the total area of the chromatogram peaks (Guarrasi et al., 2017). Triplicate analysis was carried out.

2.9. Bio-preservation of tomatoes

To evaluate the antifungal activity and mycotoxin production using CFS fermented by *L. plantarum* TR7 and TR71, tomatoes were contaminated with *A. flavus* ISPA 8111 (aflatoxin B_1 producer) and *P. expansum* CECT 2278. Tomatoes were obtained from the supermarket chain Consum Cooperative (Valencia, Spain).

The fruit was divided into triplicate batches (each batch containing 60 fruit); 20 were treated with sterile MRS broth (control), 20 with CFS fermented by *L. plantarum* TR7, and 20 with CFS fermented by *L. plantarum* TR71, respectively. First, the tomatoes were sanitised with 70% ethanol and then sterile water. A wound was made using a sterile pipette tip. One millilitre of a spore solution containing 10^4 spores/mL of fungi was sprayed on the tomatoes and dried for 1 h in a laminar flow cabinet (Telstar MH 100, Terrassa, Spain). Finally, the tomatoes were treated with 10 mL of CFS at a final concentration of 12.5 g lyophilised CFS/Kg of tomato, dried and stored in a sanitised plastic box at room temperature for 9 d. At the end of storage, 10 tomatoes of each treatment were separated and frozen for the determination of mycotoxins, and 10 were examined for viable spores. All experiments were repeated three times.

2.10. Determination of the fungal population

The spore count assay was performed as described by Luz et al. (2019) with some modifications. Ten tomatoes were homogenised with sterile buffered peptone water in a 1:10 (w/v) ratio in a Stomacher (IUL, Barcelona, Spain) for 30 s. From that, three serial decimal dilutions were prepared in glass tubes with 9 mL of peptone water. Subsequently, 100 μL of each tube was plated out in PDA culture medium plates. The plates were incubated at 26 °C, and the number of viable colonies was counted at 72 h of incubation (Dal Bello et al., 2007).

2.11. Statistical analysis

Data were statistically evaluated using the InfoStat software version 2008. The differences between the groups were analysed by one-way ANOVA, followed by the Tukey HSD post hoc test for multiple comparisons. The significance level was set at $p \leq 0.01$.

Table 1
Antifungal activity of CFS against *Penicillium, Aspergillus, Fusarium* and *Alternaria* species by diffusion agar method.

Fungi	Antifungal activity								
	Lactic Acid Bacteria								
	IRK751	IRK81	IRK82	SMF76	POM	TR7	TR71	TR14	TR2
Penicillium camemberti CECT 2267	-	-	-	-	-	-	-	-	-
Penicillium expansum CECT 2278	+	-	+	+	+	+	++	++	+
Penicillium roqueforti CECT 2905	-	-	-	-	-	-	-	-	-
Penicillium verrucosum VTT D-01847	-	-	-	-	+	++	++	++	++
Penicillium verrucosum CECT 2912	-	-	-	+	+++	++	++	+++	++
Penicillium digitatum CECT 2954	-	-	-	-	-	-	-	-	-
Penicillium brevicopactum CECT 2316	+	-	+	++	++	++	++	++	++
Penicillium nordicum CECT 2320	+	-	+	++	+++	++	++	+++	++
Penicillium commune CECT 20767	+	-	+	+	+	++	++	++	++
Penicillium solitum CECT 20818	-	-	-	-	-	+	+	+	+
Aspergillus parasiticus CECT 2681	-	-	-	-	-	-	-	-	-
Aspergillus flavus ITEM 8111	-	-	-	-	-	+	+	-	-
Aspergillus niger CECT 2088	-	-	-	-	-	-	-	-	-
Aspergillus steynii CECT 20510	-	-	-	-	-	+	+	+	+
Aspergillus carbonarius ITEM 5010	-	-	-	-	-	-	-	-	-
Aspergillus brasiliensis CECT 2574	-	-	-	-	-	-	-	-	-
Aspergillus sclerotioniger CECT 20583	-	-	-	-	-	-	-	-	-
Aspergillus tubingensis CECT 20543	-	-	-	-	-	-	-	-	-
Aspergillus tubingensis CECT 20544	-	-	-	-	-	-	-	-	-
Aspergillus lacticoffeatus CECT 20581	-	-	-	-	-	-	-	-	-
Fusarium graminearum ITEM 126	+	-	+	+	+	++	++	++	+
Fusarium graminearum ITEM 6352	-	-	-	-	-	+	-	-	-
Fusarium graminearum ITEM 6415	+	-	+	+	++	+	++	++	+
Fusarium proliferatum ITEM 12072	-	-	-	-	-	+	+	+	-
Fusarium verticillioides ITEM 12052	+	-	+	++	++	+	++	++	+
Fusarium verticillioides ITEM 12043	+	-	+	+	+	++	++	++	+
*Fusarium verticillioides*ITEM 12044	+	-	+	+	+	++	++	++	++
Fusarium sporotrichioides ITEM 12168	-	-	-	-	-	+	+	+	-
Fusarium langsethiae ITEM 11031	+	-	+	+	+	++	++	++	+
Fusarium poae ITEM 9151	+	-	+	+	+	++	++	++	+
Alternaria alternata ITEM 8121	-	-	-	-	+	+	++	++	++
Alternaria alternata ITEM 8122	-	-	-	++	++	-	-	++	-
Alternaria alternata ITEM 8123	-	-	-	-	+	+	++	++	+

3. Results and discussion

3.1. Isolation and identification of LAB strains

A total of nine LAB were isolated from tomatoes and sourdoughs. The full sequence of the 16S rRNA obtained and compared with the online tool BLAST confirmed the identity of the isolates at the species level; *Leuconostoc pseudomesenteroides* IRK751, *Leuconostoc pseudomesenteroides* SMF76, *Fructobacillus ficulneus* IRK81, *Lactobacillus brevis* IRK82, *Lactobacillus brevis* POM, *Lactobacillus plantarum* TR7, *Lactobacillus plantarum* TR71, *Lactobacillus plantarum* TR14 and *Lactobacillus ghanensis* TR2. All comparisons showed > 99% of 16S rRNA sequence similarity.

3.2. Antifungal activity in vitro

Several CFS of LAB showed antifungal activity against toxigenic fungi in the solid medium diffusion agar test (Table 1) and by the overlay method (Table 2). The highest antifungal activity was observed in the CFS of *L. plantarum* TR7 and *L. plantarum* TR71, which inhibited the growth of the three tested fungi genera in solid medium. Fig. 1 shows the antifungal capacity in the overlay test of *L. plantarum* TR71 and *L. plantarum* TR7 against *P. nordicum* CECT 2320 and *P. verrucosum* CECT 2912, respectively. It also shows that there was no antifungal activity of strain *L. pseudomesenteroides* SMF76 on *A. alternate* ITEM 8121. Some strains, such as *L. pseudomesenteroides* IRK751, *F. ficulneus* IRK81 and *L. brevis* IRK82, evidenced weak antifungal activity in solid medium. Several authors have described the antifungal activity of LAB against different fungi species using the diffusion agar method (Saladino et al., 2016) and overlay method, respectively (Guimarães et al., 2018). However, none of these studies used such a wide selection of fungal species.

In order to quantify the potential antifungal activity of CFS, the MIC and MFC values were determined (Table 3). The MIC values of CFS on *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp. and *Alternaria* spp. were in the ranges 1.6–100, 3.1–100, 0.8–50 and 3.1–50 g/L, respectively. The MFC results were 6.3–100 g/L for *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp., and 3.1–100 g/L for *Fusarium* spp. The *Fusarium* genus was the most sensitive to the compounds present in the fermented CFS, presenting the lowest average MIC and MFC values. In correlation with the studies of antifungal activity in solid medium, again *L. plantarum* TR7 and *L. plantarum* TR71 showed activity against a greater fungal spectrum and lower MIC and MFC values in comparison to the rest of the LAB isolated. Rizzello, Cassone, Coda, and Gobbetti (2011) evidenced comparable MIC values (2.5–15.2 g/L) for the water-soluble extract from sourdough fermented by LAB when tested against *Penicillium* spp.

3.3. Identification of antifungal compounds in CFS

The organic acids in lyophilised CFS fermented by LAB are shown in Table 4a. A total of four organic acids were determined, namely, lactic acid, acetic acid, succinic acid and malic acid. All isolated LAB produced lactic acid and acetic acid, with concentration ranges of 24–282

Table 2
Antifungal activity of isolated LAB against *Penicillium, Aspergillus, Fusarium* and *Alternaria* species by overlay assay.

Fungi	Antifungal activity								
	Lactic Acid Bacteria								
	IRK751	IRK81	IRK82	SMF76	POM	TR7	TR71	TR14	TR2
Penicillium camemberti CECT 2267	+	–	–	–	–	++	++	+	–
Penicillium expansum CECT 2278	+	–	–	++	–	++	++	++	–
Penicillium roqueforti CECT 2905	–	–	–	–	–	+	+	–	–
Penicillium verrucosum VTT D-01847	–	–	–	–	+	++	++	++	++
Penicillium verrucosum CECT 2912	–	–	–	–	+	++	++	++	++
Penicillium digitatum CECT 2954	–	–	–	–	–	–	+	–	–
Penicillium brevicopactum CECT 2316	++	–	–	+	–	++	++	++	++
Penicillium nordicum CECT 2320	–	–	–	–	++	++	++	++	++
Penicillium commune CECT 20767	+	–	–	–	–	++	++	+	–
Penicillium solitum CECT 20818	–	–	–	–	–	–	+	–	–
Aspergillus parasiticus CECT 2681	–	–	–	–	–	+	+	–	–
Aspergillus flavus ITEM 8111	–	–	–	–	–	–	–	–	–
Aspergillus niger CECT 2088	–	–	–	–	+	–	–	–	–
Aspergillus steynii CECT 20510	+	–	–	+	++	++	++	–	+
Aspergillus carbonarius ITEM 5010	+	–	–	+	++	++	++	–	+
Aspergillus brasiliensis CECT 2574	–	–	–	–	+	–	–	–	–
Aspergillus sclerotioniger CECT 20583	+	–	–	+	+	+	+	–	–
Aspergillus tubingensis CECT 20543	–	–	–	–	–	–	–	–	–
Aspergillus tubingensis CECT 20544	+	–	–	+	++	++	++	–	+
Aspergillus lacticoffeatus CECT 20581	+	–	–	+	+	+	++	+	–
Fusarium graminearum ITEM 126	+	–	–	–	++	+++	++	+++	–
Fusarium graminearum ITEM 6352	+	–	–	–	++	+++	++	+++	–
Fusarium graminearum ITEM 6415	+	–	–	–	++	+++	++	+++	–
Fusarium proliferatum ITEM 12072	+	–	–	–	++	+++	+++	+++	–
Fusarium verticillioides ITEM 12052	–	–	–	–	++	+++	++	+++	–
Fusarium verticillioides ITEM 12043	–	–	–	–	+	++	+	+	–
*Fusarium verticillioides*ITEM 12044	–	–	–	–	++	+++	++	+++	–
Fusarium sporotrichioides ITEM 12168	–	–	–	–	–	–	–	–	–
Fusarium langsethiae ITEM 11031	–	–	–	–	–	–	–	–	–
Fusarium poae ITEM 9151	–	–	–	–	–	+	++	++	–
Alternaria alternata ITEM 8121	–	–	+	–	+	+++	+	+++	–
Alternaria alternata ITEM 8122	–	–	–	–	–	+++	+	++	–
Alternaria alternata ITEM 8123	–	–	–	–	–	+++	+	++	–

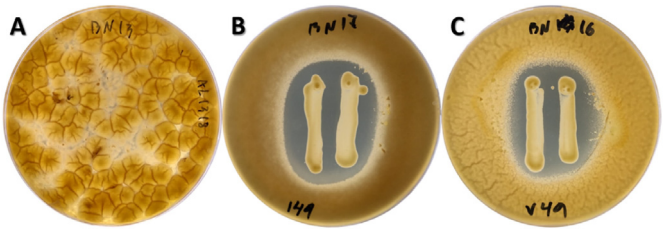

Fig. 1. Example of antifungal activity of (A) *Leuconostoc pseudomesenteroides* SMF76 against *Alternaria alternata* ITEM 8122, (B) *Lactobacillus plantarum* TR71 against *Penicillium nordicum* CECT 2320 and (C) *Lactobacillus plantarum* TR7 against *Penicillium verrucosum* VTT D-01847 determined by overlay test. Fungal growth inhibition was observed in *Lactobacillus plantarum* TR71 and *Lactobacillus plantarum* TR7. No effects were evidenced for *Leuconostoc pseudomesenteroides* SMF76.

and 21–77 g/kg, respectively. The LAB with the highest lactic acid production were *L. plantarum* TR14 (282 g/kg), *L. plantarum* TR71 (228 g/kg), *L. plantarum* TR7 (200 g/kg) and *L. ghanensis* TR2 (194 g/kg). Production of succinic acid (14–45 g/kg) and malic acid (6–13 g/kg) was determined only in five and six CFS, respectively. In addition, these two acids had a lower average concentration than lactic acid and acetic acid.

The phenolic acids detected in fermented CFS are listed in Table 4b. Among the 18 targeted compounds, four phenolic acids were detected and quantified in CFS (PLA, hydrocaffeic acid, benzoic acid, vanillic acid). These compounds have been reported in other research as antifungal agents produced by LAB (Omedi, Huang, & Zheng, 2019). PLA was quantified in each CFS, and its concentration range was 8E-05–2E-03 g/L. The highest concentration of PLA was detected in *L. plantarum* TR2. Hydrocaffeic acid, benzoic acid and vanillic acid were quantified only in four CFS, with ranges of 4E-04–6E-04, 5E-04, and 6E-05–8E-05 g/L, respectively. However, no significant ($p > 0.01$) differences were observed between the different CFS. Like organic acid production, the phenolic acids data correlated with the observed antifungal activity of CFS. Previous authors showed synergism between lactic acid and acetic acid (Dagnas, Gauvry, Onno, & Membré, 2015), and between these and PLA in the potential antifungal effect (Lavermicocca, Valerio, & Visconti, 2003).

A total of 51 VOCs was identified in the lyophilised CFS fermented by LAB and control medium (MRS broth). However, the control medium showed a relatively lower number of compounds. The profile of VOCs quantified in CFS and the control medium is given in Table 4c. The compounds were classified into six groups according to their chemical class; alcohols, aldehydes, acids, ketones, pyrazines and others. The fermentation of culture medium evidenced a significant increase in acids and pyrazines and a decrease in alcohols, aldehydes, ketones and others. The level of pyrazines was 3.7- and 7.5-fold higher than in CFS fermented by *L. brevis* POM and *L. ghanensis* TR2, respectively. Other fermented CFS showed intermediate increments. The main VOC belonging to the group of acids was acetic acid, accounting for 9.2–20.8% of the total. The inhibition of fungal growth by VOCs produced by bacteria is already well-described (Morita et al., 2019). One study mentioned the antimicrobial activity of VOC (1-octen-3-ol and 2,5-

Table 3
Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) for lyophilised CFS (g/L) fermented by isolated LAB against: a) *Penicillium*, b) *Aspergillus*, c) *Fusarium* and *Alternaria* species.

a)

Fungi	Antifungal activity																	
	Lactic Acid Bacteria																	
	IRK751		IRK81		IRK82		SMF76		POM		TR7		TR71		TR14		TR2	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium camemberti CECT 2267	50	nd	nd	nd	nd	nd	25	100	12.5	12.5	12.5	50	12.5	25	6.3	25	12.5	25
Penicillium expansum CECT 2278	100	100	nd	nd	nd	nd	100	100	50	50	25	50	12.5	25	12.5	50	25	25
Penicillium roqueforti CECT 2905	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	50	nd	100	nd	25	100	25	100
Penicillium verrucosum VTT D-01847	25	25	nd	nd	nd	nd	12.5	25	6.3	6.3	6.3	12.5	3.1	6.3	3.1	6.3	6.3	12.5
Penicillium verrucosum CECT 2912	25	50	nd	nd	nd	nd	12.5	25	12.5	12.5	6.3	12.5	1.6	12.5	1.6	12.5	6.3	12.5
Penicillium digitatum CECT 2954	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	25	nd	12.5	100	25	100	50	100
Penicillium brevicopactum CECT 2316	25	100	nd	nd	nd	nd	25	100	25	25	12.5	25	12.5	12.5	12.5	12.5	6.3	12.5
Penicillium nordicum CECT 2320	25	25	nd	nd	nd	nd	12.5	25	6.3	6.3	6.3	12.5	3.1	6.3	1.6	12.5	1.6	12.5
Penicillium commune CECT 20767	nd	nd	nd	nd	nd	nd	50	100	50	50	12.5	25	25	50	12.5	25	6.3	12.5
Penicillium solitum CECT 20818	100	nd	nd	nd	nd	nd	50	100	25	25	25	50	25	100	12.5	100	6.3	100

b)

Fungi	**Antifungal activity**																	
	Lactic Acid Bacteria																	
	IRK751		**IRK81**		**IRK82**		**SMF76**		**POM**		**TR7**		**TR71**		**TR14**		**TR2**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus parasiticus CECT 2681	50	nd	nd	nd	nd	nd	50	100	50	100	12.5	50	12.5	25	12.5	50	12.5	12.5
Aspergillus flavus ITEM 8111	50	100	nd	nd	nd	nd	50	100	25	25	25	25	25	25	12.5	12.5	12.5	25
Aspergillus niger CECT 2088	50	100	nd	nd	nd	nd	50	100	50	50	25	50	25	25	12.5	25	12.5	25
Aspergillus steynii CECT 20510	50	50	nd	nd	nd	nd	25	50	6.3	6.3	6.3	25	12.5	12.5	12.5	25	12.5	25
Aspergillus carbonarius ITEM 5010	50	50	nd	nd	nd	nd	50	50	3.1	12.5	25	25	25	50	25	25	25	25
Aspergillus brasiliensis CECT 2574	50	100	nd	nd	nd	nd	25	50	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25	12.5	12.5
Aspergillus sclerotioniger CECT 20583	50	100	nd	nd	nd	nd	50	50	25	25	12.5	25	12.5	25	12.5	25	12.5	25
Aspergillus tubingensis CECT 20543	50	nd	nd	nd	nd	nd	50	nd	25	nd	25	50	12.5	25	12.5	25	12.5	25
Aspergillus tubingensis CECT 20544	50	nd	nd	nd	nd	nd	50	100	50	50	25	50	12.5	50	12.5	25	25	50
Aspergillus lacticoffeatus CECT 20581	50	nd	nd	nd	nd	nd	50	100	100	100	25	100	25	25	25	100	25	100

c)

Fungi	**Antifungal activity**																	
	Lactic Acid Bacteria																	
	IRK751		**IRK81**		**IRK82**		**SMF76**		**POM**		**TR7**		**TR71**		**TR14**		**TR2**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Fusarium graminearum ITEM 126	12.5	50	nd	nd	nd	nd	12.5	12.5	6.3	6.3	6.3	12.5	6.3	12.5	6.3	12.5	6.3	25
Fusarium graminearum ITEM 6352	50	100	nd	nd	nd	nd	6.3	12.5	0.8	3.1	12.5	25	3.1	12.5	0.8	3.1	12.5	25
Fusarium graminearum ITEM 6415	25	50	nd	nd	nd	nd	25	25	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25	25
Fusarium proliferatum ITEM 12072	25	25	nd	nd	nd	nd	12.5	25	1.6	3.1	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25
Fusarium verticillioides ITEM 12052	25	50	nd	nd	nd	nd	25	50	12.5	12.5	12.5	25	12.5	25	12.5	25	12.5	50
Fusarium verticillioides ITEM 12043	25	50	nd	nd	nd	nd	25	50	3.1	12.5	12.5	25	12.5	25	12.5	25	12.5	25
*Fusarium verticillioides*ITEM 12044	12.5	50	nd	nd	nd	nd	6.3	50	1.6	6.3	6.3	25	6.3	25	6.3	25	6.3	50
Fusarium sporotrichioides ITEM 12168	25	50	nd	nd	nd	nd	25	25	3.1	3.1	12.5	12.5	12.5	25	12.5	12.5	6.3	100
Fusarium langsethiae ITEM 11031	25	50	nd	nd	nd	nd	12.5	25	12.5	3.1	3.1	12.5	6.3	12.5	6.3	12.5	6.3	12.5
Fusarium poae ITEM 9151	25	25	nd	nd	nd	nd	25	100	6.3	6.3	6.3	25	6.3	25	6.3	50	6.3	12.5
Alternaria alternata ITEM 8121	50	100	nd	nd	nd	nd	25	100	12.5	50	12.5	12.5	6.3	12.5	6.3	25	25	100
Alternaria alternata ITEM 8122	25	100	nd	nd	nd	nd	12.5	50	12.5	50	6.3	6.3	3.1	6.3	6.3	25	12.5	50
Alternaria alternata ITEM 8123	50	100	nd	nd	nd	nd	25	100	12.5	50	6.3	12.5	12.5	12.5	12.5	12.5	25	100

dimethyl pyrazine) against fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases (Haidar et al., 2016). Rybakova et al. (2016) described the VOCs produced by *Paenibacillus*, in particular, pyrazine derivatives, as potential biocontrol agents in agriculture.

3.4. Lactobacillus plantarum TR7 and TR71 as a tomato bio-preservative

Fig. 2 demonstrates the bio-preservation effect of CFS fermented by *L. plantarum* TR7 and *L. plantarum* TR71 on tomatoes inoculated with *A. flavus* and *P. expansum*, during storage. In particular, the visible shelf-life of tomato inoculated with *A. flavus* did not present a significant ($p > 0.01$) increase compared with the control. All treatment evidenced > 70% infected tomatoes after incubation for 7 d. Microbiological analysis of the population of the fungal confirmed the absence of inhibition of fungal growth in tomatoes treated with CFS. However, tomatoes inoculated with *P. expansum* and treated with CFS showed a visibly improved shelf-life (Fig. 3). In the control experiment, the percentage of infected tomatoes on day 9 of incubation was 100%, whereas, when CFS fermented by *L. plantarum* TR7 and *L. plantarum* TR71 was used, the values were 29% and 65%, respectively. The observed shelf-life data of tomatoes inoculated with *P. expansum* and treated with CFS were confirmed by the microbiological analysis. The control experiment at 9 d of incubation, presented a fungal population of 7.56 $\log_{10}$ spores/g, whereas in the tomatoes treated with CFS fermented by *L. plantarum* TR7, significant ($p < 0.01$) fungal growth of

Table 4
Identification and quantification of compounds produced by LAB in CFS: a) Organic acids (g/Kg), b) Phenolic acids (g/L) and c) VOCs (%). The results are expressed as mean ± standard deviation. Statistically significant differences for each fermentation are indicated with different letters, $p < 0.01$.

a)

Organic acids	Lactic Acid Bateria								
	IRK751	IRK81	IRK82	SMF76	POM	TR7	TR71	TR14	TR2
Lactic acid	160.10 ± 4.91^{bc}	24.04 ± 0.08^{a}	143.68 ± 1.06^{b}	143.83 ± 0.45^{b}	125.10 ± 0.12^{b}	200.26 ± 0.33^{cd}	227.74 ± 16.29^{d}	282.31 ± 18.40^{e}	194.46 ± 0.57^{cd}
Acetic acid	42.53 ± 4.94^{cd}	76.93 ± 0.78^{f}	20.77 ± 0.28^{a}	29.90 ± 0.35^{ab}	30.50 ± 0.49^{b}	37.49 ± 0.78^{bc}	52.02 ± 1.41^{d}	66.14 ± 1.49^{e}	43.18 ± 0.49^{cd}
Succinic acid	nd	nd	28.17 ± 0.56^{b}	31.97 ± 0.48^{b}	nd	45.46 ± 1.76^{c}	nd	32.31 ± 1.44^{b}	13.77 ± 0.40^{a}
Malic acid	nd	nd	5.75 ± 0.20^{a}	nd	6.85 ± 0.26^{a}	9.17 ± 0.74^{ab}	13.40 ± 2.15^{b}	9.83 ± 1.81^{ab}	8.03 ± 0.34^{a}

b)

Phenolic acids	Lactic Acid Bacteria								
	IRK751	IRK81	IRK82	SMF76	POM	TR7	TR71	TR14	TR2
Phenyllactic acid	5E-04 ± 2E-05^{b}	6E-04 ± 1E-05^{b}	9E-05 ± 1E-05^{a}	6E-04 ± 1E-05^{b}	8E-05 ± 1E-05^{a}	1E-03 ± 4E-05^{c}	1E-03 ± 3E-05^{c}	2E-03 ± 3E-05^{c}	2E-03 ± 3E-05^{c}
Hydrocaffeic acid	nd	nd	nd	nd	nd	6E-04 ± 6.E-05^{b}	5E-04 ± 4E-05^{b}	4E-04 ± 1E-05^{b}	5E-04 ± 5E-05^{b}
Benzoic acid	nd	nd	nd	nd	nd	5E-04 ± 5E-05^{b}	5E-04 ± 8E-05^{b}	5E-04 ± 1E-05^{b}	5E-04 ± 2E-05^{b}
Vanillic acid	nd	nd	nd	nd	nd	6E-05 ± 1E-05^{a}	5E-05 ± 1E-05^{a}	7E-05 ± 1E-05^{a}	8E-05 ± 1E-05^{a}

c)

VOC	Lactic Acid Bacteria									
	MRS	IRK751	IRK81	IRK82	SMF76	POM	TR7	TR71	TR14	TR2
ALCOHOLS	7.51 ± 0.02^{h}	4.92 ± 0.01^{g}	1.89 ± 0.01^{c}	2.37 ± 0.07^{d}	4.84 ± 0.03^{g}	3.34 ± 0.01^{f}	2.75 ± 0.02^{e}	0.72 ± 0.01^{b}	0.51 ± 0.01^{a}	0.48 ± 0.01^{a}
ethanol	nd	0.63 ± 0.01	nd	1.54 ± 0.04	1.32 ± 0.01	0.7 ± 0.01	nd	nd	nd	nd
3-methyl-1-butanol	nd	nd	0.77 ± 0.01	nd	1.24 ± 0.01	nd	nd	nd	nd	nd
3-methylacetate-1-butanol	nd	nd	nd	nd	0.25 ± 0.01	nd	nd	nd	nd	nd
2-ethyl-1- hexanol	7.51 ± 0.02	4.29 ± 0.01	1.12 ± 0.01	0.08 ± 0.01	1.72 ± 0.02	1.95 ± 0.01	2.56 ± 0.02	0.53 ± 0.01	0.51 ± 0.01	0.34 ± 0.01
2-nonanol	nd	nd	nd	0.76 ± 0.03	0.33 ± 0.01	0.7 ± 0.01	nd	nd	nd	0.15 ± 0.01
2-undecanol	nd	nd	nd	nd	nd	nd	0.19 ± 0.01	0.19 ± 0.01	nd	nd
ALDEHYDES	42.32 ± 0.09^{h}	11.89 ± 0.04^{e}	7.67 ± 0.03^{d}	17.86 ± 0.5^{g}	11.93 ± 0.04^{e}	14.21 ± 0.05^{f}	6.88 ± 0.07^{c}	6.04 ± 0.08^{b}	3.97 ± 0.03^{a}	4.44 ± 0.04^{a}
exanal	1.84 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-methyl butanal	7.51 ± 0.01	0.67 ± 0.01	1.21 ± 0.01	1.34 ± 0.04	1.45 ± 0.01	0.83 ± 0.01	0.97 ± 0.02	0.22 ± 0.01	0.12 ± 0.01	0.29 ± 0.01
2-methyl butanal	5 ± 0.04	0.35 ± 0.01	0.48 ± 0.01	0.57 ± 0.03	0.55 ± 0.01	0.41 ± 0.01	0.54 ± 0.01	0.2 ± 0.01	0.12 ± 0.01	0.19 ± 0.01
3-methyl-2-hexanal	nd	nd	nd	1 ± 0.03	0.15 ± 0.01	1 ± 0.01	0.17 ± 0.01	nd	nd	0.58 ± 0.01
2- ethyl- hexanal	1.91 ± 0.01	0.67 ± 0.01	nd	nd	0.14 ± 0.01	nd	0.37 ± 0.01	nd	nd	0.14 ± 0.01
benzaldehyde	14.14 ± 0.01	4.58 ± 0.01	2.57 ± 0.01	6.41 ± 0.16	3.82 ± 0.02	4.96 ± 0.01	2.12 ± 0.04	2.32 ± 0.03	1.6 ± 0.02	1.63 ± 0.02
benzeneacetaldehyde	8.43 ± 0.03	4.13 ± 0.03	2.19 ± 0.02	6.35 ± 0.19	4.2 ± 0.02	4.94 ± 0.02	2.37 ± 0.01	2.55 ± 0.03	1.46 ± 0.01	1.41 ± 0.01
methional	nd	0.95 ± 0.01	0.67 ± 0.01	1.61 ± 0.05	1.2 ± 0.01	1.47 ± 0.01	nd	nd	0.43 ± 0.01	nd
nonanal	3.52 ± 0.01	0.57 ± 0.01	0.57 ± 0.01	0.62 ± 0.03	0.46 ± 0.01	0.63 ± 0.01	0.37 ± 0.01	0.78 ± 0.01	0.27 ± 0.01	0.22 ± 0.01
ACIDS	nd	9.45 ± 0.01^{a}	11.12 ± 0.04^{b}	18.75 ± 0.46^{f}	13.36 ± 0.02^{d}	18.14 ± 0.09^{f}	20.81 ± 0.22^{g}	13.96 ± 0.13^{e}	12.71 ± 0.09^{c}	9.2 ± 0.08^{a}
acetic acid	nd	6.5 ± 0.01	7.97 ± 0.01	11.69 ± 0.29	9.45 ± 0.01	11.53 ± 0.04	14.25 ± 0.2	9.78 ± 0.06	8.22 ± 0.04	7.31 ± 0.07
3-methyl-butanoic acid	nd	1.66 ± 0.01	1.73 ± 0.03	3.4 ± 0.07	2.15 ± 0.01	3.02 ± 0.04	3.36 ± 0.02	1.6 ± 0.02	2.09 ± 0.04	1.13 ± 0.01
2-methyl-butanoic acid	nd	1.29 ± 0.01	1.23 ± 0.01	2.37 ± 0.06	1.56 ± 0.01	2.2 ± 0.01	2.27 ± 0.01	1.2 ± 0.02	1.51 ± 0.01	0.78 ± 0.01
octanoic acid	nd	nd	0.2 ± 0.01	1.3 ± 0.04	0.22 ± 0.01	1.4 ± 0.01	0.47 ± 0.01	1.4 ± 0.05	0.91 ± 0.01	nd
2-methyl propanoic acid	nd	nd	nd	nd	nd	nd	0.48 ± 0.01	nd	nd	nd
KETONES	1.6 ± 0.01^{d}	nd	0.16 ± 0.01^{a}	nd	nd	0.4 ± 0.01^{b}	1.51 ± 0.02^{d}	1.29 ± 0.01^{c}	2.44 ± 0.04^{e}	0.11 ± 0.01^{a}
2-heptanone	nd	nd	nd	nd	nd	0.4 ± 0.01	0.98 ± 0.01	0.65 ± 0.01	1.41 ± 0.03	nd
acetophenone	1.6 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd

(*continued on next page*)

Table 4 (*continued*)

c)

VOC	Lactic Acid Bacteria									
	MRS	IRK751	IRK81	IRK82	SMF76	POM	TR7	TR71	TR14	TR2
2-undecanone	nd	nd	0.16 ± 0.01	nd	nd	nd	0.54 ± 0.01	0.65 ± 0.01	1.04 ± 0.01	0.11 ± 0.01
PYRAZINES	9.82 ± 0.01[a]	50.31 ± 0.04[c]	64.61 ± 0.17[e]	39.14 ± 3.35[b]	47.79 ± 0.11[c]	36.47 ± 0.24[b]	53.55 ± 0.29[d]	62.04 ± 0.3[e]	70.77 ± 0.69[f]	74.01 ± 0.77[f]
methyl-pyrazine	nd	1.79 ± 0.01	3.06 ± 0.02	2.73 ± 0.08	1.5 ± 0.02	2.9 ± 0.03	2.02 ± 0.02	2.18 ± 0.02	1.75 ± 0.01	1.66 ± 0.01
2,5-dimethyl pyrazine	nd	5.61 ± 0.01	6.12 ± 0.01	10.73 ± 0.27	3.51 ± 0.01	13.39 ± 0.07	3.8 ± 0.02	4.53 ± 0.04	3.17 ± 0.05	2.98 ± 0.01
2,6-dimethyl pyrazine	nd	9.56 ± 0.01	16.83 ± 0.08	7.57 ± 0.19	7.33 ± 0.01	9.37 ± 0.09	4.7 ± 0.03	5.22 ± 0.01	0.49 ± 0.03	3.36 ± 0.03
2-ethyl- 6-methylpyrazine	nd	1.57 ± 0.01	2.67 ± 0.01	2.89 ± 0.08	3.48 ± 0.03	1.07 ± 0.02	1.6 ± 0.03	1.21 ± 0.01	0.88 ± 0.01	0.53 ± 0.01
2-ethyl- 5-methylpyrazine	nd	1.37 ± 0.01	10.62 ± 0.02	2.24 ± 0.06	0.95 ± 0.01	1.98 ± 0.01	8.58 ± 0.06	10.64 ± 0.02	9.09 ± 0.03	10.42 ± 0.1
trimethyl pyrazine	nd	1.03 ± 0.01	nd	1.53 ± 0.04	1.34 ± 0.02	1.88 ± 0.01	nd	nd	nd	nd
2-ethenyl-6-methylpirazine	nd	0.49 ± 0.01	0.52 ± 0.01	0.48 ± 0.01	0.4 ± 0.01	0.34 ± 0.01	nd	0.46 ± 0.01	0.11 ± 0.01	nd
3-ethyl-2,5-dimethylpyrazine	3.11 ± 0.01	6.97 ± 0.01	1.66 ± 0.01	4.85 ± 0.14	21.17 ± 0.01	3.19 ± 0.01	3.52 ± 0.02	3.19 ± 0.02	2.89 ± 0.02	1.17 ± 0.01
tetramethylpyrazine	nd	nd	11.84 ± 0.01	0.2 ± 0.01	0.26 ± 0.01	nd	7.87 ± 0.04	10.97 ± 0.08	12.8 ± 0.08	21.4 ± 0.13
2 - ethyl-3,5-dimethylpyrazine	nd	nd	nd	2.12 ± 2.43	0.13 ± 0.01	nd	1.94 ± 0.02	2.25 ± 0.03	4.07 ± 0.05	0.46 ± 0.01
2,3-diethyl-5-methylpyrazine	nd	0.24 ± 0.01	0.05 ± 0.01	0.13 ± 0.01	0.39 ± 0.01	0.1 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.27 ± 0.01	0.03 ± 0.01
3,5-diethyl-2-methylpyrazine	nd	0.69 ± 0.01	0.16 ± 0.01	0.44 ± 0.01	2.48 ± 0.01	0.25 ± 0.01	7.08 ± 0.01	0.36 ± 0.01	18.41 ± 0.39	0.09 ± 0.01
2,3,5-trimethyl-6-ethylpyrazine	nd	0.26 ± 0.01	5.26 ± 0.02	0.27 ± 0.01	0.49 ± 0.01	0.2 ± 0.01	0.07 ± 0.01	6.13 ± 0.02	0.12 ± 0.01	13.6 ± 0.37
2,5-dimethyl-3-isobutylpyrazine	1.62 ± 0.01	0.62 ± 0.01	0.49 ± 0.01	0.74 ± 0.03	0.43 ± 0.01	0.58 ± 0.01	0.45 ± 0.01	0.65 ± 0.01	0.35 ± 0.01	0.25 ± 0.01
2-acetyl-3,5-dimethylpyrazine	nd	nd	nd	nd	n	nd	0.35 ± 0.01	0.53 ± 0.01	0.41 ± 0.01	0.43 ± 0.01
2-isoamyl-6-methylpyrazine	0.6 ± 0.01	0.71 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.68 ± 0.01	0.32 ± 0.01	0.42 ± 0.01	0.53 ± 0.01	0.2 ± 0.01	0.08 ± 0.01
3,5,6-trimethylpyrazine	nd	nd	0.28 ± 0.01	nd	0 ± 0	nd	0.7 ± 0.01	0.7 ± 0.01	1.21 ± 0.01	nd
2,6-dimethyl-3 -isopenthylpyrazine	nd	0.51 ± 0.01	0.16 ± 0.01	0.31 ± 0.01	0.15 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.13 ± 0.01	0.09 ± 0.01
2,5-dimethyl-3-isopenthylpyrazine	3.29 ± 0.01	18.96 ± 0.01	1.13 ± 0.01	1.36 ± 0.02	3 ± 0.02	0.75 ± 0.01	2.1 ± 0.02	2.73 ± 0.02	1.07 ± 0.01	0.38 ± 0.01
2,3-dimethyl-5 iso-penthylpyrazine	nd	nd	nd	nd	nd	nd	0.91 ± 0.01	1.18 ± 0.01	0.8 ± 0.01	0.84 ± 0.01
2,3,5-trimethyl-6-isopenthylpyrazine	1.22 ± 0.01	0.02 ± 0.01	nd	0.12 ± 0.01	0.18 ± 0.01	nd	nd	nd	nd	nd
2,3,5-trimethyl-6-propylpyrazine	nd	nd	3.49 ± 0.02	0.17 ± 0.01	nd	nd	7.17 ± 0.04	8.35 ± 0.06	12.64 ± 0.06	16.33 ± 0.08
OTHERS	38.77 ± 0.02[h]	23.45 ± 0.15[f]	14.24 ± 0.02[c]	21.91 ± 0.52[e]	21.95 ± 0.05[e]	27.48 ± 0.05[g]	13.72 ± 0.59[c]	15.97 ± 0.1[d]	8.43 ± 0.04[a]	10.27 ± 0.11[b]
2,4, di tert- butylphenol	28.29 ± 0.01	16.82 ± 0.05	12.19 ± 0.01	18.37 ± 0.47	17.43 ± 0.04	22.91 ± 0.03	9.87 ± 0.52	9.74 ± 0.04	7.55 ± 0.03	7.96 ± 0.09
2-t-butyl-phenol	10.49 ± 0.01	4.79 ± 0.03	1.63 ± 0.01	1.81 ± 0.01	3.56 ± 0.01	1.8 ± 0.01	1.97 ± 0.05	4.54 ± 0.06	0.27 ± 0.01	0.51 ± 0.01
Di tert-butyl-benzene	nd	1.13 ± 0.01	0.43 ± 0.01	nd	0.83 ± 0.01	1.34 ± 0.01	1.51 ± 0.02	1.25 ± 0.01	0.44 ± 0.01	0.59 ± 0.01
1,5-cyclooctadien, 1-6, dichloro	nd	0.07 ± 0.08	nd	0.16 ± 0.01	0.15 ± 0.01	0.06 ± 0.01	nd	nd	nd	nd
ethylacetate	nd	0.68 ± 0.01	nd	1.58 ± 0.05	nd	0.92 ± 0.01	nd	nd	nd	nd
1-nonene	nd	nd	nd	nd	nd	0.47 ± 0.01	0.39 ± 0.01	0.46 ± 0.01	0.19 ± 0.01	1.22 ± 0.02

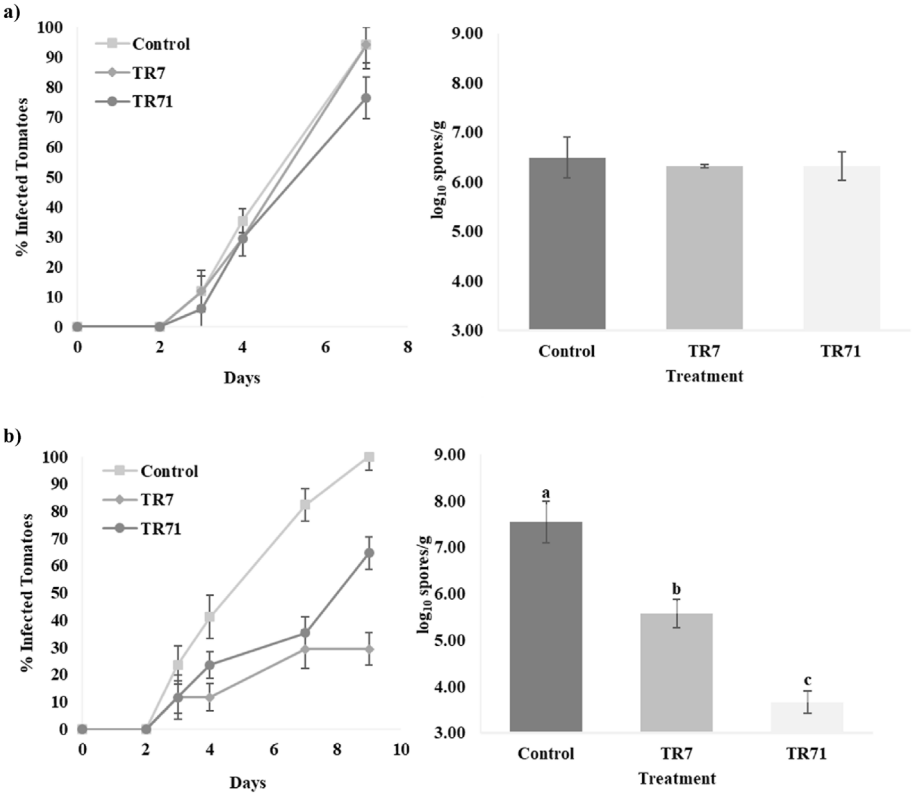

Fig. 2. Effects of *Lactobacillus platarum* TR7 and *Lactobacillus plantarum* TR71 CFS in growth of a) *Aspergillus flavus* ITEM 8111 and b) *Penicillium expansum* CECT 2278 on tomatoes. Results expressed in % infected tomatoes and microbiological count (log_{10} spores/g). Statistically significant differences for each treatment are indicated with different letters, $p < 0.01$. The results are expressed as mean ± standard deviation.

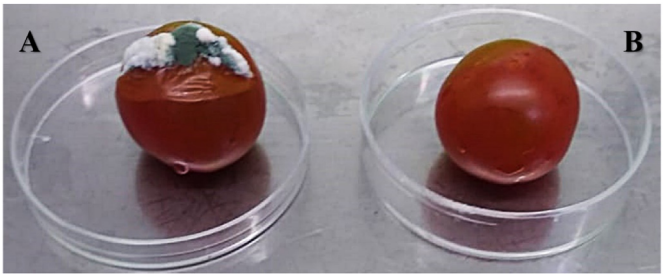

Fig. 3. Fungal growth of *Penicillium expansum* CECT 2278 on tomato treated with (A) sterile MRS Broth and (B) *Lactobacillus plantarum* TR71 CFS at 9 d of incubation.

5.58 log_{10} spores/g was observed. In the treatment with CFS fermented by *L. plantarum* TR7, infected tomatoes incubated for 9 d, presented a significant ($p < 0.01$) fungal count of 3.67 log_{10} spores/g. There are no prior publications on tomato bio-preservation using LAB and their fermentation products. Nevertheless, several studies performed tomato bio-preservation experiments using other compounds. For instance, Tian et al. (2015) reported the antifungal activity of perillaldehyde *in vitro* and *in vivo* against spoilage fungi (*A. flavus, Aspergillus oryzae, A. niger* and *A. alternata*). In that work, the production of aflatoxin B_1 by *A. flavus* in tomatoes was reduced 100% when treated with high concentrations of perillaldehyde.

4. Conclusions

Antifungal *in vitro* experiments demonstrated that CFS fermented by isolated LAB from tomatoes and sourdough has significant antifungal activity against a broad spectrum of toxigenic fungi. Furthermore, the application of CFS as a novel bio-preservative in tomatoes evidenced a reduction in the spoilage associated with *P. expansum* growth. Thus, the promising application presented in this study to increase the post-harvest shelf-life of tomatoes contributes to meeting the demand of consumers to reduce the agricultural use of synthetic compounds and increase that of natural alternatives.

CRediT authorship contribution statement

C. Luz: Conceptualization, Methodology, Writing - original draft, Investigation. **V. D'Opazo:** Investigation, Methodology, Writing - original draft. **J.M. Quiles:** Writing - review & editing. **R. Romano:** Visualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration. **J. Mañes:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **G. Meca:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors 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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