Sourdoughs as a source of lactic acid bacteria and yeasts with technological characteristics useful for improved bakery products

Alessandra Martorana[1] • Angelo Maria Giuffrè[1] • Marco Capocasale[1] • Clotilde Zappia[1] • Rossana Sidari[1]

Rossana Sidari
rossana.sidari@unirc.it

[1]Department of Agraria, *Mediterranea* University of Reggio Calabria, Loc. Feo di Vito, 89122, Reggio Calabria, Italy

Abstract In the present research, lactic acid bacteria (LAB) and yeasts which were isolated from sourdough samples were investigated for their technologically useful properties for the production of improved food products. LAB and yeasts isolates were cultured and the DNA was extracted; restriction analyses were applied to obtain profile groups and representative strains were sequenced. *Lactobacillus sanfranciscensis* was the most isolated species followed by *Lactobacillus namurensis*, *Lactobacillus pentosus*, *Lactobacillus paralimentarius*, *Lactobacillus sakei*, *Lactobacillus crustorum*, *Pediococcus parvulus*, *Leuconostoc citreum*, and *Weissella cibaria*. *Saccharomyces cerevisiae* was the most frequently detected yeast species. Minor yeast species were *Kazachstania humilis* (*Candida milleri*) and *Wickerhamomyces anomalus*. The majority of the LAB strains produced CO_2; after 4 hours of fermentation the two strains of *L. citreum* B435 and B521 reached pH values below 5.00, 19 strains reached values below 4.00 after 24 hours of fermentation, while after 72 hours of fermentation all the strains lowered their pH below 3.60. Two strains, *L. citreum* B435 and *L. sanfranciscensis* B450, produced exopolysaccharides. All the LAB strains were able to degrade gluten with different intensity; the strain of *L. sakei* B433 and the strains of *L. pentosus* B506, B508, and B512 exhibited the highest intensity of degradation. All the yeast strains were able to grow at a pH value of 2.5. *S. cerevisiae* L973 and *W. anomalus* L1081, showed amylolytic properties; excluding the *C. milleri* L999 all the strains were maltose-positive. According to the technological features, LAB and yeasts strains which are thus isolated are potential starters to be used for improved bakery products.

Keywords Sourdough microbiota • Technological characterisation • Starters • ITS-RFLP • ARDRA

Introduction

Consumers are forever careful in their choice of food and they expect to find a wide range of foods produced without the use of preservatives and characterised by good taste, texture, long shelf-life, and functional attributes. In the production of fermented foods, the food industry is interested in new strains which can enhance food quality, thus offering consumers a wider and healthier choice.

There is growing interest in sourdough preparations since the associated microbiota of this type of dough confers positive features - nutritional, organoleptic, texture, shelf-life - to the final products [1–4].

The sourdough microbiota is composed of yeasts and lactic acid bacteria (LAB) in a ratio of 1:100 [5] that co-exist and, throughout back-slopping steps, establish a dynamic equilibrium that determines the sourdough bread peculiarity as well as its prolonged shelf-life.

LAB produced organic acids are mainly lactic and acetic acids - that result in a lower pH - as well as reduced CO_2, ethanol, and aroma compounds. They can also produce bacteriocins and exopolysaccharides [6, 7]. Yeasts primarily produce ethanol, CO_2 - which contributes to the dough leavening [4] - aroma precursors and aroma compounds [8], and contribute to the dough rheology [9].

Lactobacillus sanfranciscensis is the typical lactic acid bacterium isolated from the sourdough and it has a significant role in the sourdough production [10]; also *Lactobacillus brevis* and *Lactobacillus plantarum* [11], *Lactobacillus paralimentarius* [12], and *Lactobacillus mindensis* [13] were reported.

Yeasts such as *Saccharomyces cerevisiae*, *Candida milleri*, *Candida humilis*, *Kazachstania exigua*, *Pichia kudriavzevii*, and *Wickerhamomyces anomalus* can shelter in the sourdough environment, while the most present is *S. cerevisiae* [14–16].

LAB and yeasts can possess technological properties that confer peculiar final characteristics to the sourdough. Important technological characters for LAB are the acidification rate, CO_2 production, starch hydrolysis ability [17–19], exopolysaccharides production [20, 21], and proteolytic activity [22, 23]. In regard to yeasts, the technological characters are the ability to hydrolyse starch [18], the capacity to assimilate carbohydrates such as maltose, glucose, fructose, and sucrose, the tolerance to low pH, and the ability to grow in the presence of acetic acid [24]. These properties are useful parameters to consider in the choice of LAB and yeasts as starters to produce improved products.

Furthermore, LAB and yeasts in sourdough are responsible for the production of non-volatile - including organic acids - and volatile organic compounds (VOCs) such as alcohols, aldehydes,

ketones, and esters that together confer better taste to sourdough bread compared to the other types of bread [6].

The aim of this research was to isolate, identify, and asses the technological properties of the microbiota present in sourdoughs from the Calabria region (Italy) to select the best autochthonous strains to be used as starter for improved bakery products.

Materials and methods

Strains isolation

LAB and yeasts were isolated from ten mature sourdough products sampled immediately prior to the back-slopping (Table 1). Two batches of each sourdough, kindly supplied by artisanal bakeries from the Calabria region (Italy), were collected, stored at 4 °C, and transported to the laboratory for chemical, microbiological, and molecular analyses.

Sourdough samples (10 g) were homogenised in a solution of 0.9% NaCl by a Stomacher (Astori) for 2 min at maximum speed. Then, tenfold dilution were prepared and plated in triplicate onto Petri plates containing: de Man-Rogosa-Sharpe (MRS) agar (VWR) and Sourdough Bacteria (SDB) agar [25] supplemented with 100 mg/L cycloheximide (Oxoid) - to enumerate and isolate LAB - and Yeast Peptone Dextorse (YPD) agar (Amresco) supplemented with 100 mg/L chloramphenicol (Liofilchem Diagnostici) to enumerate and isolate yeasts. LAB and yeasts were incubated at 30 °C for 48 h anaerobically and aerobically, respectively. After enumeration, colonies from each media were randomly selected from the highest dilution plates - to increase the probability of collecting a dominant species [26, 27]; then, the isolates were purified. The presumptive LAB were tested for catalase and for Gram by KOH method [28]. All the purified isolates were stored at – 80 °C by Microbank^{TM} (Pro-Lab Diagnostics).

Reference strains

The microorganisms used in this study as reference strains were: *Lactobacillus plantarum* subsp. *plantarum* LMG 06907^{T} (BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent, Belgium), *Lactobacillus paraplantarum* LMG 16673^{T}, *Lactobacillus pentosus* LMG 10755^{T}, *Lactobacillus sanfranciscensis* LMG 16002^{T}, *Lactobacillus brevis* LMG 07944^{T}, *Lactobacillus buchneri* LMG 06892^{T}, *Lactobacillus fructivorans* LMG 09201^{T}, *Lactobacillus reuterii* LMG 09213^{T}, *Pediococcus pentosaceus* LMG 11488^{T}, *Lactobacillus pontis* LMG 14187^{T}, *Lactobacillus acidophilus* LMG 09433^{T}, and *Pediococcus acidilactici* LMG 11384^{T},

Saccharomyces pastorianus CBS 1538^T (Centraalbureau voor Schimmelcultures, Baarn, The Netherlands), *Candida milleri* CBS 6897^T, *Kazachstania exigua* CBS 379^T, *Saccharomyces bayanus* var. *bayanus* CBS 380^T, *Kluyveromyces lactis* var. *lactis* CBS 683^T, *Torulaspora delbrueckii* CBS 817^T, *Kluyveromyces marxianus* CBS 834^T, *Saccharomyces cerevisiae* CBS 1171^T, *Pichia kudriavzevii* CBS 5147^T, *Candida humilis* CBS 5658^T, *Wickerhamomyces anomalus* CBS 5759^T, and *Pichia terricola* CBS 8131^T.

LAB and yeast restriction analyses

DNA from LAB (157 isolates) and yeasts (154 isolates) was extracted by InstaGene Matrix (Bio-Rad Laboratories) according to the manufacturer's instructions. Then, LAB were analysed by PCR-Y1/Y2 and Amplified Ribosomal DNA Restriction Analysis (ARDRA) of the 16S rRNA gene while yeasts by PCR-ITS and RFLP analysis of the 5.8S ITS rRNA region. The amplification reactions were performed in a MasterCycler Nexux GX2 (Eppendorf). The GeneRuler 100 bp Plus (Thermo Fisher Scientific) and the 50 bp DNA ladder (Biotechrabbit) were used as ladders for PCR-ITS and PCR-Y1/Y2 amplicons and restriction analyses, respectively. The gels, stained with RealSafe Nucleic Acid Staining Solution (0.5 µL/100 mL) (Real) were checked under UV transillumination and documented using the MicroDoc system (Cleaver Scientific).

In detail, the primer Y1 (5'-TGGCTCAGAACGAACGCTGGCGGC-3') corresponds to positions 20 to 43 in the *Escherichia coli* 16S rRNA sequence and Y2 (5'-CCCACTGCTGCCTCCCGTAGGAGT-3') corresponds to *E. coli* positions 361 to 338 were used to amplify the 16S rRNA gene [29]. The reaction mixture (35 µL) contained 10 ng DNA template, 1x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µM of each primer (Thermo Fisher Scientific), and 0.6 U of Taq DNA Recombinant (Biotechrabbit). The amplification program was: initial denaturation at 94 °C for 3 min; 30 cycles of 45 s at 94 °C for denaturing, 45 s at 55 °C for annealing, 1 min at 72 °C for extension, and a final extension step of 7 min at 72 °C [30]. The products were digested with *Alu*I, *Fok*I, and *Hae*III restriction enzymes (Sigma-Aldrich) at 37 °C for 4 h and then analysed on 2.5% (w/v) agarose gels.

The 5.8S ITS rRNA region was amplified and the ITS amplicons were analysed by Restriction Fragment Lenght Polymorphism (RFLP) [31] using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') - amplifying the region that includes the 5.8S rRNA gene and the two non-coding regions designated the internal transcribed spacers (ITS1 and ITS2) - [32, 33] under the following conditions: each 35 µL reaction mixture contained 100 ng DNA template, 1x reaction buffer, 1.5 mM $MgCl_2$, 0.125

mM dNTP mix, 0.25 μM of each primer (Thermo Fisher Scientific), and 1 U of Taq DNA Recombinant (Biotechrabbit). The amplification program was: initial denaturation at 95 °C for 2 min; 35 cycles of 30 s 95 °C for denaturing, annealing for 30 s at 55 °C, extension for 1 min at 72 °C, and a final extension step of 10 min at 72 °C. Each PCR-amplified product was separately digested by *Hae*III, *Hinf*I, and *Cfo*I restriction enzymes (Sigma-Aldrich). Restriction mixtures were incubated at 37 °C for 2 h and then analysed on 2.5% (w/v) agarose gel. LAB and yeasts restriction profiles were compared with reference strain profiles and with those reported in research papers.

16S and 26S D1/D2 sequencing

The ARDRA/RFLP profiles of the isolates from batches of each sourdough sample were confirmed by the analysis of the isolates from the other batches, and then strains which were isolated from each single batch (71 LAB and 84 yeasts) were further analysed.

A representative for each PCR-ARDRA and PCR-RFLP profile was chosen for the sequence analysis of 16S and 26S D1/D2 rRNA regions for LAB and yeasts, respectively.

The 16S amplification was carried out using fD1 (5' CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG3') and rD1 (5' CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC 3') primers (Thermo Fisher Scientific) according to Weisburg et al. [34].

The D1/D2 domain of the 26S rRNA gene was amplified using NL1 (5' GCATATCAATAAGCGGAGGAAAAG 3') and NL4 (5' GGTCCGTGTTTCAAGACGG 3') primers (Thermo Fisher Scientific) according to Kurtzman and Robnett [35].

All the amplified products were purified by Illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare), according to the manufacturer's instructions, and sequenced by Sanger method (Eurofins Genomics). The sequences were compared with those available at NCBI using Blast search tool [36] and submitted to GenBank (https://submit.ncbi.nlm.nih.gov/subs/genbank/) for accession numbers.

LAB and yeasts technological characterisation

LAB (30 strains) and yeasts (21 strains), chosen according to the species and to the sourdough origin, were grown overnight, harvested by centrifugation (5000 rpm for 10 min), washed once in 0.9% NaCl solution and re-suspended to OD_{600} of 1.0 in the same solution. Then, the

204 microorganism suspensions were inoculated in triplicate in either different broths or media
205 according to the tests performed.
206 The tests performed on LAB were the production of CO_2 [19], the acidifying activity [17], the
207 tolerance to 3% NaCl, the starch hydrolysis [19], the exopolysaccharides production [21], and the
208 proteolytic activity on Bovine Serum Albumin (BSA), gelatine [17], gluten, and Gluten Base
209 Medium - GBM [37]. For the gluten test, the BSA and gelatine method was used.
210 The test performed on yeasts analysed their ability to hydrolyse starch [18], to grow in the
211 presence of glucose, fructose, saccharose, maltose, to tolerate pH values of 2.5, 3.5, and 5.0, and to
212 grow in the presence of acetic acid [24].
213
214 **Sourdough chemical characteristics determined by microbiota**
215
216 The pH was determined in three different parts of the samples using a spin electrode pH-meter
217 (HI99161, Hanna Instruments). The total titratable acidity (TTA) was determined in triplicate using
218 0.1 N NaOH to a final pH of 8.5 and the value was expressed as mL of NaOH.
219 The organic acids extraction was carried out in triplicate according to Ventimiglia et al. [38]
220 slightly modified. In particular, 10 g of sourdough were homogenised with 90 mL of distilled water
221 using a bag mixer (Interscience). The obtained mixture was centrifuged at 5000 g for 15 min at
222 room temperature and the supernatant was filtered with 0.45 µm PTFE filter (Supelco). Then, the
223 obtained water/salt-soluble extract was analysed by HPLC equipped with an Acclaim OA 5 µm (4 x
224 250 mm) working at 30 °C and a UV detector operating at 210 nm, with a flow rate of 0.6 mL/min;
225 the isocratic mobile phase was 100 mM Na_2SO_4 acidified to a pH of 2.65 with methansulfonic acid.
226 The quantification was obtained with the external standard method and each compound was
227 expressed as mg/g. The quotient of fermentation (QF) was calculated as the molar ratio between
228 D,L-lactic and acetic acids.
229 The VOCs from sourdough samples were carried out in triplicate. They were extracted by
230 headspace solid-phase micro-extraction (HS-SPME) and analysed using gas chromatography as
231 described by Ripari et al. [39], modified as follows: an aliquot (2 g ± 0.1) of sourdough was placed
232 in a 20 mL vial covered with a septum silicone/PTFE cap. Samples were subjected directly to HS-
233 SPME. The SPME fibre used was 50/30 µm DVB/CAR/PDMS (Supelco). The vial was heated at
234 20 °C for 30 min in a water bath to obtain the stabilisation of the headspace; then, the SPME was
235 placed into the headspace where the extraction lasted for 20 min with heating at 20 °C. Next, the
236 fibre was desorbed in a GC-MS injector at 270 °C for 4 min. GC/MS analysis was carried out using
237 a GC Thermo Trace 1310 apparatus (Waltham) equipped with Single Quadrupole Mass

Spectrometer ISQ LT system and a fused-silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness (Thermo Scientific), TG-5MS 5% phenyl phase. The oven temperature was 30 °C held for 15 min, then from 30°C to 260 °C at 10°C/min, and held isothermally at 260 °C for 5 min. MS transfer line temperature was 270 °C and ion source temperature was 260 °C. Mass range was from 45 to 500 m/z. The samples were injected in the split-less mode, using helium as the carrier gas (1 mL/min). VOCs identification was based on the spectra comparison with those of NIST/EPA/NIH Mass spectral library Version 2.0 and they were expressed as a percentage of the relative peak areas (peak area of each compound/total area) × l00.

Statistical analysis

Data was subjected to the *Least Significant Differences* of Fisher, confidence level of 95%, and Principal Component Analysis (PCA) using StatGraphics Centurion XVI from StatPoint. Biochemical characteristics - pH, TTA, LAB and yeasts cell density, concentrations of organic acids, and concentration of categories of VOCs were used as variables for PCA.

Results

LAB and yeasts count

The microbial loads of the Calabrian sourdough are reported in Table 2. The highest LAB counts both for MRS and SDB media were observed for PF4 while the lowest were reported for PF9 and PF10 on MRS and SDB, respectively. The majority of the sourdough samples had a level of LAB in the range of 10^3–10^9 CFU/g while the range of yeasts were 10^5–10^8. Sourdoughs were statistically different for LAB count in both MRS and SDB media where they were distributed into 4 and 6 homogeneous groups (a homogeneous group defined as a group of means within which there are no statistically significant differences), respectively. Furthermore, the yeasts count in YPD showed significant differences among the samples and they were distributed into 7 homogeneous groups. Seven out of ten sourdough samples exhibited a ratio pro LAB; in the other sourdoughs - PF2, PF6, PF10 - the yeast were more present than LAB.

LAB and yeasts identification

The presumptive LAB was catalase-negative and Gram-positive. Table 3 details the LAB isolates for each sample and their identification. For all the LAB tested, an expected fragment of approximately 360–380 bp was obtained. Ten patterns of ARDRA profiles were observed. The

sequencing analysis of representative of these isolates and of the other LAB that did not match any reference strains or profiles reported in literature established their identity. On a total of 71 LAB isolates, 45 were identified as *L. sanfranciscensis*, 7 as *Pediococcus parvulus*, 6 as *L. pentosus*, 3 as *W. cibaria*, 2 as *Lactobacillus sakei*, 2 as *Leuconostoc citreum*, 2 as *Lactobacillus namurensis*, 1 as *Lactobacillus crustorum*, 2 as *Lactobacillus paralimentarius*, and 1 as *Streptococcus salivarius*. The identity of *L. pentosus* was confirmed by multiplex PCR according to Torriani et al. [40].

The accession numbers of the LAB strains sequenced and deposited to GenBank are: MF504009 *L. sanfranciscensis* B415, MF541648 *L. sakei* B434, MF540541 *L. citreum* B435, MF540542 *P. parvulus* B469, MF540543 *L. crustorum* B481, MF540544 *S. salivarius* B504, MF540545 *W. cibaria* B522, MF567401 *L. namurensis* B501, MF540546 *L. paralimentarius* B503, and MF540547 *L. pentosus* B512.

The 5.8S ITS region amplicons of the yeast reference strains showed, as expected, size diversity (data not shown). Whilst unlikely for the LAB, less diversity was observed for the ITS region and for the restriction profiles of the yeast isolated from the sourdough samples (Table 4). Three restriction patterns were obtained. In particular, the majority of sourdough samples sheltered *S. cerevisiae*; in PF9 and PF10 were detected strains of *Kazachstania humilis* (synonims in Blast *C. milleri*, *C. humilis*) and *W. anomalus*, respectively. Of a total of 84 yeast isolates, 78 were identified as *S. cerevisiae*, 4 as *Kazachstania humilis* or *C. milleri* according to the *Hae*III restriction profile as reported by Pulvirenti et al. [41], and 2 as *W. anomalus*. The sequencing analysis of representative strains confirmed the identification reported above. The accession numbers of the yeast strains sequenced and deposited to GenBank are: MF498873 *S. cerevisiae* L1018, MF526974 *K. humilis* (*C. milleri*) L999, and MF526975 *W. anomalus* L1081.

LAB and yeasts technological features

Twenty LAB strains out of thirty produced CO_2 and only two were not able to grow in the presence of 3% of NaCl.

With regard to the LAB acidification activity, Fig. 1 reports the pH values measured at 2 h intervals for the first 8 h of incubation, and then after 24, 48, and 72 h from the inoculum (Fig. 1a) and the strains grouped as class frequency according to their acidification activity (Fig. 1b). After 4 h, the strains B521 and B435 exhibited a pH value below 5.00. After 6 h, 8 strains (B433, B434, B435, B504, B506, B508, B512, and B521) had a pH values below 4.70. At 24 h, 19 strains (B423, B425, B433, B434, B435, B442, B445, B450, B457, B462, B472, B504, B506, B508, B512, B521, B522, B555, and B556) exhibited a pH in the range 3.51–3.92 with the minimum value for the

strain B435. All the pH values were below 3.60 after 72 h of fermentation with the minimum 3.21 for the strain B506 and the maximum 3.56 for the strain B425.

All the strains tested were able to grow on the media supplied with starch. While growth in the presence of different carbohydrates and the related production of exopolysaccharides, 6 (B423, B433, B434, B435, B450, B504), 3 (B434, B435, B450), and 2 (B415, B417) strains exhibited good biomass growth on the media with glucose, saccharose, and maltose, respectively; a medium biomass growth was observed for 17 (B506, B508, B415, B417, B425, B442, B445, B457, B462, B463, B469, B472, B479, B480, B489, B493; B500), 21 (B415, B417, B425, B433, B442, B445, B457, B462, B463, B469, B472, B479, B480, B489, B493, B500, B504, B506, B508, B521, B522), 22 (B423, B425, B433, B434, B435, B442, B445, B450, B457, B462, B463, B469, B472, B479, B480, B489, B493, B500, B504, B506, B508, B512), and 8 (B433, B434, B450, B469, B472, B504, B506, B508) strains in the media with glucose, saccharose, maltose, and lactose, respectively. Moreover, in the presence of saccharose the strains B435 and B450 exhibited a highly mucous colony, while the strains B462, B469, B472, and B504 showed lightly mucous properties. The strains B450, B457, B469, B472, and B500 - on media with maltose - together with the strains B469, B472 - on media with lactose - showed lightly mucous properties.

With reference to the proteolytic activity (Table 5), the LAB strains tested demonstrated different behaviour according to the media used. They showed two possible visible responses on Petri plates: the presence of footprint visualised after staining the plates was often coupled with the presence of a halo surrounding the footprint. According to the clear footprint left on the BSA medium, 15 strains exhibited high proteolytic activity, 12 strains medium activity, and 3 strains low proteolytic activity. Thirteen out of 30 strains, other than footprint, showed a halo surrounding the footprint with high, medium, and low intensity. On the media supplemented with gelatine, 8, 16, and 6 strains showed high, medium, and low activity, respectively, considering their footprint. On MRS/SDB supplemented with gluten, the footprints of the strains highlighted their diversity; in particular, 12, 14, and 4 strains showed high, medium, and low degrading activity, respectively. On GBM medium, 10 strains exhibited their ability to degrade gluten showing only footprint or footprint coupled with halo.

In relation to the yeasts, all the strains assimilated glucose and saccharose; only two strains did not grow in the presence of fructose while only one strain - the L999 - was maltose-negative. Two strains were amylase-positive. Moreover, except for one strain, the others grew in the presence of acetic acid. All the strains were able to tolerate low pH values (Table 6).

Sourdough chemical profiles

The pH and the TTA of the sourdough samples are reported in Table S1 (in the Online Resources 1). The pH ranged from 3.77 (PF3) to 5.59 (PF2) with a mean value of 4.53. Sourdough PF1, PF3, PF4, PF8, and PF10 exhibit lower pH (3.77–3.98) than the others. For seven out of ten sourdough samples the TTA values confirms the pH values. Among the samples there are significant differences both for pH - 4 homogeneous groups - and TTA - 6 homogeneous groups.

Organic acids concentration, expressed as mg/g, and the QF of the 10 sourdough samples are reported in Table S2 (in Online Resources 2). The lactic and acetic acid concentration ranged from 0.18 (PF6) to 10.42 (PF1) and from 0.07 (PF4) to 1.70 (PF10). Regarding the QF, the lowest value was reported for the PF2 while the highest for the PF4. Six samples had a QF above 4.00. For tartaric acid, 5 out of 10 sourdough samples exhibited a higher concentration. Malic and succinic acids were detected in low concentration. The sourdough samples exhibited significant differences for organic acids; in particular, they were distributed in 5, 7, 5, 3, and 7 homogeneous groups for acetic, lactic, tartaric, malic, and succinic acids, respectively.

Table S3 (in Online Resources 3) reports the VOCs characterising the sourdough samples. The analysis revealed 33 compounds: 18 esters, 8 alcohols, 3 hydrocarbons, 2 aldehydes, and 2 ketones. Ethanol, ranging from 20.84% (PF5) to 58.30% (PF4); ethyl acetate, ranging from 9.04% (PF4) to 44.70% (PF8); 3-methyl-1-butanol, ranging from 7.00% (PF3) to 28.14% (PF7); 3-methyl-1-butanol acetate, ranging from 1.39% (PF1) to 7.99% (PF3); phenyl ethyl alcohol, ranging from 0.03% (PF9) to 2.47% (PF2); and octanoic acid ethyl ester, ranging from 0.06% (PF10) to 3.37% (PF6) were the VOCs present in all the samples and in high content. VOCs present in less concentration and not characterising all the sourdough samples were 1-hexanol, acetoin, ethyl lactate, and hexanoic acid ethyl ester. The sourdough samples exhibited significant differences and they are distributed into 2 homogeneous groups (propionic acid ethyl ester, 1-pentanol, 2-3 butanediol, 1-butanal-3-methyl propionate, 3-octanon, para cymene, decanoic acid ethyl ester, 2-methyl butyl acetate, pentanoic acid ethyl ester) to 8 (ethyl acetate).

Multivariate analysis

PCA allowed a visualisation of the effect of different variables on the distribution of the samples studied (Fig. 2). The criterion of selecting the number of principal components to extract was to select components for which the eigen-values were at least 1.00. Five principal components were extracted showing that the variables explain 88.27% of the total variance with the first three accounting for 38.46%, 19.34%, and 13.62%, respectively. The first component was weighted most

heavily in a positive direction for TTA, MRS, SDB, esters, aldehydes, malic and succinic acids; the second component was weighted most heavily in a positive direction for YPD, MRS, alcohols, malic and succinic acids, while the third component for pH, MRS, SDB, alcohols, ketones, hydrocarbons, tartaric and lactic acids. Moreover, the figure shows the distribution of the samples as a function of the three components. Component 1 discriminates the PF1, PF3, PF5, PF7, PF8, and PF9; component 2 the PF1, PF2, PF3, PF7, and PF8, while component 3 the PF2, PF4, PF5, PF6, and PF7.

Discussion

The selection of novel strains of LAB and yeasts with properties which are useful to producing improved products for their organoleptic properties and which can possibly provide additional functional benefits is a great challenge.

We tested 30 LAB and 21 yeast strains which were isolated from the samples because of their useful characteristics for fermented bakery products and other fermented food production.

The LAB densities were similar to those reported in research papers [38, 42] whilst the yeast densities were similar to results reported by Valmorri et al. [16] and Yağmur et al. [42]. We isolated mainly *L. sanfranciscensis* - 100% of the LAB population of PF1 and PF3, and 57.14%, 20%, 88.88%, 83.33%, 50%, and 50% of the population of PF2, PF4, PF5, PF8, PF9, and PF10, respectively. The almost dominant presence of these species is in accordance with research on sourdough [42, 43]. Furthermore, *W. cibaria* (PF6, 66.66%), *L. namurensis* (PF7, 50%) were detected as major species. Other minor species detected were *L. paralimentarius*, *L. sakei*, *L. crustorum*, *P. parvulus*, and *L. citreum*. All these species detected are in agreement with results previously reported [18, 43].

Regarding the yeasts, we isolated mainly *S. cerevisiae*; this finding is in agreement with previous research [18, 44] and confirms the high competitiveness of *S. cerevisiae* in wheat flour sourdough [45] and the possible contamination of the bakery environment with commercial bakers' yeast [24]. In reality, this explanation could fit with bakeries involved in this study since they produce other types of bread, other than sourdough bread, that shelters *S. cereivisiae*. Other species isolated were *K. humilis* (*C. milleri*) (PF9) and *W. anomalus* (PF10).

Sourdough is a very interesting source of microorganism variability, in terms of species and the technological characteristics of the strains, connected to various factors - among which the geographical origin. LAB and yeasts possessing interesting technological properties should be used as starter [21, 46, 47].

This study permits the selection of autochthonous strains with interesting technological features. As regards to the yeasts, we selected the strains *S. cerevisiae* L973 and *W. anomalus* L1081 which are able to hydrolyse starch. This is of applicative interest as the amylase activity produces maltose from the damaged grain of starch which is used as a fermentable source. Moreover, these can be used as a source of amylases that find application in many sectors; such as the food, paper, detergent, medical, and pharmaceutical industries [48, 49].

The majority of the yeast strains were able to use maltose. The utilisation of various carbohydrates is important for the fermentation process; in particular, of great applicative significance is the use of maltose which is the most available fermentable substrate following the depletion of glucose. Such strains possessing maltase activity guarantee fermentation continuity and, therefore, an appropriate leavening of the fermented products. All the selected yeast strains tolerated low pH values, and this feature could be used in high acidic food preparation.

The selected LAB strains gave rapid fermentation with CO_2 production - determining leavening and influencing yeast metabolism and thus yeast-leavening ability [50]. The high acidification activity after the first hours of fermentation is desired as it acts on bread structure [51] and has a positive effect on gluten, starch and endogenous enzymes [52]. Among the strains tested, two strains of *L. citreum* B435 and B521 were able to reach pH values below 5.00 after 4 hours of fermentation; consequently, within the pH parameter they could be useful in bakery fermentation. All the LAB strains selected showed various rate of proteolysis and were able to degrade gluten with different intensity - having an impact on nutritional and health aspects [2] since they can be used to produce functional food for celiac consumers.

Two strains, *L. citreum* B435 and *L. sanfranciscensis* B450, produced exopolysaccharides - having a pre-biotic role and effect on bread structure [2, 53]. Therefore, these strains can be used in food technology to replace hydrocolloids and plant polysaccharides used as texturing, anti-staling, or pre-biotic additives.

The majority of the LAB strains tested showed the ability to grow in the presence of NaCl as a percentage of 3%, this value was reported as the limit by local bakeries. The NaCl has an impact in improving flavour, acting on gluten and, due to the action on amylase, supplies yeasts with maltose [54]. The NaCl is also a factor to consider given its role in controlling the rapidity of microorganism fermentation and the CO_2 production [55]. In fact, the lack of NaCl determines a high rapidity of CO_2 production which is detrimental to the product structure.

The LAB and the yeasts which were isolated and tested for various properties have the potential to be used as a multiple-starter. Obviously, strains coming from different sourdough microbiotas could outcompete each other when used in a new formulation. It could be that a

LAB/yeast strain exhibiting useful characteristics when used as a single starter modifies its behaviour positively or negatively when used in combination with other strains. Therefore, strain compatibility tests must be performed to obtain a multiple-starter system composed of strains able to exhibit their desired traits when in co-existence.

Concerning sourdoughs' chemical characteristics, the microbiota determines the decrease in pH and the rise in TTA. The pH values are in agreement with those previously reported [15, 42] and the majority of them showed a correlation with the TTA; therefore, and consistent with other research, there is not always a correlation between pH and TTA among sourdoughs [16, 56].

In the sourdough fermentation, lactic acid is produced by homofermentative and heterofermentative LAB and acetic acid by both heterofermentative LAB and yeasts [1]. In our sourdoughs, lactic acid was the organic acid present in greater quantity, with some exception regarding the tartaric acid. For the majority of the sourdough samples, the concentration of lactic acid was similar to values reported by Valmorri et al. [16]. Similar values both for lactic and acetic acids were reported by Ventimiglia et al. [38]. Concerning the QF, the value of some sourdough was higher than the 1.50–4.00 range and resulted positively for both bread aroma and structure [57] indicating low acetic acid concentration compared to lactic acid concentration; this could be due to a major presence of homofermentative and facultative heterofermentative LAB. Values lower than 4.00 were observed in sourdoughs such as PF2, PF6, PF7 that harbour obligate heterofermentative species such as *L. sanfranciscensis,* while values higher than 4.00 were found in samples such as PF4 harbouring obligate homofermentative specie such as *P. parvulus* and PF8 and PF9 harbouring facultative heterofermentative specie such as *L. paralimentarius* and *L. pentosus*. However, the lactic acid determines a more elastic gluten structure [18]. Other research has reported high QF values of up to 8.00 [47], 14.50 [15], 17.70 [58], and 18.47 [42]. Also in this case, the wide range of QF observed is due to the natural variability of the microbiota characterising the sourdough environment. The other organic acids, deriving from diverse microorganism metabolic pathways, showed similar values among the sourdough samples with some limited exceptions. Generally, tartaric acid gave the major contribution contrarily to succinic acid. This latter was found in little quantity as previously reported by Scheirlinck et al. [43] for Belgian sourdoughs.

A volatile organic compounds profile of a sourdough is the effect of different microbiological, enzymatic and chemical reactions during fermentation. The microorganism present in the sourdough determined the complex aroma profile and organic acids, alcohols, esters, and carbonyl compounds which gave the main contribution [59]. They, primarily yeasts, produced a high content of ethanol. Yeasts [60] and LAB [61] were responsible for the presence of 3-methyl-1-butanol, and LAB for the ethyl acetate [60]. These findings are in agreement with those reported for Belgian and

Italian sourdoughs [43, 62]. Compounds detected in minor concentration were previously reported by Scheirlinck et al. [43] - 3-methyl-1-butanol acetate, and by Settanni et al. [62] - phenyl ethyl alcohol, 1-hexanol, and 3-methyl-1-butanol acetate.

Taking into account the results of the PCA analysis, LAB and yeasts present in the PF1, PF3, PF7, and PF8 characterize these sourdough samples mainly for esters, aldehydes, alcohols, and malic and succinic acids; those present in the PF2 characterise this sample mainly for alcohols, ketons, hydrocarbons, and organic acids; those present in the PF4 and PF6 characterise these sourdough samples mainly for alcohols, ketons, hydrocarbons, and tartaric and lactic acids; those present in the PF5 characterise this sample for esters, aldehydes, alcohols, ketons, hydrocarbons, and organic acids; those present in the PF9 characterise this sample mainly for esters, aldehydes, and malic and succinic acids.

The LAB and yeasts isolated from the 10 sourdough samples produce a variety of compounds that confer aroma to the final products. Furthermore, they showed some technological properties tested here qualitatively that need to be verified in experimental bakery preparation. It would also be of interest to evaluate the compatibility of the different strains deriving from the various sourdoughs and demonstrating those characteristics which would be useful for bakery fermentation when used in combination. Future research will be carried out to further investigate the usefulness of the strains here selected in order to use them in novel preparations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This research does not contain any studies with human or animal subjects.

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Figure captions

Fig. 1 pH determined by the lactic acid bacteria strains isolated from the 10 sourdough samples. (a) kinetic of acidification; (b) class frequency of the strains according to their acidification activity

Fig. 2 Biplot resulting from PCA of the variables determined on the 10 sourdough samples

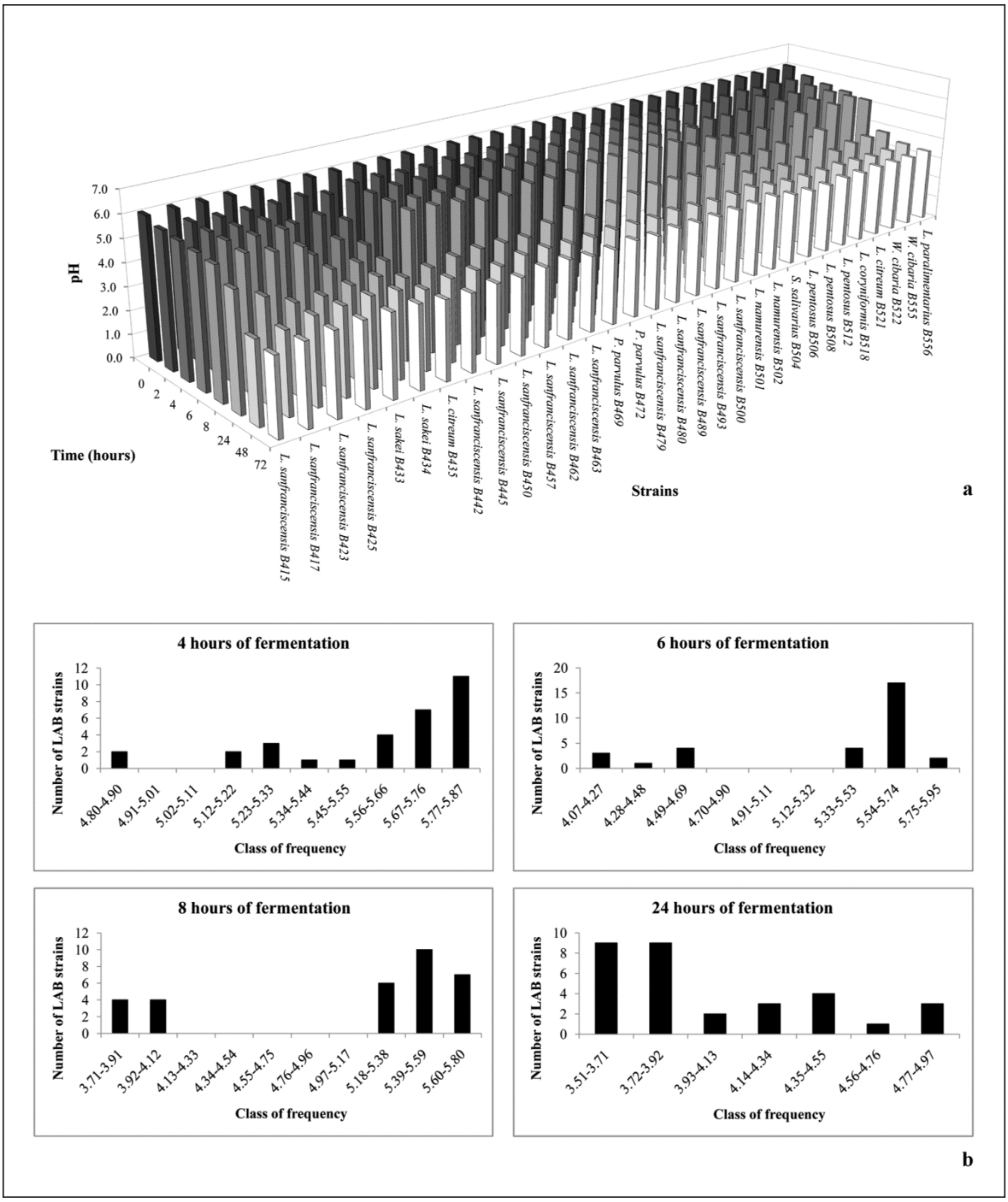
pH
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Time (hours)
0
2
4
6
8
24
48
72
Strains
L. sanfranciscensis B415
L. sanfranciscensis B417
L. sanfranciscensis B423
L. sanfranciscensis B425
L. sakei B433
L. sakei B434
L. citreum B435
L. sanfranciscensis B442
L. sanfranciscensis B445
L. sanfranciscensis B450
L. sanfranciscensis B457
L. sanfranciscensis B462
L. sanfranciscensis B463
P. parvulus B469
P. parvulus B472
L. sanfranciscensis B479
L. sanfranciscensis B480
L. sanfranciscensis B489
L. sanfranciscensis B493
L. sanfranciscensis B500
L. namurensis B501
L. namurensis B502
S. salivarius B504
L. pentosus B506
L. pentosus B508
L. pentosus B512
L. coryniformis B518
L. citreum B521
W. cibaria B522
W. cibaria B555
L. paracalimentarius B556
a
4 hours of fermentation
Number of LAB strains
Class of frequency
4.80-4.90
4.91-5.01
5.02-5.11
5.12-5.22
5.23-5.33
5.34-5.44
5.45-5.55
5.56-5.66
5.67-5.76
5.77-5.87
6 hours of fermentation
4.07-4.27
4.28-4.48
4.49-4.69
4.70-4.90
4.91-5.11
5.12-5.32
5.33-5.53
5.54-5.74
5.75-5.95
8 hours of fermentation
3.71-3.91
3.92-4.12
4.13-4.33
4.34-4.54
4.55-4.75
4.76-4.96
4.97-5.17
5.18-5.38
5.39-5.59
5.60-5.80
24 hours of fermentation
3.51-3.71
3.72-3.92
3.93-4.13
4.14-4.34
4.35-4.55
4.56-4.76
4.77-4.97
b

Fig.1

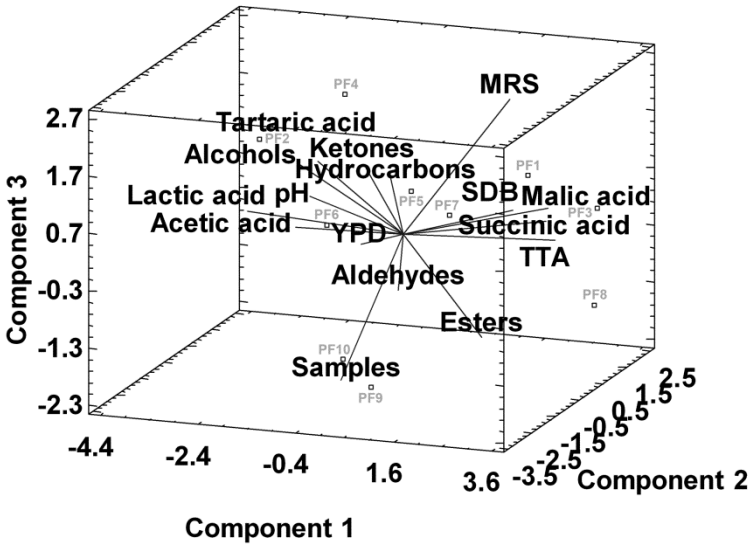
MRS
Tartaric acid
Alcohols
Ketones
Hydrocarbons
Lactic acid pH
Acetic acid
SDB
Malic acid
Succinic acid
YPD
TTA
Aldehydes
Esters
Samples
PF1
PF2
PF3
PF4
PF5
PF6
PF7
PF8
PF9
PF10
Component 3
2.7
1.7
0.7
-0.3
-1.3
-2.3
Component 1
-4.4
-2.4
-0.4
1.6
3.6
Component 2
2.5
1.5
0.5
-1.5
-2.5
-3.5

Fig. 2

727 **Table 1 Sourdough samples used to isolate LAB and yeasts**

Sourdoughs	Typical name	Flour	Bakeries
PF1	Pane tradizionale	Reground durum wheat semolina	Panificio S. Antonio - Simeri Crichi (CZ)[a]
PF2	Pitta	Soft wheat flour	Panificio Il fornaretto - Catanzaro Lido (CZ)
PF3	Pane tradizionale	Durum wheat semolina	Panificio Il fornaretto - Catanzaro Lido (CZ)
PF4	Pane tradizionale	Durum wheat semolina	Panificio Il forno a legna - Fortuna (CZ)
PF5	Pane tradizionale	Soft wheat flour	Panificio L'antico sapore (RC)
PF6	Pane tradizionale	Soft wheat flour	Panificio Gramuglia - Pellegrina di Bagnara Calabra (RC)
PF7	Pane tradizionale	Soft wheat flour and whole	Colacchio Food - San Costantino Calabro (VV)
PF8	Pane tradizionale	Soft wheat flour	Panificio S. Filippo - Favelloni (VV)
PF9	Pane tradizionale	Soft wheat flour	Panificio La Scala Salvatore - Arzona di Filandari (VV)
PF10	Pane tradizionale	Reground durum wheat semolina	Panificio Circosta - Gioiosa Ionica (RC)

[a]CZ: Catanzaro; RC: Reggio Calabria; VV: Vibo Valentia

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734 **Table 2** Means, standard deviations, and homogeneous groups of microbial loads of the 10 sourdough samples

Sourdoughs	**Microbial loads (Log CFU/g)**		
	MRS	SDB	YPD
PF1	8.9 ± 0.7^{b}	7.9 ± 0.7^{cde}	7.6 ± 0.7^{a}
PF2	6.5 ± 0.1^{a}	6.6 ± 0.2^{ab}	8.3 ± 0.1^{f}
PF3	8.6 ± 0.2^{a}	9.1 ± 0.1^{e}	7.4 ± 0.0^{g}
PF4	9.0 ± 0.1^{c}	9.0 ± 0.1^{de}	7.5 ± 0.1^{h}
PF5	8.7 ± 0.3^{d}	8.8 ± 2.4^{bc}	5.4 ± 0.1^{g}
PF6	6.3 ± 0.1^{a}	6.5 ± 0.5^{a}	6.7 ± 1.8^{ab}
PF7	7.9 ± 1.3^{b}	7.9 ± 0.4^{cd}	8.0 ± 0.0^{de}
PF8	8.9 ± 0.4^{b}	9.1 ± 5.4^{e}	7.1 ± 0.1^{e}
PF9	3.3 ± 0.4^{a}	9.2 ± 0.1^{f}	6.9 ± 1.4^{bcd}
PF10	3.6 ± 0.1^{a}	6.3 ± 0.4^{a}	7.9 ± 1.1^{cd}

MRS: de Man-Rogosa-Sharpe agar; SDB: Sourdough Bacteria agar; YPD: Yeast Peptone Dextrose agar

Superscript letters indicate the homogeneous groups (*Least Significant Difference* of Fisher, confidence level of 95 %)

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742 **Table 3** LAB species isolated from sourdough samples, restriction profiles, representative strains sequenced (in italic),

743 and percentage of similarity by Blast

Sourdoughs	ARDRA profile	Species	Strains	% similarity[a] (accession no. of the closest relative by Blast)
PF1	A	*Lactobacillus sanfranciscensis*	From *B415* to B419; from B422 to B426	99 (CP002461)
PF2	A	*Lactobacillus sanfranciscensis*	B442, B444, B445, B446	
	B	*Lactobacillus sakei*	B433, *B434*	99 (CP025839)
	C	*Leuconostoc citreum*	*B435*	99 (CP024929)
PF3	A	*Lactobacillus sanfranciscensis*	B450, B451, B452, B455, B457; from B459 to B463	
PF4	A	*Lactobacillus sanfranciscensis*	B479, B480	
	D	*Pediococcus parvulus*	From *B469* to B473; B482, B483	99 (AB601176)
	E	*Lactobacillus crustorum*	*B481*	99 (AM285453)
PF5	A	*Lactobacillus sanfranciscensis*	From B489 to B493; from B498 to B500	
	F	*Streptococcus salivarius*	*B504*	99 (CP015283)
PF6	C	*Leuconostoc citreum*	B521	
	G	*Weissella cibaria*	*B522*, B558	99 (CP020928)
PF7	H	*Lactobacillus namurensis*	*B501*, B502	100 (KX649189)
	I	*Lactobacillus paralimentarius*	*B503*	99 (NR114844)
	G	*Weissella cibaria*	B555	
PF8	A	*Lactobacillus sanfranciscensis*	B505, B523, B551, B554, B559	
	I	*Lactobacillus paralimentarius*	B556	
PF9	A	*Lactobacillus sanfranciscensis*	B552, B553, B557	
	L	*Lactobacillus pentosus*	B506, B507, B508	

PF10	A	*Lactobacillus sanfranciscensis*	B509, B510, B511	
	L	*Lactobacillus pentosus*	*B512*, B513, B514	99 (CP022130)

Restriction fragment (ARDRA):

A - *HaeIII*: 130+230; *Alu*I: 50+90+210; *Fok*I: 120+250

B - *HaeIII*: 320; *Alu*I: 80+300; *Fok*I: 120+250

C - *HaeIII*: 300; *Alu*I: 350; *Fok*I: 350

D - *HaeIII*: 320; *Alu*I: 100+180; *Fok*I: 120+250

E - *HaeIII*: n.d.; *Alu*I: n.d; *Fok*I: n.d.

F - *HaeIII*: 300; *Alu*I: 80; *Fok*I: 100+160

G - *HaeIII*: 340; *Alu*I: 70+310; *Fok*I: 380

H - *HaeIII*: 340; *Alu*I: 120+260; *Fok*I: 120+260

I - *HaeIII*: 310; *Alu*I: 80+100+180; *Fok*I: 120+240

L - *Hae*III: 315; *Alu*I: 120+260; *Fok*I: 120+260

[a]Data refers to the strains reported in italic

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753 **Table 4** Yeasts species isolated from sourdough samples, restriction fragments, representative strains sequenced (in italic), and percentage of

754 similarity by Blast

Sourdoughs	RFPL profile	Species	Strains	% similarity[a] (accession no. of the closest relative by Blast)
PF1	A	*Saccharomyces cerevisiae*	From L1014 to *L1018*; from L1019 to L1023	99 (KY109409)
PF2	A	*Saccharomyces cerevisiae*	From L1026 to L1035; L1062, L1063, L1068	
PF3	A	*Saccharomyces cerevisiae*	From L1036 to L1043; L1045, L1064, L1065, L1069	
PF4	A	*Saccharomyces cerevisiae*	From L1046 to L1055; L1066, L1067, L1071, L1072, L1073, L1074	
PF5	A	*Saccharomyces cerevisiae*	From L1056 to L1061	
PF6	A	*Saccharomyces cerevisiae*	L948, L950, L953, L955, L988, L1024, L1025	
PF7	A	*Saccharomyces cerevisiae*	L958, L962, L967, L992, L993	
PF8	A	*Saccharomyces cerevisiae*	L968, L973, L975, L995, L996	
PF9	B	*Kazachstania humilis* (*Candida milleri*)	L985, L986, *L999*, L1076	99 (FJ468468)
PF10	A	*Saccharomyces cerevisiae*	L1080, L1085, L1087, L1089	
	C	*Wickerhamomyces anomalus*	*L1081*, L1084	99 (JX049429)

Restriction fragment length polymorphism (RFLP) fragment:

A - *HaeIII*: 130+160+230+330; *Hinf*I: 145+355; *Cfo*I: 140+340+360

B - *HaeIII*: 230+430; *Hinf*I: 280+350; *Cfo*I: 180+250

C - *HaeIII*: 600; *Hinf*I: 300; *Cfo*I: 550

[a]Data refers to the strains reported in italic

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Table 5 Proteolytic activity of the LAB strains isolated from the 10 sourdough samples

Sourdoughs	LAB strains	Proteolytic activity			
		BSA (footprint/halo)	Gelatine	Gluten	GBM (footprint/halo)
PF1	*Lactobacillus sanfranciscensis* B415	+++/-	+	+++	-/-
	Lactobacillus sanfranciscensis B417	++/-	+	+++	-/-
	Lactobacillus sanfranciscensis B423	++++/+++	++	+++	-/-
	Lactobacillus sanfranciscensis B425	++/-	++	+++	-/-
PF2	*Lactobacillus sakei* B433	++++/-	++++	+++++	-/-
	Lactobacillus sakei B434	++++/-	+++	++++	-/-
	Leuconostoc citreum B435	++++/-	++++	++++	+/-
	Lactobacillus sanfranciscensis B442	++++/+	+++	+++	-/-
	Lactobacillus sanfranciscensis B445	++++/++	+++	+++	-/-
PF3	*Lactobacillus sanfranciscensis* B450	+++/-	++	+++	++/-
	Lactobacillus sanfranciscensis B457	+++/-	+	++++	-/-
	Lactobacillus sanfranciscensis B462	++++/++	+++	+++	-/-
	Lactobacillus sanfranciscensis B463	+++/+++	+++	+++	-/-
PF4	*Pediococcus parvulus* B469	++/-	+	++++	-/-
	Pediococcus parvulus B472	++/-	+	++++	-/-
	Lactobacillus sanfranciscensis B479	++++/+++	++	+	-/-
	Lactobacillus sanfranciscensis B480	++++/++	+++	+	-/-
PF5	*Lactobacillus sanfranciscensis* B489	++++/++	+++	+++	-/-
	Lactobacillus sanfranciscensis B493	++++/+++	+++	+++	-/-
	Lactobacillus sanfranciscensis B500	+++/-	+++	++++	-/-
	Streptococcus salivarius B504	+++/-	+++	++	++++/+++
PF6	*Leuconostoc citreum* B521	++++/-	+++++	++++	+/-
	Weissella cibaria B522	++/-	+++	+	-/-
PF7	*Lactobacillus namurensis* B501	+/-	+	++++	-/-
	Lactobacillus namurensis B502	+/-	++	++	-/-

	Weissella cibaria B555	++/-	++++	+	+/-
PF8	*Lactobacillus paralimentarius* B556	+/+	+++++	+++	+/-
PF9	*Lactobacillus pentosus* B506	+++++/+	+++++	+++++	+/-
	Lactobacillus pentosus B508	+++++/+	+++++	+++++	+/-
PF10	*Lactobacillus pentosus* B512	+++++/+	+++++	+++++	+/-

BSA: Bovine Serum Albumine; GMB: Gluten Base Medium; +++++,++++: high; +++,++: medium; +: low; -: none

773 **Table 6** Starch hydrolysis, carbohydrates and acetic acid assimilation, and growth with low pH of the yeasts strains tested

Sourdoughs	**Yeast strains**	**Technological tests**								
		Starch hydrolysis	Carbohydrates assimilation				Tolerance to low pH			Growth with acetic acid
			Glucose	Fructose	Saccharose	Maltose	2.5	3.5	5.0	
PF1	*Saccharomyces cerevisiae* L1014	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1018	-	+	-	+	+	+	+	+	+
	Saccharomyces cerevisiae L1023	-	+	-	+	+	+	+	+	+
PF2	*Saccharomyces cerevisiae* L1026	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1031	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1063	-	+	+	+	+	+	+	+	+
PF3	*Saccharomyces cerevisiae* L1037	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1040	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1042	-	+	+	+	+	+	+	+	+
PF4	*Saccharomyces cerevisiae* L1046	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1049	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1071	-	+	+	+	+	+	+	+	+
PF5	*Saccharomyces cerevisiae* L1057	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1059	-	+	+	+	+	+	+	+	+
	Saccharomyces cerevisiae L1061	-	+	+	+	+	+	+	+	+
PF6	*Saccharomyces cerevisiae* L955	-	+	+	+	+	+	+	+	+
PF7	*Saccharomyces cerevisiae* L993	-	+	+	+	+	+	+	+	+
PF8	*Saccharomyces cerevisiae* L973	+	+	+	+	+	+	+	+	+
PF9	*Candida milleri* L999	-	+	+	+	-	+	+	+	+
PF10	*Saccharomyces cerevisiae* L1080	-	+	+	+	+	+	+	+	+
	Wickerhamomyces anomalus	++	+	+	+	+	+	+	+	-

L1081

+: growth; -: no growth

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Table S1 Means, standard deviations, and homogeneous groups of pH and total titratable acidity (TTA) of the 10 sourdough samples

Sourdoughs	pH	TTA
PF1	3.93 ± 0.06^{abc}	9.60 ± 0.07^{f}
PF2	5.59 ± 0.03^{f}	2.00 ± 0.14^{ab}
PF3	3.77 ± 0.04^{a}	10.80 ± 0.21^{g}
PF4	3.96 ± 0.05^{abc}	7.10 ± 0.14^{de}
PF5	4.59 ± 0.27^{d}	6.10 ± 0.28^{cd}
PF6	5.55 ± 0.02^{f}	2.80 ± 0.14^{b}
PF7	5.40 ± 0.06^{f}	5.25 ± 0.57^{c}
PF8	3.98 ± 0.01^{bc}	10.30 ± 0.57^{fg}
PF9	4.06 ± 0.05^{c}	7.65 ± 0.92^{e}
PF10	3.86 ± 0.01^{ab}	5.50 ± 0.85^{c}

Table S2 Organic acids characterising the 10 sourdough samples

Sourdoughs	Tartaric acid (mg/g)	Malic acid (mg/g)	Succinic acid (mg/g)	Lactic acid (mg/g)	Acetic acid (mg/g)	QF
PF1	2.84±0.100^{e}	0.19±0.001^{a}	0.13±0.008^{de}	10.42±0.372^{g}	1.20±0.062^{d}	5.80
PF2	1.30±0.058^{c}	0.48±0.040^{c}	0.35±0.041^{f}	0.26±0.029^{a}	0.54±0.060^{b}	0.32
PF3	1.95±0.043^{d}	0.13±0.000^{a}	0.07±0.001^{abc}	7.73±0.207^{e}	1.12±0.044^{d}	4.61
PF4	0.92±0.024^{b}	0.15±0.004^{a}	0.11±0.002^{cde}	6.83±0.258^{d}	0.07±0.001^{a}	63.16
PF5	1.81±0.103^{d}	0.19±0.010^{a}	0.13±0.003^{de}	6.69±0.360^{cd}	0.48±0.103^{b}	9.29
PF6	0.55±0.080^{a}	0.35±0.090^{bc}	0.11±0.050^{e}	0.18±0.050^{a}	0.09±0.030^{a}	1.32
PF7	0.62±0.022^{a}	0.34±0.065^{b}	0.45±0.018^{g}	0.36±0.009^{a}	0.40±0.017^{b}	0.60
PF8	1.22±0.042^{c}	0.12±0.004^{a}	0.04±0.002^{ab}	6.29±0.072^{c}	0.73±0.044^{c}	5.72
PF9	0.97±0.040^{b}	0.13±0.009^{a}	0.03±0.029^{a}	4.60±0.280^{b}	0.42±0.033^{b}	7.30
PF10	0.91±0.083^{b}	0.14±0.013^{a}	0.08±0.009^{bcd}	8.75±0.243^{f}	1.70±0.139^{e}	3.40

Table S3 Volatile organic compounds characterising the 10 sourdough samples

Chemical compounds	Sourdoughs									
	PF 1	PF 2	PF 3	PF 4	PF 5	PF 6	PF 7	PF 8	PF 9	PF 10
Ethanol	46.38±3.02^{e}	55.13±0.18^{f}	39.86±1.24^{d}	58.30±1.28^{g}	20.84±0.29^{a}	39.45±0.20^{d}	32.34±1.75^{c}	29.58±1.33^{b}	22.68±1.70^{a}	38.54±1.25^{d}
Ethyl acetate	9.60±0.11^{a}	14.31±0.97^{b}	37.21±0.32^{f}	9.04±0.10^{a}	35.13±0.60^{e}	26.52±0.70^{c}	27.82±0.31^{d}	44.7±1.07^{h}	42.19±0.08^{g}	42.85±1.61^{g}
Isobutyl alcohol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Propionic acid ethyl ester	n.d.	n.d.	n.d.	n.d.	n.d.	0.33±0.01^{a}	1.40±0.15^{b}	0.29±0.09^{a}	n.d.	0.34±0.02^{a}
Acetoin	n.d.	n.d.	n.d.	2.78±.01^{b}	4.22±0.10^{c}	n.d.	n.d.	n.d.	n.d.	1.14±0.08^{a}
3-methyl-1-butanol	9.46±1.07^{b}	20.77±1.88^{e}	7.00±0.29^{a}	16.75±0.76^{d}	11.69±0.35^{c}	21.51±1.13^{e}	28.14±2.41^{f}	7.25±0.26^{a}	7.26±0.21^{a}	12.14±0.31^{c}
1-pentanol	n.d.	n.d.	n.d.	n.d.	0.78±0.01^{a}	n.d.	n.d.	n.d.	1.14±0.22^{b}	n.d.
Isobutyl acetate	n.d.	0.29±0.11^{b}	n.d.	n.d.	n.d.	0.38±0.11^{b}	0.53±0.02^{c}	0.17±0.01^{a}	0.47±0.06^{c}	0.33±0.03^{b}
2-3 butanediol	n.d.	n.d.	n.d.	n.d.	n.d.	0.17±0.09^{a}	n.d.	n.d.	n.d.	0.40±0.02^{b}
Butanoic acid ethyl ester	n.d.	0.24±0.02^{a}	n.d.	n.d.	n.d.	0.51±0.14^{b}	0.70±0.31^{c}	0.10±0.02^{a}	1.94±0.03^{d}	n.d.
Ethyl lactate	2.30±0.10^{d}	n.d.	2.97±0.38^{e}	1.65±0.12^{c}	2.32±0.18^{d}	0.81±0.09^{b}	0.29±0.04^{a}	7.29±0.67^{g}	5.33±0.41^{f}	n.d.
1-hexanol	0.74±0.12^{ab}	0.39±0.12^{a}	1.73±0.14^{b}	1.24±0.05^{ab}	4.54±0.14^{c}	0.68±0.16^{a}	0.39±0.16^{a}	1.72±0.03^{b}	13.50±1.85^{d}	0.75±0.09^{ab}
3-methyl-1-butanol acetate	1.39±0.03^{a}	1.57±0.52^{ab}	7.99±1.19^{f}	4.01±0.47^{de}	2.70±0.20^{bc}	1.94±0.79^{abc}	2.04±1.62^{abc}	4.26±0.34^{e}	2.90±0.19^{cd}	2.43±0.10^{abc}
2-methyl butyl acetate	n.d.	0.44±0.17^{a}	n.d.	n.d.	n.d.	0.58±0.20^{a}	2.35±1.43^{b}	n.d.	n.d.	n.d.
Pentanoic acid ethyl ester	n.d.	n.d.	n.d.	n.d.	n.d.	0.12±0.05^{a}	0.51±0.32^{b}	0.07±0.01^{a}	n.d.	0.06±0.01^{a}
Pinene alpha	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13±0.09^{b}	n.d.	0.02±0.01^{a}	n.d.
Acetic acid penthyl ester	n.d.	n.d.	n.d.	0.04±0.01^{a}	0.24±0.04^{b}	n.d.	n.d.	n.d.	0.24±0.02^{b}	0.46±0.02^{c}
1-pentene	n.d.	n.d.	n.d.	0.58±0.03^{c}	0.47±0.02^{b}	n.d.	n.d.	n.d.	n.d.	0.26±0.03^{a}
1-butanal-3-methyl propionate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05±0.02^{a}	n.d.	0.08±0.03^{b}	n.d.
Heptanal	n.d.	n.d.	n.d.	n.d.	n.d.	0.10±0.05^{ab}	0.08±0.02^{a}	0.13±0.01^{b}	0.35±0.01^{c}	n.d.
1-octen-3-ol	0.06±0.02^{a}	n.d.	0.22±0.04^{d}	n.d.	0.20±0.02^{cd}	0.12±0.02^{b}	0.07±0.02^{a}	0.17±0.01^{c}	0.29±0.02^{e}	n.d.
3-octanon	n.d.	n.d.	0.24±0.04^{a}	0.26±0.01^{a}	0.46±0.06^{b}	n.d.	n.d.	n.d.	n.d.	n.d.
2-pentyl furan	n.d.	n.d.	n.d.	0.07±0.02^{a}	0.71±0.03^{d}	n.d.	n.d.	0.16±0.05^{b}	0.38±0.01^{c}	n.d.

Hexanoic acid ethyl ester	n.d.	1.87±0.58^{d}	0.52±0.07^{a}	2.11±0.21^{d}	1.43±0.04^{c}	1.40±0.34^{c}	0.92±0.07^{b}	1.34±0.20^{c}	0.23±0.01^{a}	n.d.
Acetic acid ethyl ester	n.d.	n.d.	0.17±0.02^{b}	0.11±0.01^{a}	0.27±0.01^{c}	n.d.	n.d.	n.d.	n.d.	n.d.
Para cymene	n.d.	n.d.	0.03±0.01^{a}	0.06±0.02^{a}	n.d.	0.30±0.15^{b}	0.09±0.01^{a}	n.d.	0.03±0.01^{a}	n.d.
Isoamyl lactate	0.09±0.01^{ab}	n.d.	0.24±0.02^{d}	0.10±0.01^{b}	0.47±0.02^{e}	n.d.	n.d.	0.07±0.01^{a}	0.22±0.03^{c}	0.08±0.01^{ab}
Eptanoic acid ethyl ester	n.d.	n.d.	n.d.	0.17±0.02^{b}	n.d.	0.28±0.08^{c}	0.08±0.00^{a}	0.17±0.02^{b}	n.d.	n.d.
Phenyl ethyl alcohol	1.01±0.05^{c}	2.47±0.46^{e}	1.47±0.07^{d}	1.51±0.10^{d}	0.6±0.01^{b}	1.43±0.32^{d}	1.25±0.01^{cd}	0.27±0.03^{a}	0.03±0.01^{a}	0.10±0.01^{a}
Octanoic acid ethyl ester	0.38±0.05^{ab}	1.81±0.56^{e}	0.28±0.04^{a}	1.05±0.02^{d}	0.67±0.04^{bc}	3.37±0.08^{f}	0.81±0.02^{cd}	2.07±0.19^{e}	0.11±0.02^{a}	0.06±0.01^{a}
Nonanoic acid ethyl ester	0.22±0.04^{d}	0.09±0.02^{bc}	0.04±0.01^{a}	0.07±0.01^{b}	0.10±0.01^{c}	n.d.	n.d.	n.d.	n.d.	0.03±0.01^{a}
Decanoic acid ethyl ester	0.05±0.01^{a}	0.59±0.14^{b}	0.03±0.02^{a}	0.10±0.01^{a}	0.08±0.01^{a}	n.d.	n.d.	n.d.	n.d.	0.05±0.02^{a}

n.d. Not determined