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# Selection of Algerian lactic acid bacteria for use as antifungal bioprotective cultures and application in dairy and bakery products

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ABSTRACT

In the context of a demand for "preservative-free" food products, biopreservation appears as a promising alternative to either replace or reduce the use of chemical preservatives. The purpose of this study was to evaluate the antifungal activity of a collection of lactic acid bacteria (n = 194), and then to evaluate the applicability and efficacy of selected ones used as bioprotective cultures against mold spoilers in dairy and bakery products. First, lactic acid bacteria were isolated from various Algerian raw milk samples and Amoredj, a traditional fermented product. Secondly, *in vitro* screening tests against *Mucor racemosus* UBOCC-A-109155, *Penicillium commune* UBOCC-A-116003, *Yarrowia lipolytica* UBOCC-A-216006, *Aspergillus tubingensis* AN, *Aspergillus flavus* T5 and *Paecilomyces formosus* AT allowed for the selection of 3 active strains, namely *Lactobacillus plantarum* CH1, *Lactobacillus paracasei* B20 and *Leuconostoc mesenteroides* L1. *In situ* tests were then performed to validate their activity in actual products (sour cream and sourdough bread) challenged with fungal spoilers. These tests showed that antifungal LAB could slow the fungal target growth and could be candidates of interest for industrial applications. Finally, organic acids and various antifungal compounds produced in sour cream and sourdough bread by the selected LAB, and thus potentially supporting the observed antifungal activity, were identified and quantified by HPLC and LC-QTOF.

## 1. Introduction

Molds and yeasts are responsible for the spoilage of different food products and therefore cause important economic losses for manufacturers and food waste for consumers. In addition, some fungi can constitute a danger to human health due to their ability to produce mycotoxins in the finished product. Currently, among the various methods available for food preservation, chemical preservatives are widely used and are effective. However, consumers are requesting less processed and "preservative-free" products. This trend is supported by the authorities and has led both the industrials and scientists to search for natural alternatives. In this context, biopreservation with the use of lactic acid bacteria is a promising and developing method (Leyva-Salas et al., 2017). Moreover, LAB are generally considered as safe (GRAS) by the US Food and Drug Administration in the United States, and many of them have been granted a QPS (Qualified Presumption of Safety) status within the European Union (EFSA, 2012; EC, 2008). Noteworthy, despite these status, safety assessment concerning potential biogenic amine production and antibiotic resistance should be performed before use in food production (Coton et al., 2018).

The use of lactic acid bacteria in different types of food as bioprotective antifungal cultures has been the subject of numerous studies over the years. For example, the study by Dal Bello et al. (2007) showed that sourdough bread fermented with *Lactobacillus plantarum* FST 1.7 delayed the growth of *Fusarium culmorum* and *Fusarium graminearum* and had the potential to increase the shelf life of wheat bread. Other studies like that of Leyva-Salas et al. (2018) has reported that using a combination of antifungal strains *Lactobacillus plantarum* L244 with *Lactobacillus harbinensis* L172 (A1) could slow the growth of *Penicillium commune*, *Mucor racemosus* and *Rhodotorula mucilaginosa* in sour cream for 2–24 days without affecting the organoleptic properties at various inoculum levels.

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Antifungal LAB are widely used in various fermented foods and their antifungal potential is due to their ability to produce different antifungal compounds, including different organic acids such as lactic or acetic acids which also reduce the pH (Moore et al., 2008; Crowley et al., 2013; Ahlberg et al., 2015; Hassan et al., 2005), but also fatty acids (Bergsson et al., 2001; Sjögren et al., 2003), reuterin (Axelsson et al., 1989, Magnusson et al., 2003a) and cyclic dipeptides (Niku-Paavola et al., 1999; Ström et al., 2002).

In this study, the antifungal activity of 194 lactic acid bacteria isolates from different Algerian products (goat, cow, sheep and camel raw milks and Amoredj, a fermented olive oil derivate) was evaluated *in vitro* on different media (MRSm, WFH and yogurt) and against various molds. Then, the applicability and efficacy of the most active strains was evaluated on actual food products (sour cream and sourdough bread produced at the lab scale). Finally, compounds potentially supporting the observed antifungal activity were identified and quantified by chromatographic methods.

## 2. Materials and methods

### 2.1. Microorganisms and culture conditions

Lactic bacteria were isolated from various raw milk samples (cow's milk, camel's milk, goat's milk, sheep's milk) and traditional fermented products (Amoredj) from Algeria using different media, Man, Rogosa and Sharpe (MRS at pH 5.4), Mayeux Sandine and Elliker (MSE at pH 6.8) and M 17 at pH 6.8, targeting the *Lactobacillus*, *Leuconostoc* and *Lactococcus* genera, respectively.

Target molds for antifungal activity evaluation corresponded to strains from the Université de Bretagne Occidentale culture collection (UBOCC, Plouzané, France, https://www.univ-brest.fr/ubocc), *Mucor racemosus* UBOCC-A-109155, *Penicillium commune* UBOCC-A-116003 and *Yarrowia lipolytica* UBOCC-A-216006 while *Aspergillus tubingensis* AN, *Aspergillus flavus* T5, *Aspergillus fumigatus* AF, *Aspergillus ochraceus* AAB, *Fusarium graminearum* FAB, *Fusarium verticillioides* F1M, *Talaromyces stollii* P1M and *Paecilomyces formosus* AT were from Oran 1 University culture collection.

### 2.2. Molecular identification of isolates

Lactic acid bacteria were firstly identified presumptively using phenotypic methods, including fermentative type, tests for catalase, arginine dehydrolase, citrate, exopolysaccharide production (dextran), carbohydrates fermentation, growth at different temperatures (15 °C and 45 °C) and NaCl concentrations (4% and 6.5%). LAB were then identified by sequencing of the gene encoding the 16S rRNA. DNA was extracted from MRS cultures using the FastDNA Spin kit (MP Biomedicals, France) and then amplified through PCR using the 16S-F (5′-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′) and 16S-R (5′-CCCGGGATCCAAGCTTACGGCTACCTTGTTACGACTT-3′) primers. PCR was then performed using the following conditions: 95 °C for 5 min; 29 cycles: 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1 min 30 s, and a final extension at 72 °C for 7 min.

Sequencing was performed by Eurofins Scientific (Germany) with the same primers as those used during the PCR. The obtained sequences were compared with the GenBank database using the “Basic Local Alignment Search Tool” (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST).

### 2.3. In vitro antifungal screening tests

#### 2.3.1. Preliminary tests

Antifungal activity screening of the isolated lactic acid bacteria was done using 3 methods. The first one corresponded to the confrontation method according to Gerbaldo et al. (2012). The second one was the double layer method as described by Magnusson et al. (2003b), while the third method corresponded to the well-diffusion method proposed by Cizeikiene et al. (2013). Each method was used for LAB isolates screening against *Aspergillus tubingensis* AN, *Aspergillus flavus* T5, *Aspergillus fumigatus* AF, *Aspergillus ochraceus* AAB, *Fusarium graminearum* FAB, *Fusarium verticillioides* F1M, *Talaromyces stollii* P1M and *Paecilomyces formosus* AT. The antifungal activity of the 30 most active isolates was then evaluated *in vitro* and *in situ* on different matrices.

#### 2.3.2. In vitro screening

Antifungal activity was first confirmed on MRSm agar, a medium without acetate (Le Lay et al., 2016a). After 2 successive LAB cultures at 30 °C for 24h in MRS broth (AES Laboratoire, France), 10 µL of bacterial culture at a $10^9$ CFU/mL concentration were added to each well of a 24-well plate and then 1 mL of MRSm agar, a sodium acetate-free modified MRS supplemented with 1.4% glycerol (150 mM), 20 mg/L bromocresol green and 0.7% agar, was added. After incubation at 30 °C for 48h, the surface pH was measured (Eutech instruments, Singapore) in another 24-well control plate to confirm bacterial growth. Then, an artificial contamination was performed on the surface of the culture media using 50 spores or cells/well of *Mucor racemosus* UBOCC-A-109155, *Penicillium commune* UBOCC-A-116003, *Yarrowia lipolytica* UBOCC-A-216006, *Aspergillus tubingensis* AN, Aspergillus *flavus* T5 or *Paecilomyces formosus* AT which were prepared as previously described (Delavenne et al., 2012). In parallel, a negative control plate without bacteria was inoculated.

Wheat flour hydrolysate WFH was used to simulate bakery product (sourdough). Briefly, 20% wheat flour (w/v in tap water) was incubated on a rotary shaker at 30 °C for 4h at 90 rpm. The supernatant obtained after decantation was supplemented with glucose, maltose, sucrose and fructose (1.25 g/L of each), 1% yeast extract and 0.7% agar, followed by sterilization at 104 °C for 20 min (Gobbetti et al., 1994) before distribution on 24-well plates. After 2 successive cultures at 30 °C for 24h in MRS broth (AES Laboratoire, France), 10 µL of LAB culture at a concentration of $10^9$ CFU/mL were added to each well of a 24-well plate followed by addition of 1 mL of WFH agar. After incubation at 30 °C for 48h, surface pH was measured to confirm bacterial growth. Then, an artificial fungal contamination was made by surface inoculation of 50 spores of either *Aspergillus tubingensis* AN, *Aspergillus flavus* T5 or *Paecilomyces formosus* AT.

As for dairy product simulating model, miniaturized yogurts were produced as described previously (Leyva-Salas et al., 2018) by fermentation of semi-skimmed UHT milk (Grand lait, Candia, France) enriched by 4% skimmed milk powder (Lait écrémé en poudre, Carrefour, France), pasteurized for 30 min at 85 °C and then rapidly cooled to 45 °C. The milk was then supplemented with a sterile pH indicator, Litmus dye at 0.4‰ and inoculated with commercial LAB starters MY800 (composed of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* - Danisco, Sassenage, France) according to the manufacturer's recommendations. After distribution of the inoculated milk into 24-wells plates (2mL/well), 100 µL of the bacterial solutions at a concentration of $10^8$ CFU/mL were deposited per well. Each analysis was made in duplicate. The miniaturized yogurts were incubated at 42 °C to allow for the development of the technological starter thermophile strains, until a pH of 5. At the end of the fermentation, the exudate was removed and 50 mold spores or yeast cells were deposited on the surface of the yogurts, the fungal targets were *M. racemosus* UBOCC-A-109155, *P. commune* UBOCC-A-116003, *Y. lipolytica* UBOCC-A-216006 (Leyva-Salas et al., 2018).

For each method, positive controls corresponded to the matrix without protective cultures and inoculated with the fungal targets; while negative controls were devoid of target.

MRSm and WFH plates were incubated at 25 °C and yogurt at 12 °C and fungal growth was visually assessed after 7 and 14 days and compared to the negative controls. Inhibition of the fungal targets was evaluated according to the following scoring system: 3, for no target

growth; 2, for a strong reduction effect; 1 for a delay effect (slow target growth) and 0, for no growth delay as compared to the control (Le Lay et al., 2016a).

### 2.4. In situ tests

#### 2.4.1. Challenge tests on sourdough bread

The two most active strains and one weakly active control strain were selected for *in situ* tests and were cultured twice successively in MRS broth at 30 °C for 24h. Cells were washed twice with tryptone salt in a centrifuge at 5000 × *g* for 5 min and resuspended in 40 mL of sterile tap water. In order to prepare 1 kg of sourdough, 410 g of organic T80 flour (Golden Oatmeal, France), 60 g of sucrose, 30 g of NaCl, 460 mL of sterile tap water and 40 mL of bacterial inoculum containing $2.5 \times 10^8$ CFU/mL were homogenized manually for 5 min then incubated at 30 °C for 48h according to Dal Bello et al. (2007). pH was determined on 10 g of sourdough before and after 48h of fermentation at 30 °C. LAB and potential yeasts were enumerated after 48h of fermentation on MRS and YGC media, respectively. After homogenization of the various bread ingredients (Supplementary Table 1) for 3 min, the dough was kneaded for 20 min in a bread machine (Moulinex OW240E30, France) then covered and left for a fermentation of 1.5 h at room temperature. Afterwards, the dough was cut to the desired size (18 cm × 9 cm x 5 cm) then covered again for a second fermentation period of 1.5 h at room temperature. Finally, the bread was baked in an oven (Carbolite-Gero, France) at 220 °C for 40 min (Russo et al., 2017a), then cut into 4 × 1 × 4 cm pieces and put into 140 mm Petri dishes. pH and water activity were measured 2 h after cooking. The bread pieces were then challenged by surface inoculation of 50 spores of the fungal targets. Petri dishes were then sealed with parafilm paper, transferred into a box (with a lid) containing distilled water at 20% of glycerol in order to maintain a stable $a_w$ in the bread, and incubated at 25 °C for 5–10 days. Control breads corresponded to bread without antifungal cultures for positive control and without fungal targets for negative controls, 4 bread pieces were used for each analysis. Time-to-visible-growth of each fungal target was evaluated and photographs were taken using a digital camera during incubation.

#### 2.4.2. Challenge tests on sour cream

A 30% fat liquid pasteurized cream (Fleurette de Carrefour, France) was used. First, 10 mL of the LAB starter cultures MM100 (*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacteylactis*) at $10^8$ CFU/mL and 10 mL of the selected antifungal LAB (except for positive controls) at $10^9$ CFU/mL were inoculated into 1 L of cream. Then, 50 mL of the inoculated cream were distributed into sterile containers and finally incubated at 24 °C for 20h–24h, the fermentation was stopped once pH ≈ 4.4 was reached.

After fermentation, the sour cream surface was artificially contaminated by 50 spores of the fungal targets. Assays were performed in quadruplicate for each condition. The containers were then incubated at 12 °C for 7 days and up to 15 days. Time-to-visible-growth of the fungal target was evaluated and photographs were taken using a digital camera. Moreover, in order to evaluate the growth of the antifungal strains after 15 days of fermentation, lactobacilli were enumerated on LAMVAB medium (MRS agar, cysteine hydrochloride 0.5 mg/L and vancomycin 20 mg/L).

#### 2.4.3. Durability tests

For sour cream, 20 g of fermented cream were distributed into Petri dishes. Then, they were exposed in the open air for 1 h in an office room to allow for natural fungal contamination, and incubated at 12 °C for 8 days. As for bread pieces, they were also exposed for 1 h in the open air and incubated under the same conditions as those used for bread challenge tests.

### 2.5. Analytical methods

#### 2.5.1. Fungal quantification

For *in situ* tests on bread and sour cream samples, fungal biomass was measured using ergosterol quantification as described by Delavenne et al. (2013).

#### 2.5.2. Metabolite quantification

Lactic, acetic and succinic standards were prepared at different concentrations 0.01, 0.1, 0.5, 1, 2 g/L. To extract these organic acids, 1 g of the samples (bread, cream and sourdough) were diluted in 9 mL of sulfuric acid (0.01 N) and stirred for 1h, the obtained mixtures were then centrifuged at 9056 g for 5 min at 4 °C and filtered with 0.45 µm filter and kept at 4 °C. These 3 acids were quantified with an HPLC Agilent 1100 series (Agilent technologies, Santa Clara, CA) equipped with a Rezex ROA column (Phenomenex, France) using both UV (set at 210 nm) and refractometer detectors. Concerning other targeted antifungal compounds, they were extracted and quantified by LC-QTOF as described by Le Lay et al. (2016b).

#### 2.5.3. Sensorial tests

Sensorial analysis was done on the produced sour creams and sourdough breads. Thirty people (untrained panel) evaluated both products according to the following criteria: color, smell, texture in mouth, taste and global appreciation, product acceptability and will of consumption. The first 5 criteria were scored on a hedonic scale of 1–8 (1 = dislike and 8 = really appreciated).

#### 2.5.4. Statistical analyses

Quantitative results for ergosterol, antifungal compounds and sensorial tests were subjected to an analysis of variance (ANOVA) using STATGRAPHICS Centurion XVII (Statistical Graphics Corp., VA, USA). Tukey's test was used for mean comparison, and differences were considered significant at a $P \leq 0.05$.

## 3. Results

### 3.1. In vitro antifungal screening tests

The isolation process led to the creation of a working collection of 194 isolates. These LAB were preliminary screened for their antifungal activity against *A. tubingensis* AN, *A. flavus* T5, *A. fumigatus* AF, *A. ochraceus* AAB, *F. graminearum* FAB, *F. verticillioides* F1M, *T. stollii* P1M and *P. formosus* AT which led to the selection of the 30 most active isolates (data not shown). After a genotypic identification, it turned out that they belonged to the *Lactobacillus* and *Leuconostoc* genera including 17 *Lactobacillus paracasei,* 2 *Lactobacillus plantarum* and 11 *Leuconostoc mesenteroides*.

To confirm the observed antifungal activity and further select isolates of interest, tests on MRSm were performed. For cereal associated molds, *Leuconostoc* spp. were globally more antifungal than *Lactobacillus* spp. In particular, *A. tubingensis* AN was completely inhibited by 6 *Leuconostoc* but only 3 *Lactobacillus* (including 2 *L. plantarum* and 1 *L. paracasei*). *A. flavus* T5 was totally inhibited by 2 *L. plantarum*. For *P. formosus* AT, the studied strains generally expressed a weak antifungal activity. Indeed, *L. paracasei* and the majority of the *L. mesenteroides* exhibited a score of 1. However, 2 *L. plantarum* isolates stood out and completely inhibited the development of this mold in the tested conditions (Table 1). For the dairy model, for which the 3 chosen target molds were *P. commune, Y. lipolytica* and *M. racemosus*, isolates belonging to the *Lactobacillus* genus were the most antifungal compared to *Leuconostoc* spp. (with the exception of *L. mesenteroides* L1 which was active against the 3 targets). The results also showed that, among the 3 fungal targets, *M. racemosus* UBOCC-A-109155 was the most resistant mold. Indeed, only 2 *L. plantarum* and 1 *L. mesenteroides* could inhibit its growth with a score of 2; whereas, for example, *Y. lipolytica* UBOCC-A-

**Table 1**
*In vitro* antifungal activity of 30 selected LAB isolates against *A. tubingensis, A. flavus, P. formosus, P. commune, Y. lipolytica* and *M. racemosus*.

| Sources | Isolate number | Species | Cereal model | | | | | | Dairy model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *A. flavus* | | *A. tubingensis* | | *P. formosus* | | *M. racemosus* | | *P. commune* | | *Y. lipolytica* | |
| | | | MRSm | WFH | MRSm | WFH | MRSm | WFH | MRSm | Yogurt | MRSm | Yogurt | MRSm | Yogurt |
| Amoredj | B18 | *L. paracasei* | 0[a] | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| | B17 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | B12 | *L. paracasei* | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B9 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | B10 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | B6 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | B19 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| | B20 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 2 | 1 |
| | B13 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 1 |
| | B5 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 1 |
| | B8 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | B7 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 0 |
| | B16 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Camel milk | S8 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 0 |
| | S9[a] | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | S2 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 1 |
| | S3 | *L. paracasei* | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Amoredj | B12′ | *L. mesenteroides* | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | B4 | *L. mesenteroides* | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Camel milk | TE6 | *L. mesenteroides* | 1 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| | L1 | *L. mesenteroides* | 1 | 2 | 3 | 3 | 0 | 0 | 2 | 0 | 3 | 1 | 2 | 1 |
| | L4 | *L. mesenteroides* | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| | L8 | *L. mesenteroides* | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 |
| | L13 | *L. mesenteroides* | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | L2 | *L. mesenteroides* | 1 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cow milk | C11 | *L. mesenteroides* | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C4 | *L. mesenteroides* | 1 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C17 | *L. mesenteroides* | 0 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 0 |
| Goat milk | CH1 | *L. plantarum* | 3 | 1 | 3 | 0 | 3 | 1 | 2 | 0 | 3 | 2 | 2 | 1 |
| | CH2 | *L. plantarum* | 3 | 1 | 3 | 0 | 3 | 1 | 2 | 0 | 3 | 2 | 2 | 1 |

[a] Antifungal activity score: 0, No inhibition (growth of the fungal target identical to that of the positive control); 1, low inhibition (slightly delayed growth of the fungal target in comparison with the positive control); 2, strong inhibition (marked delayed growth of the fungal target in comparison with the positive control); 3, total inhibition.

216006 was inhibited by 5 *L. paracasei*. *P. commune* UBOCC-A-116003 was inhibited by only two *L. plantarum* strains and 1 *L. mesenteroides* (Table 1).

The next step consisted to evaluate the antifungal activity in matrices closer to actual final products; therefore, the 30 selected isolates were tested *in vitro* on WFH, for bakery products, and miniaturized yogurts, for dairy products. For WFH, the results were completely different from those obtained in MRSm as the majority of the selected antifungal strains showed low or no antifungal activity except for a few strains. *A. tubingensis* was very resistant and was only inhibited by 2 *L. mesenteroides* isolates (L1 and L2). As for *P. formosus*, almost no antifungal activity was observed except for the 2 *L. plantarum* isolates (CH1 and CH2) which only slightly slowed fungal growth (score of 1). However, for *A. flavus*, several isolates were efficient antifungal, from which 16 *L. paracasei* and 1 *L. mesenteroides* were very antifungal (score of 3) (Table 1). For miniaturized yogurts, the obtained results were also different from those obtained on MRSm, 2 of the 3 targets were very resistant to the selected LAB as weak antifungal activities were observed against *Y. lipolytica* and none against *M. racemosus*. On the contrary, *P. commune* was the most sensitive mold as it was inhibited by 17 *Lactobacilli* with scores of 1–2 (Table 1).

### 3.2. In situ tests

#### 3.2.1. Sourdough bread

Three LAB, including 2 antifungal ones, namely *L. plantarum* CH1, *L. mesenteroides* L1, and a control strain (*L. paracasei* B6) were chosen based on the *in vitro* results on MRSm and WFH. pH of the obtained sourdough varied between 3.47 and 3.92 while that of sourdough bread and control breads after baking varied from 4.62 to 5.48 for the sourdough bread containing *L. plantarum* CH1 and control bread, respectively (Supplementary Table 2). After fermentation, antifungal culture populations in the sourdough were $3.6 \times 10^8$, $5.5 \times 10^8$ and $9.7 \times 10^7$ CFU/g for *L. plantarum* CH1, *L. paracasei* B6 and *L. mesenteroides* L1, respectively (Supplementary Table 3).

The challenge tests showed that the sourdough bread containing the antifungal strains could delay the growth of both fungal targets (Table 2), despite the very favorable conditions for fungal growth such as the large number of inoculated spores, the percentage of added sourdough, high humidity and temperature. *A. tubingensis* developed from the day 3, while its growth was only visible after 5 days with *L. plantarum* CH1 and 4 days with *L. mesenteroides* L1 (Fig. 1). The results

**Table 2**
Fungal growth delay caused by the 4 selected LAB during *in situ* tests against *A. tubingensis, A. flavus, P. commune* and *M. racemosus* expressed in number of days to visible growth as compared to the positive control.

| Species | Isolate number | Growth delay (days) | | | |
|---|---|---|---|---|---|
| | | Sour cream | | Sourdough bread | |
| | | *M. racemosus* | *P. commune* | *A. flavus* | *A. tubingensis* |
| *L. plantarum* | **CH1** | 1 | 5 | 2 | 2 |
| *L. paracasei* | **B20** | 1 | 3 | NT | NT |
| *L. mesenteroides* | **L1** | NT* | NT | 1 | 1 |
| *L. paracasei* | **B6** | 0 | 0 | 1 | 1 |

*NT, Not tested.

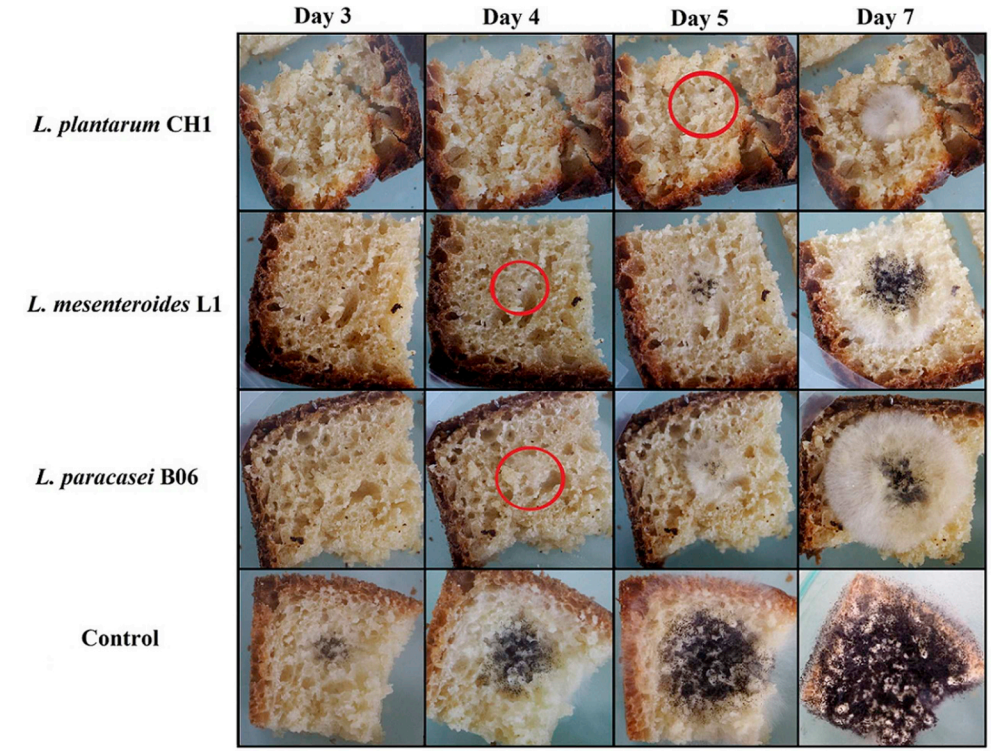


**Fig. 1.** Growth of *A. tubingensis* after 7 days of storage at 25 °C on sourdough bread obtained by fermentation with 3 different protective strains and on control bread (without protective strain). Red circles indicate visible growth of the fungal target.

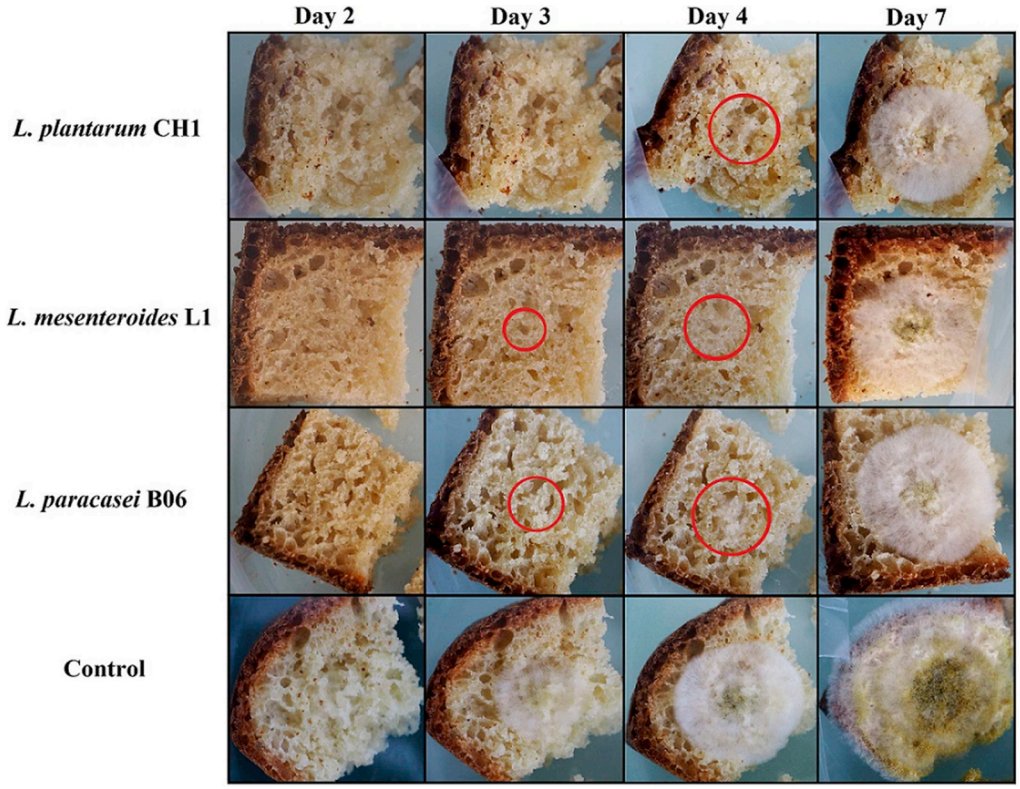


**Fig. 2.** Growth of *A. flavus* on control bread and sourdough bread obtained by fermentation with 3 LAB strains after storage for 7 days at 25 °C. Red circles indicate visible growth of the fungal target.

were similar against *A. flavus*, *L. plantarum* CH1 sourdough bread was also the most antifungal and inhibited the target for two extra days as compared to the negative control while for *L. mesenteroides*, the growth was delayed by one day (Fig. 2).

For durability tests, which simulated natural contaminations, and are more realistic especially in terms of contamination levels, bread

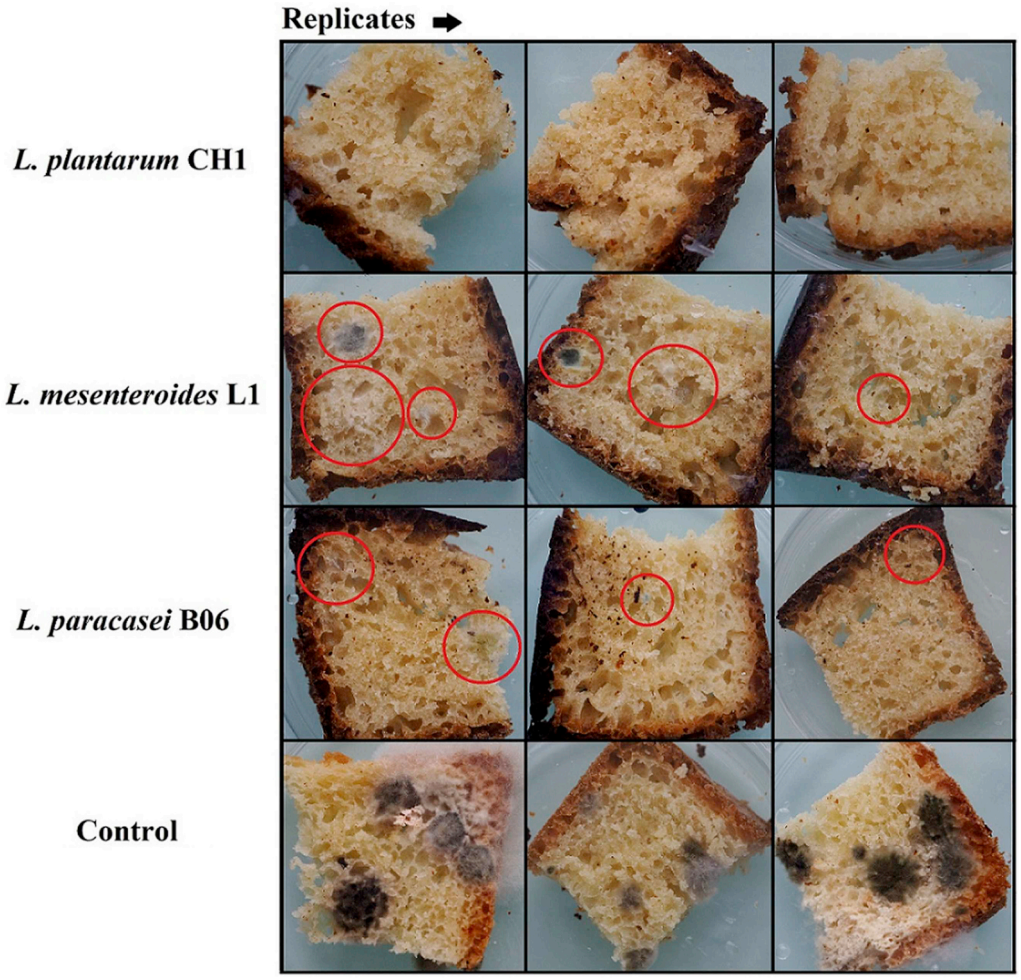


**Fig. 3.** Visual observation of bread pieces (with or without antifungal cultures) after 1h exposure to the open air and storage for 7 days at 25 °C. Red circles indicate visible growth of fungi.

pieces were exposed to the open air for 1 h during this experiment. Similar results to those of challenge tests were observed, as important fungal contaminations were observed on bread control on the day 7 (Fig. 3), whereas bread produced with *L. plantarum* CH1 was not contaminated during the 7 incubation days. On the other hand, bread produced by *L. mesenteroides* L1 did not exhibit noticeable antifungal activity and was comparable to bread containing *L. paracasei* B6 (Fig. 3).

#### 3.2.2. Sour cream

Based on the obtained *in vitro* results on MRSm and miniaturized yogurts, *L. plantarum* CH1 and *L. paracasei* B20 were selected for the challenge tests, as well as *L. paracasei* B6 as a control. Overall, as observed in *in vitro* tests, *P. commune* was the most sensitive target. *L. plantarum* CH1 and *L. paracasei* B20 completely inhibited its growth for 5 and 3 additional days as compared to the control without antifungal bacteria, respectively (Table 2). After 14 days of incubation at 12 °C, the surface of the control sour creams was completely covered by *P. commune* while on those of the antifungal LAB fermented sour creams, especially the one containing *L. plantarum* CH1, only the slight presence of unpigmented mycelium was observed (Fig. 4). As for *M. racemosus*, the target was very resistant as observed in *in vitro* tests and was only inhibited by the two selected antifungal strains for 1 additional day as compared to the control (data not shown). Concerning the durability tests, on control creams, mold had developed on almost the entire surface of the product at the end of the 8th day, whereas *L. paracasei* B20 and 2 replicates of *L. plantarum* CH1 creams were free of fungal contaminations (Fig. 5). It is worth mentioning that the replicate of *L. plantarum* CH1 cream that did not remain free of fungal contamination only presented one fungal thallus after incubation for 8 days (Fig. 5). While pH of sour creams after fermentation were in the same range (i.e., 4.4) (Supplementary Table 2), sour creams containing antifungal cultures showed a higher post-acidification than the control after storage for 14 days at 12 °C. Indeed, pH of sour creams containing antifungal cultures were 0.39 to 0.26 lower than that of the control. After storage, populations of *L. plantarum* CH1, *L. paracasei* B6 and B20 were $3.4 \times 10^8$, $2.21 \times 10^8$ and $6.75 \times 10^8$ CFU/g, respectively (Supplementary Table 3).

### 3.3. Analytical results

#### 3.3.1. Ergosterol quantification

Fungal biomass quantification through ergosterol quantification validated the observed *in situ* test results in both dairy and cereal models. *L. plantarum* CH1 was the most antifungal strain as it delayed the growth of the 4 targets *in situ*. Indeed, *L. plantarum* CH1 sourdough bread and sour cream contained the lowest amounts of ergosterol compared to the other strains (*i.e.* with *L. mesenteroides* L1, *L. paracasei* B20 and the control *L. paracasei* B6) (Table 3).

#### 3.3.2. Sensorial tests

For sourdough breads, the results expressed on the radar chart (Fig. 6) did not reveal significant differences between sourdough breads and the control bread. Statistically, the 30 taster panel found that the

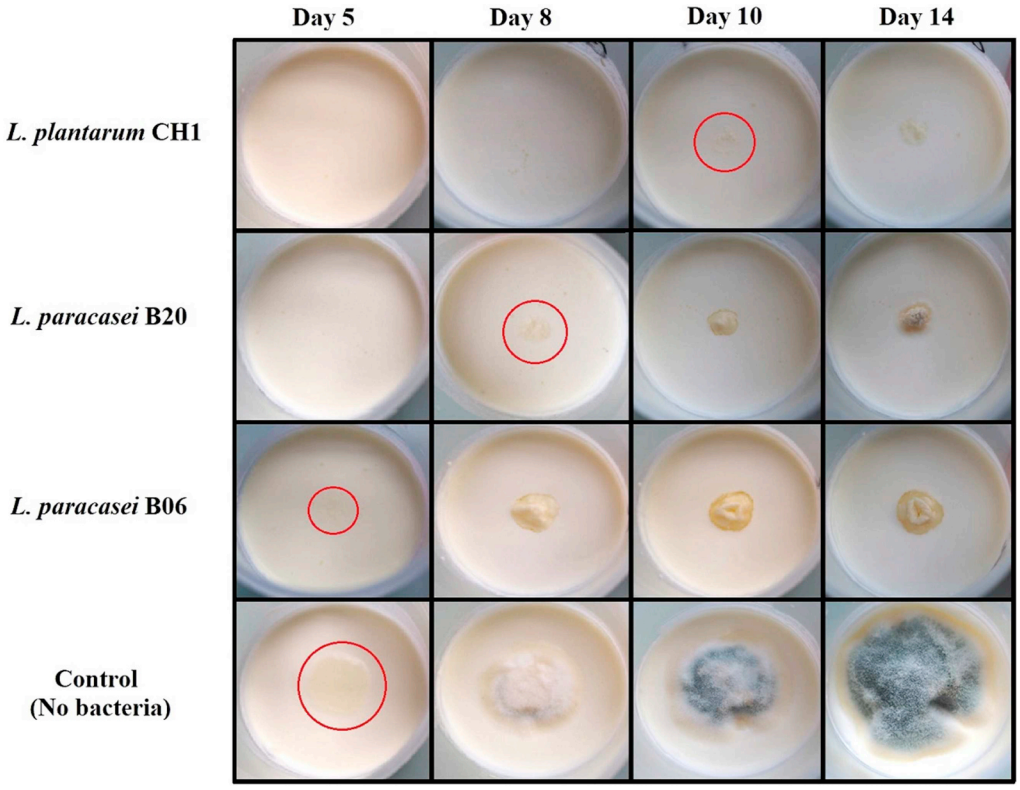


**Fig. 4.** Growth of *P. commune* on sour cream containing added antifungal bacterial strains during 14 days at 12 °C. Red circles indicate visible growth of fungi.

control bread exhibited a more attractive color and aroma than the sourdough breads. However, they considered as better the L. *plantarum* CH1 bread, the differences being associated to the taste and texture profiles (Fig. 6).

For the sour cream sensorial test results, antifungal creams were slightly less appreciated than the control sour cream. The major differences concerned the taste, texture in mouth and the aroma profile. The tasting panel considered that the creams with antifungal cultures were more acidic and had a slightly different texture than the control cream, especially in the case of *L. plantarum* CH1 cream. Statistical analyses showed that only texture was significantly less appreciated between creams with antifungal culture and the control cream (Fig. 7). Noteworthy, the other differences were not significant and did not affect significantly the desirability and quality of the antifungal sour creams.

### 3.3.3. Metabolite quantification

Lactic, acetic and succinic acids were first quantified in the products. As shown in Table 4, HPLC analyses revealed high concentrations of lactic acid in both bakery and dairy models. In fact, lactic acid concentrations were important in sourdough and bread, especially in those with *L. plantarum* CH1 that produced the highest concentration, 3.38 g/kg in bread (12.1 g/Kg in sourdough) while the control bread only contained 0.03 g/kg. In sour cream, the selected *Lactobacillus* strains produced almost twice as much as in the control. *L. paracasei* B20 was the highest producer (9 g/kg) in comparison to the control cream (5.3 g/kg). Concerning acetic acid production, *L. mesenteroides* L1 sourdough samples contained 0.64 ± 0.17 g/kg while bread samples contained 0.83 ± 0.12 g/Kg. In sour cream, *L. plantarum* CH1 produced the highest quantities (0.64 ± 0.3 g/kg), almost 4 times more than in the control cream (0.17 ± 0.02 g/kg). For succinic acid, *L. plantarum* CH1 was the strain that produced the most in bread (0.21 ± 0.03 g/kg) compared to control bread (0.03 ± 0.001 g/kg). In fresh cream, *L. plantarum* CH1 also produced the most (0.06 ± 0.01 g/kg). However, the concentrations determined for the other LAB and the control cream were only slightly lower (0.04 ± 0.01 g/kg).

In order to establish the molecule potentially supporting the observed antifungal activity, we also targeted various compounds described in the literature as antifungal using a LC-QToF method (Le Lay et al., 2016b). Eleven out of the 30 targeted compounds were not detected in either the sourdough or sour creams. Overall, *L. plantarum* CH1 strain produced the highest concentrations of the majority of the 19 detected antifungal molecules compared to *L. mesenteroides* L1 and *L. paracasei* B20. The main produced compounds corresponded to DL-hydroxyphenylacetic, phenyllactic acid, 3-(4-Hydroxyphenyl) propionic, 3-(4-hydroxy-3-methoxyphenyl) propanoic, benzoic and methylcinnamic acids (Table 4). In addition, some of these molecules were only produced by *L. plantarum* CH1, such as mevalonolactone, and 3,4-dihydroxyhydrocinnamic and 2-hydroxydodecanoic acids (Table 4).

However, other antifungal strains such as *L. mesenteroides* L1 also produced significantly higher concentrations of certain molecules such as caffeic acid and phenyl acetate in comparison to the other strains. Concerning *L. paracasei* B20, even if it did not produce as many molecules as the other LAB, the determined concentrations were only slightly lower compared to the others as observed for phenyl acetate and DL-hydroxymyristic or benzoic acids (Table 4). These analyses also showed that some compounds like azelaic acid and di-*tert*-butylphenol were present but at concentrations below their respective quantification limits.

## 4. Discussion

In this study, during the *in vitro* tests, several LAB of the *Lactobacillus* genus such as *L. plantarum* CH1 and *L. plantarum* CH2 were more active in MRSm than in WFH, this can be explained by the amount of carbohydrates in both culture media as MRSm contained 20 g/L glucose while there was only 1.25 g/L of 4 different carbohydrates (glucose, maltose, sucrose and fructose) in WFH. This lower concentration could have induced bacteria to produce less acid during fermentation thus

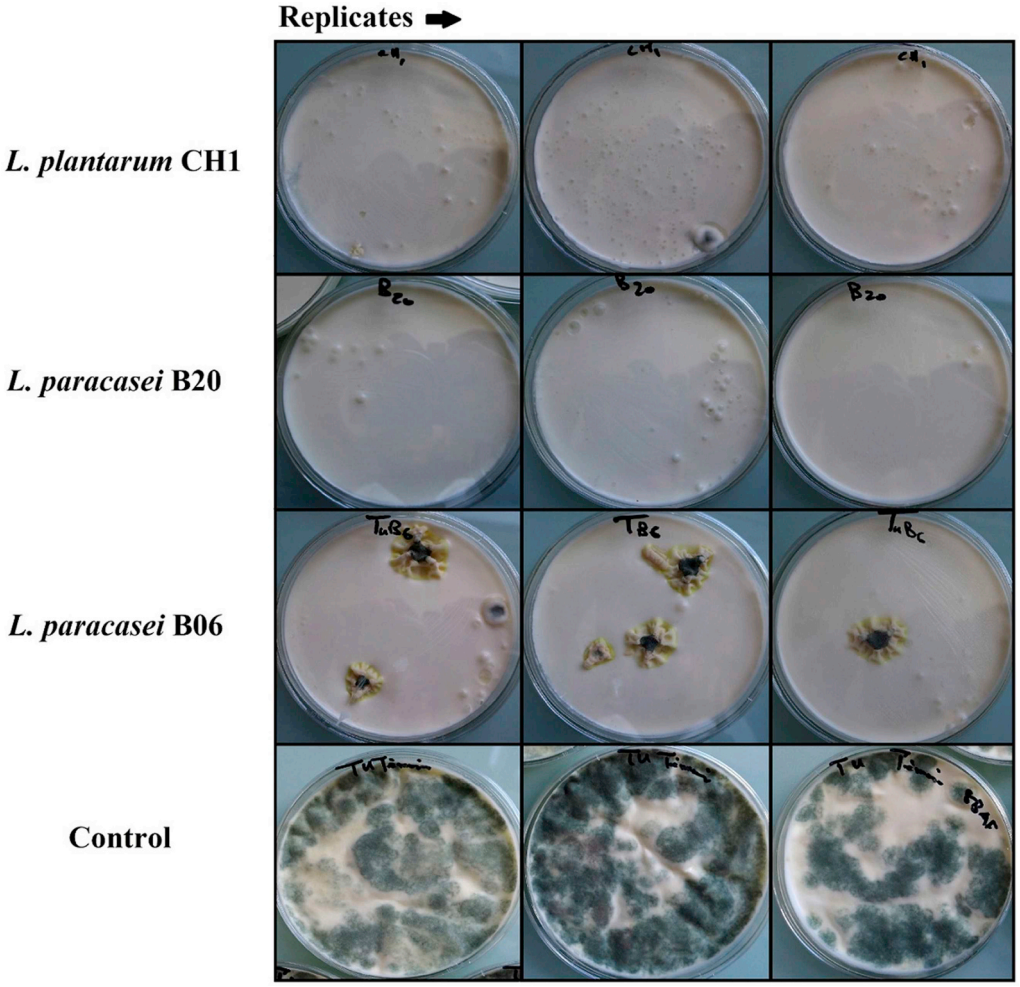


**Fig. 5.** Visual observation of sour creams (with or without antifungal cultures) after 1h exposure to the open air and storage for 8 days at 12 °C.

causing lower antifungal activity as suggested by Gobbetti et al. (1994). Similarly, we observed a better antifungal activity against *P. commune, M. racemosus* and *Y. lipolytica* on MRSm than on the miniaturized yogurts, the difference being potentially associated to the pH of the two media after fermentation (pH below 3.4 for MRSm vs 4.2–4.3 for yogurts). Moreover, the composition of the two media is completely different and yogurt also contained commercial LAB starters.

*Lactobacillus* spp. showed a better antifungal activity in *in vitro* tests than *Leuconostoc* spp. This could be explained by a slower growth of the latter compared to *Lactobacillus* spp., which did not allow the total expression of their antifungal potential, especially in miniaturized yogurts in which incubation conditions (4–6 h at 42 °C) were less favorable to the growth of members of this genus.

After this sequential screening steps, 3 LAB strains, namely *L. plantarum* CH1, *L. mesenteroides* L1 and *L. paracasei* B20, were selected for *in situ* tests (both challenge and durability tests) in bakery and dairy products. In general, the results of *in situ* tests indicated that *L. plantarum* CH1 was the most active strain in both dairy and cereal models, as supported by the ergosterol quantification results. Noteworthy, during this study, the 3 strains were especially challenged during *in situ* tests for which the incubation conditions were very favorable for fungal growth such as breads that were incubated at 25 °C in high humidity boxes, or the sour cream which were incubated at 12 °C. In addition, the number of inoculated spores during each challenge test was high (50 spores) in comparison to actual contamination events. Overall, these really challenging conditions ("worst case scenario") can therefore suggest even better results in real conditions. In this context, durability tests provide a closer look to the reality of fungal contamination (Leyva-Salas et al., 2018).

Noteworthy, other strains of the 3 selected LAB species have already been studied for food biopreservation. As for *L. plantarum*, it has already been described in various studies as a species with high antifungal activity against various molds such as those of the *Aspergillus*, *Penicillium* and *Fusarium* genera (Lavermicocca et al., 2000; Magnusson et al., 2003a; Dal Bello et al., 2007; Cheong et al., 2014; Saladino et al., 2016;

**Table 3**
Quantification of fungal targets expressed in mg of ergosterol/g of bread after storage for 7 days at 25 °C and sour cream surface after storage for 15 days at 12 °C.

| | Fungi | Control | *L. plantarum* CH1 | *L. mesenteroides*L1 | *L. paracasei* B20 | *L. paracasei* B6 |
|---|---|---|---|---|---|---|
| Bread | ***A. tubingensis*** | 100.2 ± 15.5a[a] | 11.8 ± 0.2b | 22.2 ± 1.4c | NT[b] | 33.1 ± 0.9c |
| | ***A. flavus*** | 106.2 ± 9a | 10.4 ± 1.2b | 33.9 ± 1.2c | NT | 28.9 ± 4.5c |
| Sour cream | ***M. racemosus*** | 53.1 ± 15.1a | 28.7 ± 1.4b | NT | 26.3 ± 1b | 37 ± 1.6b |
| | ***P. commune*** | 111.1 ± 4.8a | 1.3 ± 0.25b | NT | 2.71 ± 0.63b | 7.83 ± 0.6b |

[a] Values with different letters within a same line are significantly different by Tukey-HSD test ($P \leq 0.05$).
[b] NT, Not tested.

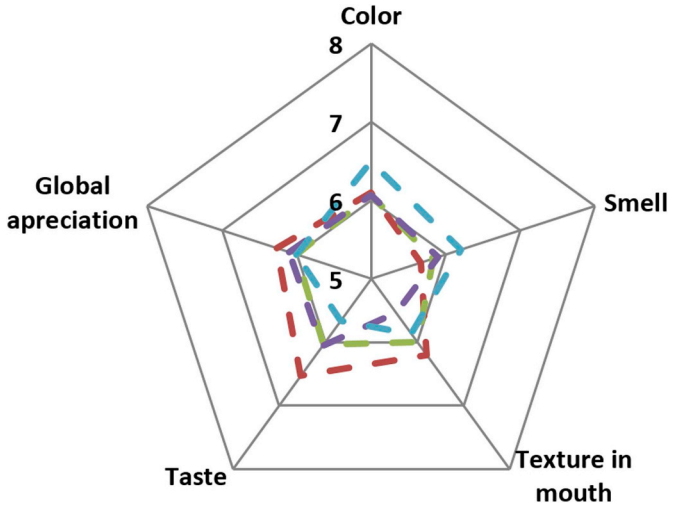


**Fig. 6.** Sensory characteristics of different breads. (▬), control bread without sourdough; (▬), bread with sourdough fermented with *L. plantarum* CH1; (▬), bread with sourdough fermented by *L. mesenteroides* L1; (▬), bread with sourdough fermented by *L. paracasei* B6, Values are means of 30 independent evaluations and are expressed using a hedonic scale of 1–8 (1 = I do not like and 8 = I like it a lot).

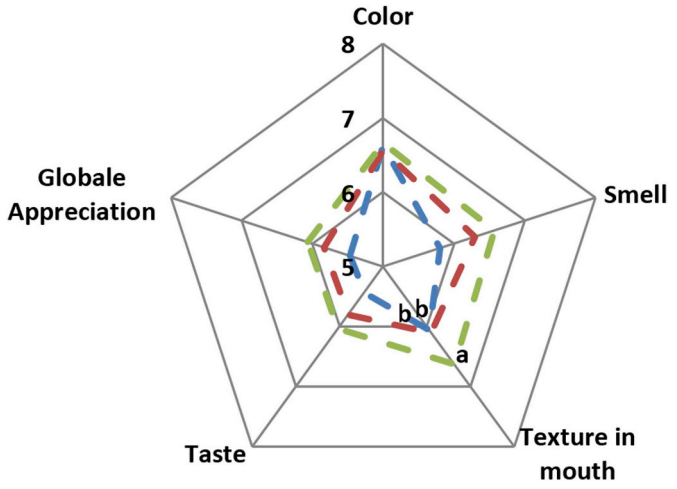


**Fig. 7.** Sensory characteristics of different sour creams fermented with or without antifungal strains. (▬), sour cream control without antifungal bacteria; (▬), sour cream with *L. plantarum* CH1; (▬), sour cream with *L. paracasei* B20. Values are means of 30 independent evaluations and are expressed using a hedonic scale of 1–8 (1 = I do not like and 8 = I like it a lot). Means with different letters are significantly different according to Tukey's HSD test ($P \leq 0.05$).

Leyva-Salas et al., 2018). For *L. paracasei*, Aunsbjerg et al. (2015) showed that it could express antifungal activity against some *Penicillium* species in yogurt. Lee and Chang (2016) also reported that *L. mesenteroides* could inhibit certain fungal targets such as *A. fumigatus* on Bacto Agar supplemented with Malt Extract Agar.

The use of sourdough fermented by antifungal lactic acid bacteria to extend the shelf life of cereal products such as bread has been extensively studied in recent years (Dal Bello et al., 2007; Gerez et al., 2009. Russo et al., 2017a; Bartkiene et al., 2018). Antifungal LAB-fermented sourdough provides significant antifungal properties due to the production of various highly active secondary metabolites during fermentation (Messens and De Vuyst, 2002). In our study, we did not use the entirety of the fermented sourdough in breads preparation (30% of the total weight), which could explain the average antifungal effects observed against both *A. tubingensis* and *A. flavus*. This suggest a dilution by the matrix of the antifungal molecules (Ryan et al., 2008). Lavermicocca et al. (2000) reported that using 100% of the sourdough would produce a highly antifungal bread against *A. niger*, while Russo et al. (2017) also showed that use of the totality sourdough fermented for 18 h could provide better antifungal properties and therefore better bread preservation. However, such bread presented some changes in elasticity, color alveolation while an improvement of the aromatic profile was observed. For further investigations, it would be of interest to study the impact of higher percentage of fermented antifungal sourdough for bread preparation. Noteworthy, sourdough bread antifungal activity does not only depend on the amount of fermented sourdough used or the antifungal properties of LAB but could also depend on other factors such as certain synergistic or non-synergistic interactions between starter yeast and the antifungal LAB as suggested by Gobbetti et al. (1994). Indeed, Gobbetti et al. (1994) reported that yeast carbohydrate metabolism could stimulate lactic acid bacteria during sourdough fermentation. This LAB-yeast co-culture could also influence lactic and acetic acid production (Gobbetti et al., 1994) that could influence the antifungal activity.

Beyond the antifungal activity of the selected biopreservation cultures, an important aspect concerns the organoleptic impact; indeed, a really effective antifungal culture that would strongly negatively impact the final product would be of no use. In this study, sensory tests performed by a 30 people untrained panel showed that the produced antifungal sourdough breads were as appreciated as bread without sourdough and biopreservation culture only slightly affected the desirability and the quality of the final product. Similar results were observed for sour creams. It would be of interest to investigate further the effect of sourdough or antifungal cultures addition on bread and sour cream organoleptic properties with a trained panel to better identify differences between products and thus, better explain the results of the hedonic tests.

Concerning the compounds potentially supporting the observed antifungal activity, we determined that the 3 antifungal strains synthesized various fungal molecules. HPLC and LC-Q-ToF analyses also showed that *L. plantarum* CH1 produced higher amounts of compounds common to the 3 selected antifungal strains but also specific compounds, which could very well explain the observed better antifungal activity.

The observed antifungal activity is therefore probably due to the various active molecules that can produce these LAB. Indeed, the various organic acids such as acetic and succinic acids have already been described as being antifungal due to their ability to cause viability loss and cell destruction as suggested in the theory of weak acids (Torino et al., 2001. Orij et al., 2012). In this context, Russo et al. (2017b) showed that *L. plantarum* UFG121 produced lactic and phenyllactic acids which are largely responsible for the antifungal activity against *Aspergillus*, *Penicillium* and *Fusarium* spp. A similar study has shown that a preparation of a sourdough bread fermented with *L. plantarum* CRL778 would increase shelf life thanks to a significant production of acetic and phenyllactic acids (Gerez et al., 2009). Our results also showed that the 3 chosen LAB not only produced these acids but also other hydroxylated compounds such as DL-hydroxyphenyl, 3,4-dihydroxyhydrocinnamic, 3-(4-hydroxyphenyl) propionic, 3-(4-hydroxy-3-methoxyphenyl) propanoic acids which have been described by Pohl et al. (2011) as compounds increasing the acid bioactivity involved in antifungal activity. *L. paracasei* (DGCC2132, DGCC11287, DGCC695) also produced hydroxylated compounds that have been shown to contribute to antifungal activity (Honoré et al., 2016).

In addition, the LC-QToF analyses showed that the selected LAB produced various antifungal fatty acids, such as decanoic acid, but also hydroxylated fatty acids such as 2-hydroxydodecanoic and (S)-(-)-2-hydroxyisocaproic acids which have been described as being able to increase the permeability of the fungal membrane, thus making fungal cells more vulnerable (Sjögren et al., 2003). Moreover, Black et al. (2013) also reported that the hydroxylated fatty acids synthesized by

**Table 4**
Identification and quantification of antifungal compounds produced in sourdough, bread and sour cream by HPLC and LC-QToF analyses.

| Compounds | Sourdough | | | Bread | |
|---|---|---|---|---|---|
| | *L. plantarum* CH1 | *L. mesenteroides* L1 | *L. paracasei* B6 | *L. plantarum* CH1 | *L. mesenteroides* L1 |
| Lactic acid (g/kg) | **12.1 ± 1.61a** | 2.6 ± 0.19b | 10.42 ± 1.35a | **3.28 ± 0.52a** | 1.04 ± 0.18a |
| Acetic acid (g/kg) | 0.13 ± 0.03a | **0.64 ± 0.17b** | 0.03 ± 0.006a | 0.55 ± 0.07a | **0.83 ± 0.12a** |
| Succinic acid (g/kg) | 0.06 ± 0.01a | 0.05 ± 0.01a | **0.14 ± 0.01a** | **0.21 ± 0.03a** | 0.09 ± 0.04a |
| DL-Hydroxyphenyllactic acid (mg/kg) | **12.41 ± 0.8a** | 4.55 ± 0.06b | 4.89 ± 0.16b | **5.29 ± 0.9a** | 4.53 ± 0.03b |
| (S)-(-)-2-Hydroxyisocaproic acid (mg/kg) | **14.48 ± 1.18a** | 4.35 ± 0.66b | 10.18 ± 4.08a | 4.01 ± 1.03a | 3.75 ± 0.17 ab |
| 3,4-Dihydroxyhydrocinnamic acid (mg/kg) | **1.33 ± 0.05** | – | – | **1.33 ± 0.44** | – |
| Vanillic acid (mg/kg) | + | 0.92 ± 0.01a | 1.11 ± 0.14a | 0.88 ± 0.02a | 1.10 ± 0.06a |
| Caffeic acid (mg/kg) | 0.93 ± 0.07a | 0.88 ± 0.01a | **3.86 ± 0.86b** | 0.86 ± 0.01 ab | **2.36 ± 0.08b** |
| Mevalonolactone (mg/kg) | **21.1 ± 1.21** | – | – | **20.43 ± 2.47** | – |
| Phenyllactic acid (mg/kg) | **19.42 ± 1.78a** | 4.43 ± 0.25b | 5.01 ± 0.41 ab | **4.88 ± 1.45a** | 3.88 ± 0.04b |
| 3-(4-Hydroxyphenyl) propionic acid (mg/kg) | **38.58 ± 4.01a** | 5.78 ± 0.56b | 7.09 ± 0.93 ab | **6.81 ± 3.26a** | 4.56 ± 0.09b |
| Phenyl acetate (mg/kg) | **12.48 ± 0.53a** | 10.7 ± 0.62a | 10.91 ± 1.73a | 10.35 ± 1.6a | **11.08 ± 1.33a** |
| 3-(4-Hydroxy-3-methoxyphenyl) propanoic acid (mg/kg) | **5.1 ± 0.55a** | 1.7 ± 0.62b | 0.06 ± 0.002c | **1.74 ± 1.45a** | 0.07 ± 0.003b |
| Benzoic acid (mg/kg) | **0.83 ± 0.68a** | 0.72 ± 0.42b | 0.78 ± 0.03b | **2.22 ± 0.37a** | 1.6 ± 0.17a |
| Azelaic acid (mg/kg) | + | + | + | + | + |
| Methylcinnamic acid (mg/kg) | **0.28 ± 0.09a** | 0.25 ± 0.05a | 0.19 ± 0.01b | **0.2 ± 0.02a** | 0.18 ± 0.006a |
| DL-Hydroxylauric acid (mg/kg) | 2.03 ± 0.01a | 2.02 ± 0.0004b | 2.02 ± 4.03c | 2.02 ± 0.001a | 2.02 ± 0.0007a |
| 2-Hydroxydodecanoic acid (mg/kg) | **4.72 ± 0.03** | – | – | – | – |
| Decanoic acid (mg/kg) | **1.23 ± 0.24a** | 0.09 ± 0.03b | 0.06 ± 0.01b | 0.43 ± 0.02a | 0.14 ± 0.06a |
| DL-Hydroxymyristic acid (mg/kg) | **2.45 ± 0.06a** | 2.41 ± 0.002b | 2.42 ± 0.001b | 2.41 ± 0.003a | 2.42 ± 0.001a |
| Di-*tert*-butylphenol (mg/kg) | + | + | + | + | + |
| D-glucuronic acid (mg/kg) | 4.61 ± 0.004a | 4.8 ± 0.02a | 4.61 ± 0.01a | 4.75 ± 0.11a | 4.69 ± 0.04a |

| Compounds | Bread | Sour cream | | | |
|---|---|---|---|---|---|
| | Control | *L. plantarum* CH1 | *L. paracasei* B20 | *L. paracasei* B6 | Control |
| Lactic acid (g/kg) | 0.03 ± 0.01b | 9.08 ± 1.25a | **9.34 ± 1.10a** | 8.06 ± 2.33a | 5.3 ± 0.26b |
| Acetic acid (g/kg) | 0.6 ± 0.03a | **0.64 ± 0.3a** | 0.48 ± 0.09a | 0.46 ± 0.11a | 0.17 ± 0.02b |
| Succinic acid (g/kg) | 0.03 ± 0.001b | **0.06 ± 0.01a** | 0.04 ± 0.02a | 0.04 ± 0.01a | 0.04 ± 0.007a |
| DL-Hydroxyphenyllactic acid (mg/kg) | 4.22 ± 0.03b | **9.86 ± 0.44a** | 6.15 ± 0.37b | 6.97 ± 0.56b | 5.08 ± 0.24b |
| (S)-(-)-2-Hydroxyisocaproic acid (mg/kg) | 2.79 ± 0.08b | **34.5 ± 3.9 ab** | 22.85 ± 1.02a | 25.24 ± 2.16 ab | 27.9 ± 7.94b |
| 3,4-Dihydroxyhydrocinnamic acid (mg/kg) | – | **0.88 ± 0.18** | – | – | – |
| Vanillic acid (mg/kg) | 0.92 ± 0.12a | 0.84 ± 0.003 | + | + | + |
| Caffeic acid (mg/kg) | 0.59 ± 0.51a | 0.87 ± 0.007a | – | 0.87 ± 0.02a | 0.66 ± 0.44a |
| Mevalonolactone (mg/kg) | – | **22.21 ± 7.12** | – | – | – |
| Phenyllactic acid (mg/kg) | 3.18 ± 0.04b | **7.5 ± 0.43a** | 5.8 ± 0.17a | 6.27 ± 0.27a | 6.42 ± 0.6a |
| 3-(4-Hydroxyphenyl) propionic acid (mg/kg) | 2.97 ± 0.11b | **12.81 ± 0.98 ab** | 8.89 ± 0.39a | 9.93 ± 0.62a | 11.98 ± 3.42b |
| Phenyl acetate (mg/kg) | 10.31 ± 0.21b | 10.2 ± 0.57 ab | 9.86 ± 0.82 ab | **11.3 ± 1.38b** | 10.04 ± 0.22a |
| 3-(4-Hydroxy-3-methoxyphenyl) propanoic acid (mg/kg) | 0.06 ± 0.002b | **0.06 ± 0.001** | – | – | – |
| Benzoic acid (mg/kg) | 1.74 ± 0.65a | 9.58 ± 0.67a | 9.62 ± 0.87a | 8.53 ± 0.37a | **13.17 ± 3.9b** |
| Azelaic acid (mg/kg) | + | + | + | + | + |
| Methylcinnamic acid (mg/kg) | – | 0.18 ± 0.005a | 0.18 ± 0.007a | 0.18 ± 0.007a | 0.18 ± 0.005a |
| DL-Hydroxylauric acid (mg/kg) | 2.02 ± 0.004a | 2.02 ± 0.001a | 2.02 ± 0.001a | 2.02 ± 0.0005b | 2.02 ± 0.001a |
| 2-Hydroxydodecanoic acid (mg/kg) | – | – | – | – | – |
| Decanoic acid (mg/kg) | 0.63 ± 0.16a | 0.31 ± 0.04a | 0.28 ± 0.12 ab | **0.32 ± 0.05 ab** | 0.30 ± 0.17b |
| DL-Hydroxymyristic acid (mg/kg) | 2.41 ± 0.003a | 2.41 ± 0.001a | 2.42 ± 0.001a | 2.41 ± 0.001a | 2.42 ± 0.001a |
| Di-*tert*-butylphenol (mg/kg) | + | + | + | + | + |
| D-glucuronic acid (mg/kg) | 4.69 ± 0.06a | – | – | – | – |

Bold characters correspond to the highest observed in each product; (-) Compounds not detected; (+) Detected compounds but not quantified.
For each matrix, values with different letters in the same line are significantly different by Tukey-HSD test ($P \leq 0.05$).

LAB in sourdough were responsible for the antifungal activity.

Beside all the antifungal compounds identified in our study, some LAB can produce other active metabolites such as peptides that may be involved in the antifungal activity (Leyva-Salas et al., 2017). Dal Bello et al. (2007) showed that the cyclic dipeptides produced by *L. plantarum* FST 1.7 were able to delay the growth of certain molds belonging to the *Fusarium* genus. Gourama and Bullerman (1997) also reported that *L. casei* subsp. *pseudoplantarum* produced a small peptide with antifungal property against *Aspergillus flavus*. Other LAB such as *L. paracasei* can produce volatile compounds with strong antifungal properties such as diacetyl (Aunsbjerg et al., 2015). For further investigations, it would be interesting to evaluate the ability of the selected antifungal strains to produce this type of molecules. In addition, it would be of interest to assess the antifungal activity of water/salt-soluble extracts after an enzymatic, heat and neutralization treatment, to elucidate whether proteinaceous compounds are involved in this antifungal activity and the actual involvement of organic acids.

## 5. Conclusion

The performed study allowed selecting 3 antifungal strains that were tested in actual bakery and dairy products in both challenge and durability tests. *L. plantarum* CH1 was clearly the most antifungal, was active whatever the matrix used and did not alter the sensory characteristics of the final products. It produced more antifungal compounds as compared to the other strains which could explain its

antifungal efficiency. Overall, the obtained data suggest that this LAB strain is a good candidate for a potential industrial application in both fermented milk and wheat flour-based products, although its safety (antibiotic resistance profile and potential biogenic amine biosynthesis) remains to be evaluated as described recently by Coton et al. (2018).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.020.

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