CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Archivio istituzionale della ricerca - Università di Palermo

Food Microbiology 51 (2015) 57–68

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Codominance of *Lactobacillus plantarum* and obligate heterofermentative lactic acid bacteria during sourdough fermentation

Giusi Ventimiglia [a], Antonio Alfonzo [a], Paola Galluzzo [b], Onofrio Corona [a], Nicola Francesca [a], Santo Caracappa [b], Giancarlo Moschetti [a], Luca Settanni [a, *]

[a] Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy
[b] Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via G. Marinuzzi 4, Palermo, Italy

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form
4 February 2015
Accepted 8 April 2015
Available online 7 May 2015

Keywords:
Bacterial codominance
Lactic acid bacteria
Lactobacillus plantarum
Sourdough
Starter selection

ABSTRACT

Fifteen sourdoughs produced in western Sicily (southern Italy) were analysed by classical methods for their chemico-physical characteristics and the levels of lactic acid bacteria (LAB). pH and total titratable acidity (TTA) were mostly in the range commonly reported for similar products produced in Italy, but the fermentation quotient (FQ) of the majority of samples was above 4.0, due to the low concentration of acetic acid estimated by high performance liquid chromatography (HPLC). Specific counts of LAB showed levels higher than 10^8 CFU g^{-1} for many samples. The colonies representing various morphologies were isolated and, after the differentiation based on phenotypic characteristics, divided into 10 groups. The most numerous group was composed of facultative heterofermentative isolates, indicating a relevance of this bacterial group during fermentation. The genetic analysis by randomly amplified polymorphic DNA (RAPD)-PCR, 16S rRNA gene sequencing and species-specific PCRs identified 33 strains as *Lactobacillus plantarum*, *Lactobacillus curvatus* and *Lactobacillus graminis*. Due to the consistent presence of *L. plantarum*, it was concluded that this species codominates with obligate heterofermentative LAB in sourdough production in this geographical area. In order to evaluate the performances at the basis of their fitness, the 29 *L. plantarum* strains were investigated for several technological traits. Twelve cultures showed good acidifying abilities *in vitro* and *L. plantarum* PON100148 produced the highest concentrations of organic acids. Eleven strains were positive for extracellular protease activity. Bacteriocin-like inhibitory substances (BLIS) production and antifungal activity was scored positive for several strains, included *L. plantarum* PON100148 which was selected as starter for experimental sourdough production. The characteristics of the sourdoughs and the resulting breads indicated that the best productions were obtained in presence of *L. plantarum* PON100148.
© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Fermented cereals have been important for humans since ancient times (Spicher, 1999; De Vuyst and Neysens, 2005). Sourdough breads were part of the European diet 5000 years ago (Währen, 1985). The technology based on sourdough is still applied to produce several kind of breads and other baked goods, both at artisanal and industrial level, throughout the world (Corsetti and Settanni, 2007). Sourdough bread production is typical for the European countries, the San Francisco bay and southern America (De Vuyst and Neysen, 2005), but it became common even for the southern-eastern Asian countries (Luangsakul et al., 2009; Zhang et al., 2011) that have not been traditional bread consumers.

Basically, sourdough is made of a mixture of cereal flour and water in which a heterogeneous population of mainly lactic acid bacteria (LAB) and yeasts is metabolically active, either by spontaneous fermentation or by fermentation initiated through the addition of a sourdough starter culture, whether or not involving backslopping (De Vuyst et al., 2009). Unlike other fermented foods, where LAB responsible for the transformation of raw materials into final products belong to the obligate homofermentative (OHo) and/or facultative heterofermentative (FHe) species, typical sourdough

* Corresponding author. Tel.: +39 091 23896043; fax: +39 091 6515531.
E-mail address: luca.settanni@unipa.it (L. Settanni).

http://dx.doi.org/10.1016/j.fm.2015.04.011
0740-0020/© 2015 Elsevier Ltd. All rights reserved.

LAB mainly belong to the genus *Lactobacillus* and include obligately and facultatively heterofermentative and OHo species (Hammes and Vogel, 1995). However, obligately heterofermentative (OHe) lactobacilli, such as *Lactobacillus sanfranciscensis*, are reported as the most important LAB (Gobbetti and Corsetti, 1997), especially in type I sourdough obtained through continuous daily refreshments at 30 °C (Böcker et al., 1995).

In general, besides *L. sanfranciscensis*, the dominating LAB species in traditional sourdoughs are *Lactobacillus brevis* and *Lactobacillus plantarum* (Corsetti and Settanni, 2007; Iacumin et al., 2009; Robert et al., 2009; Scheirlinck et al., 2009). Gobbetti (1998) reported on the *L. sanfranciscensis*/*L. plantarum* association in Italian wheat sourdough, and Scheirlinck et al. (2008, 2009) found that the species *L. paralimentarius*, *L. plantarum*, *L. sanfranciscensis*, *Lactobacillus pontis* and *Lactobacillus spicheri* were dominant in type I sourdoughs produced in a Belgian bakery. Recently, *L. plantarum* has been detected at high cell densities, even higher than those of the OHe LAB species, also in Asian sourdoughs (Luangsakul et al., 2009; Zhang et al., 2011), highlighting the relevance of this species to produce sourdoughs throughout the world.

As being characterized by a FHe metabolism, *L. plantarum* ferment hexose carbohydrates to lactic acid through glycolysis because of the presence of a constitutive fructose-1,6-diphosphate aldolase (key enzyme of glycolysis), while ferment pentoses to lactic and acetic acid through the inducible 6-phosphogluconate/phosphoketolase pathway (Axelsson, 1998). Hence, the dominance of *L. plantarum* does not ensure the same acetic acid production due to the species for which the enzyme phosphoketolase is constitutive.

L. plantarum in sourdough production is not only important for the generation of acids, but also for several other positive effects expressed during fermentation. *L. plantarum* has been reported as inhibitor of rope-producing *B. subtilis* (Pepe et al., 2003; Menteş et al., 2007). It is able to produce bacteriocins, namely plantaricin (Todorov et al., 1999), and antifungal compounds (Lavermicocca et al., 2000; Magnusson et al., 2003; Sjögren et al., 2003; Ryan et al., 2008).

The growing interest towards gluten-free products intensified the studies on the application of LAB to improve the quality of products for people affected by the celiac sprue. At this proposal, *L. plantarum* and *L. sanfranciscensis* strains showed that the corresponding gluten-free sourdoughs became significantly softer during 24 h of fermentation compared to the chemically acidified control (Moore et al., 2007). Furthermore, *L. plantarum* was found to reduce the antinutritional factors of cereals and might contribute to the conversion of toxic compounds responsible for the celiac sprue (Settanni and Moschetti, 2010).

In this study, the presence and the role of *L. plantarum* in Sicilian sourdoughs was investigated through an integrated microbiological/technological approach.

2. Materials and methods

2.1. Sample collection

Fifteen sourdough samples (Table 1) were collected from bakeries located within three bordering provinces (Agrigento, Palermo and Trapani) of western Sicily (southern Italy). Sourdoughs were sampled before refreshment, transferred into sterile Stomacher bags and placed into a portable fridge. Sample collection occurred twice from each bakery at an interval of 15 days. The samples were kept refrigerated until analyses were performed.

2.2. Chemical analyses

Sourdough pH was determined electrometrically using the pH meter BASIC 20+ (Crison Instrument S.A., Barcelona, Spain).

Total titratable acidity (TTA) was determined by titration with 0.1 N NaOH (expressed in terms of mL of NaOH) on 5 g of each sample.

Lactic and acetic acid concentration were determined on 10 g of sourdough homogenised with 90 mL distilled H_2O with a Sorvall Omni-Mixer (Dupont Instruments, Newtown, CT). Ten millilitres were added with 5 mL of 1 mmol L^{-1} $HClO_4$ solution. The mixtures were centrifuged at 4.000 × g for 15 min at 15 °C and the supernatants were acidified to pH 3.0 ± 0.1 with 1 mmol L^{-1} $HClO_4$ and brought to the final volume of 25 mL with distilled H_2O. The solutions were left in ice for 30 min and filtered through 0.45 μm cellulose filters (Millipore). HPLC analyses were conducted as reported by Alfonzo et al. (2013). PerkinElmer software specific to the HPLC instrument (TotalChrom Workstation 2008 rev. 6.3.2) was used to acquire and process data. Analyses were carried out in triplicate and the results expressed as means ± standard deviation.

2.3. Microbiological analyses

Each sample (15 g) was suspended in Ringer's solution (135 mL) (Sigma–Aldrich, Milan, Italy), homogenised in a stomacher

Table 1
Sourdoughs collected in western Sicily.

Sourdough sample	Bakery (production level)	City of production	Type of flour (company)
F1	La Piana della Bontà (industrial)	Piana degli Albanesi (PA)	Durum wheat semolina (Società Landro Di Manata Santo & C. Snc, Misilmeri – PA)
F8	Pellitteri Caterina (industrial)	Castronovo di Sicilia (PA)	Durum wheat semolina (Molini Gattuso, Castronovo di Sicilia – PA)
F9	Loria Rosa (artisanal)	Castronovo di Sicilia (PA)	Durum wheat semolina (Molini Gattuso, Castronovo di Sicilia – PA)
F13	F.lli Parrino S.N.C. (industrial)	Piana degli Albanesi (PA)	Durum wheat semolina (Società Landro Di Manata Santo & C. Snc, Misilmeri – PA)
F15	Carbone Domenica (industrial)	Piana degli Albanesi (PA)	Durum wheat semolina (Società Landro Di Manata Santo & C. Snc, Misilmeri – PA)
F21	F.lli Gangi (industrial)	Roccapalumba (PA)	Durum wheat semolina (Molisud s.r.l., Giarratana – RG)
F25	La Palerma Giuseppe (industrial)	Cammarata (AG)	Durum wheat semolina (Molino Ancona, San Giovanni Gemini – AG)
F27	Il Fornaio (industrial)	Balata di Baida (TP)	Durum wheat semolina (Molino Siragusa S.N.C., Prizzi – PA)
F32	L'arte Del Pane S.A.S. (industrial)	Marsala (TP)	Durum wheat semolina (Molini Grillo & C. S.a.S., Marsala – TP)
F36	Don Bosco Di Giarratana Nicola S.N.C. (industrial)	Cammarata (AG)	Durum wheat semolina (Molino Ancona, San Giovanni Gemini – AG)
F37	Russo Francesco (industrial)	San Giovanni Gemini (AG)	Durum wheat semolina (Molino Ancona, San Giovanni Gemini – AG)
F38	Cutrò Carmelo (industrial)	San Giovanni Gemini (AG)	Durum wheat semolina (Molino Ancona, San Giovanni Gemini – AG)
F54	La Piana della Bontà (industrial)	Piana degli Albanesi (PA)	Durum wheat semolina (Salvia, Partinico – PA)
F55	La Piana della Bontà (industrial)	Piana degli Albanesi (PA)	Soft wheat flour (Molitoria San Paolo, Siracusa)
F59	La Bottega del Pane (industrial)	Castelvetrano (TP)	Tumminia wheat flour (Molini Del Ponte S.R.L., Castelvetrano – TP)

(BagMixer® 400; Interscience, Saint Nom, France) for 2 min at maximum speed, and then serially diluted. Decimal dilutions were plated and incubated as follows: on MRS, pour plated and incubated anaerobically at 30 °C for 48 h, for rod LAB; on M17, pour plated and incubated anaerobically at 30 °C for 48 h, for coccus LAB; on Sour Dough Bacteria (SDB) (Kline and Sugihara, 1971), spread plated and incubated aerobically at 30 °C for 48 h, for sourdough LAB; on Wallerstein laboratory (WL) nutrient agar, spread plated and incubated aerobically at 28 °C for 72 h, for total yeasts. To avoid fungal growth, cycloheximide (10 mg mL^{-1}) was added to MRS, M17 and SDB. All media and chemical components were purchased from Oxoid (Milan, Italy). Plates counts were performed in triplicate.

2.4. LAB isolation and phenotypic grouping

The different presumptive LAB were collected (at least 4 colonies characterized by the same colour, morphology, edge, surface and elevation) from the highest plated dilution following their growth on MRS, M17 and SDB. After Gram determination (Gregersen KOH method) and catalase test (determined by transferring fresh colonies from an agar medium to a glass slide and adding 5% H_2O_2), the Gram-positive cultures incapable of converting H_2O_2 to H_2O and O_2 were transferred into the corresponding broth media. The isolates were then purified with successive sub-culturing and stored at −80 °C in broth containing 20% (v/v) glycerol.

The presumptive LAB were initially subjected to a phenotypic grouping based on cell morphology type, determined by an optical microscope, growth at 15 and 45 °C and metabolism type, testing the ability to produce CO_2 from glucose. The final assay was carried out with the same growth media used for isolation, without citrate because its fermentation by certain LAB can result in gas formation. Furthermore, M17 contained glucose in place of lactose, and SDB was prepared with glucose instead of maltose. Cells of pure cultures, grown overnight in the corresponding optimal media, were centrifuged at 5000 × g for 5 min, washed twice in physiological solution (NaCl 0.85%, w/v) and re-suspended in the same solution till reaching an optical density at 600 nm (OD_{600}), measured with a 6400 Spectrophotometer (Jenway Ltd., Felsted, Dunmow, UK), of ca. 1.00 which approximately corresponds to a concentration of 10^9 CFU mL^{-1} as evaluated by plate count. LAB were inoculated (1%, v/v) into test tubes, sealed with H_2O agar (2%, w/v) and, after incubation for 48 h, the test was scored positive for CO_2 production (OHe metabolism) if a rising of the agar cap was detected. LAB strains that scored negative in the assay were inoculated into test tubes containing the optimal growth media prepared with a mixture of pentose carbohydrates (xylose, arabinose, and ribose; 8 g L^{-1} each) in place of glucose. Strains that grew in this media were defined as having a FHe metabolism, and strains that were unable to grow were defined as having an OHo metabolism. For coccus isolates, sub-grouping also included the examination of growth at pH 9.6 and in the presence of 6.5% (w/v) NaCl.

2.5. Genetic typing and identification of FHe LAB

All FHe isolates were genetically characterized. Genomic DNA for PCR assays was prepared from LAB cultures after their overnight growth in broth media at 30 °C. Cells were harvested, subjected to DNA extraction using the InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol, and the crude cell extracts were used as templates for PCR.

Strain differentiation was performed with random amplification of polymorphic DNA (RAPD)-PCR analysis in a 25 μL reaction mix using the single primers M13, AB111, and AB106 as previously described by Settanni et al. (2012). Resultant PCR products were separated by electrophoresis on a 2% (w/v) agarose gel (Gibco BRL, Cergy Pontoise, France), stained with SYBR® Safe DNA gel stain (Molecular Probes, Eugene, OR, USA), and subsequently visualised by UV transillumination. The GeneRuler 100 bp Plus DNA Ladder (M-Medical S.r.l, Milan, Italy) was used as a molecular size marker. RAPD patterns were analysed using Gelcompar II software, version 6.5 (Applied-Maths, Sint-Martens-Latem, Belgium).

Genotypic characterisation of the different strains of FHe LAB was carried with 16S rRNA gene sequencing. Some isolates representative for the phenotypic groups of OHe LAB, including at least 1 isolate per sourdough, were also identified at species level. PCR reactions were performed as described by Weisburg et al. (1991). PCR products were visualised and amplicons of approximately 1600 bp were purified with a GFX PCR DNA and gel band purification kit (GE Healthcare, Buckinghamshire, UK). DNA fragments were sequenced by the Big Dye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies, Monza, Italy) and the resulting products were purified by Illustra Autoseq G-50 Dye terminator Removal kit (GE Healthcare) and analysed by ABI3130 Genetic Analyzer (Life Technologies). The sequences were compared with those available in the GenBank/EMBL/DDBJ (http://www.ncbi.nlm.nih.gov) (Altschul et al., 1997) and EzTaxon-e (http://eztaxon-e.ezbiocloud.net/) (Chun et al., 2007) databases. The last database compares a given sequence to those of type strains only. All strains belonging to the *L. plantarum* group were subjected to the *recA* gene based multiplex PCR described by Torriani et al. (2001) to distinguish unequivocally among *L. plantarum*, *Lactobacillus paraplantarum* and *Lactobacillus pentosus*. The strains of *Lactobacillus curvatus* were confirmed by the species-specific PCR assay of Berthier and Ehrlich (1998). *L. brevis*, *L. sanfranciscensis* and *Lactobacillus rossiae* were verified by the multiplex PCR strategy reported by Settanni et al. (2005a). *Weissella cibaria* and *Weissella confusa* were discriminated with a species-specific PCR as reported by Fusco et al. (2011).

2.6. Evaluation of the technological aptitudes of FHe LAB

2.6.1. Acidification capacity

The kinetics of acidification of LAB were evaluated *in vitro* using a sterile flour extract (SFE) broth prepared as described by Alfonzo et al. (2013). Overnight LAB cultures were harvested by centrifugation at 5000 × *g* for 5 min, washed with Ringer's solution, and, to standardise bacterial inocula, resuspended in the same solution to an OD_{600} of 1.00. LAB were individually inoculated (1%, v/v) in 20 mL of SFE and the test tubes incubated for 24 h at 30 °C. pH was measured at 2 h intervals for the first 8 h of incubation, and then at 24, 48, and 72 h after inoculation.

2.6.2. Proteolytic activity

The extracellular protease activity of LAB was determined on agar plates, with the method described by Vermelho et al. (1996). Bovine serum albumin (BSA) and gelatine (Sigma–Aldrich) were used as protease substrates, incorporated into each medium at 1% (w/v).

2.6.3. Antimicrobial activity

Lactobacillus sakei LMG2313, *Listeria innocua* 4202, and *Listeria monocytogenes* ATCC 19114, that are reported as strains highly sensitive to bacteriocins (Corsetti et al., 2008; Hartnett et al., 2002), were used as indicators for the determination of the antibacterial activity of sourdough LAB. Inhibitory activity was first evaluated with the agar-spot deferred method, and strains displaying antimicrobial properties were further subjected to the well diffusion assay (WDA) (Schillinger and Lücke, 1989), with the method modifications described by Corsetti et al. (2008). All tests were carried out in triplicate. Active supernatants were treated with

proteinase K (12.5 U mg^{-1}), protease B (45 U mg^{-1}), and trypsin (10.6 U mg^{-1}), each diluted to 1 mg mL^{-1} in phosphate buffer (pH 7.0), for 2 h at 37 °C, following which any remaining activity was quantified with the WDA (Settanni et al., 2005b). Enzymes were purchased from Sigma–Aldrich.

The antifungal activity of LAB was evaluated against *Alternaria alternata* Ra56, *Aspergillus carbonarius* Sc13, *Aspergillus ochraceus* Ra49, *Cladosporium cladosporioides* Sc71, *Penicillium roquefortii* R1 and *Penicillium verrucosum* Sc29 using the method described by Villani et al. (1993), with the modifications reported by Alfonzo et al. (2012).

2.7. Production of experimental sourdoughs

Among LAB, one strain showing the best technological performances and one strain characterized by the worst aptitudes in bread making were selected to be used in sourdough production, alone or in combination with *L. sanfranciscensis* LMG 17498^T. Cells were centrifuged and washed as described above and re-suspended in Ringer's solution. The inocula were added to a final concentration of approximately 10^6 CFU g^{-1} in doughs. Each dough of 200 g was produced following the protocol reported by Settanni et al. (2013) with a dough yield (weight of the dough/weight of the flour × 100) of 160 adding 75 mL of tap H_2O, containing the cell suspension, to 125 g of commercial durum wheat flour (Molino Gaspare Salvia, Partinico, Italy). A dough without LAB inocula was used as control. An additional control dough (CY) was produced with 1% (w/w) of fresh baker's yeast (La Parisienne, AB Mauri Italy S.p.A., Casteggio, Italy) composed of *Saccharomyces cerevisiae* cells.

Each dough was divided in two portions: one portion of 80 g was placed in a stainless steel circular baking pan (10 cm diameter) covered with aluminium foil to be baked after fermentation performed at 30 °C for 8 h for all doughs, except CY fermented at 25 °C for 2 h; the other portion of 120 g was placed in a sterile plastic beaker covered with parafilm and incubated at 30 °C for 21 h.

Sourdough productions were carried out in duplicate over two consecutive weeks.

2.8. Analysis of experimental sourdoughs

Sourdough fermentation was followed by pH, TTA, lactic and acetic acid production, microbiological analyses conducted as reported above. In addition, volatile organic compound (VOC) generation was also estimated for experimental sourdoughs.

Determinations of pH and TTA were carried out just after dough preparation (T_0), at 2 h intervals for the first 8 h and then again after 21 h. Microbial counts were performed at T_0, at 8 and 21 h of fermentation on MRS and SDB. Colonies from the highest dilution of sample suspensions were isolated, microscopically inspected and analysed by RAPD analysis to confirm the presence of the LAB added as starter cultures.

The concentrations of organic acids and VOC were estimated at T_0 and after 8 h from production. VOCs within the experimental sourdoughs were identified using the solid phase micro extraction (SPME) isolation technique applying the conditions reported by Settanni et al. (2013) with the following modifications: 5 g of each sourdough were heated to 60 °C, during which the headspace was collected with DBV-Carboxen-PDMS fibres (Supelco, Bellefonte, PA) for 40 min. The SPME fibre was directly inserted into a Finnegan Trace MS for GC/MS (Agilent 6890 Series GC system, Agilent 5973 Net Work Mass Selective Detector; Milan, Italy) equipped with a DB-WAX capillary column (Agilent Technologies; 30 m, 0.250 mm i.d., film thickness 0.25 μm, part no 122-7032). Individual peaks were identified by comparing their retention indices to those of control samples and by comparing their mass spectra with those within the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d, build 2005). VOCs were expressed as relative peak areas (peak area of each compound/total area) × l00. All solvents and reagents were purchased from WWR International (Milan, Italy). Chemical and physical tests were performed in triplicate, with the results expressed as mean ± standard deviation.

2.9. Analyses of experimental breads

Sourdoughs were baked in the industrial convection oven Modular 80012 DH (Tornati Forno S.r.l., Montelabbate, Italy), at 218 °C for 20 min. Bread weight and the other attributes were evaluated after cooling at ambient temperature. The height of the breads was evaluated after cutting each sample transversely in two halves and measuring the central slice (Schober et al., 2005). Colour was determined on three points of the crumb of the central slices and five points of the crust by means of a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan) measuring L*, a* and b* parameters of the Hunter's scale.

The hardness of crumb was determined as reported by Corsetti et al. (2000) by means of the Instron-5564 (Instrom Corp., Canton, MA).

The image analysis of the breads was conducted on the two central slices of each sample. The slices were scanned (Epson HP-4535, Seiko Epson Corp., Japan) with 350 dpi of resolution and the images were saved in TIFF format. Each image, analysed with the ImageJ software (National Institutes Health, Bethesda, Md, USA), was cropped to a square of 207 × 207 pixels (representing 15 × 15 mm of the slice area) and converted to grey-level image (8 bit). The Otsu's threshold algorithm was applied to obtain a binary image to calculate void fraction (the fraction of the total area corresponding to the bread pores), cell density (number of cells/cm^2) and mean cell area in mm^2.

2.10. Statistical analyses

Data of acidification, TTA, microbial load and organic acid concentration of sourdoughs and height, colour, firmness, void fraction, cell density and mean cell area of the resulting breads were statistically analysed using the ANOVA procedure with the software STATISTICA, version 10 StatSoft Inc., Tulsa, OK, USA). The *post-hoc* Tukey method was applied for pairwise comparison. Significance level was $P < 0.05$.

3. Results

3.1. Sourdough characteristics

The main chemical and microbiological characteristics of the sourdoughs collected in western Sicily are reported in Table 2. pH ranged between 3.81 and 4.77, with a majority of samples showing values below 4.50. TTA values increased inversely with pH. The HPLC analyses revealed different concentrations of lactic and acetic acid among sourdough samples, with sample F36 showing the highest concentration of lactic acid. However, the concentration of acetic acid in sample F36 was quite low and, as a consequence, it was characterized by almost the highest value of FQ. Acetic acid did not exceed 1.36 mg g^{-1} and FQ was in the range 0.90–6.80. Nine samples displayed a FQ above 4.00, indicating a low concentration of acetic acid with respect to lactic acid.

The highest counts were reported on SDB for 10 samples and on MRS for five samples, while only the sample F27 showed the highest cell density on M17. The levels of sourdough LAB estimated on SDB were above 10^7 CFU g^{-1} for almost all samples except F54 that showed concentrations of about 10^4–10^5 CFU g^{-1} and for

Table 2
Characteristics of sourdoughs.

Sourdough samples	pH	TTA[a]	Lactic acid (mg g^{-1})	Acetic acid (mg g^{-1})	FQ	Microbial loads (Log CFU g^{-1})			
						MRS	M17	SDB	WL
F1	4.42 ± 0.03^{H}	7.50 ± 0.00^{E}	2.82 ± 0.19^{BD}	0.38 ± 0.02^{A}	4.95	8.7 ± 0.2^{C}	7.1 ± 0.7^{F}	7.7 ± 0.1^{C}	6.6 ± 0.1^{E}
F8	3.90 ± 0.04^{BD}	12.30 ± 0.00^{L}	2.93 ± 0.09^{B}	0.39 ± 0.01^{A}	5.01	5.1 ± 0.4^{A}	5.7 ± 0.8^{BC}	8.5 ± 0.6^{D}	3.2 ± 0.3^{A}
F9	4.09 ± 0.04^{EF}	10.60 ± 0.20^{I}	3.98 ± 0.13^{C}	0.49 ± 0.02^{A}	5.41	5.6 ± 0.2^{A}	6.3 ± 0.1^{ED}	7.9 ± 0.3^{D}	3.5 ± 0.6^{B}
F13	4.17 ± 0.04^{E}	9.30 ± 0.10^{G}	5.00 ± 0.03^{D}	0.49 ± 0.03^{A}	6.80	6.5 ± 0.3^{B}	5.8 ± 0.6^{BC}	8.4 ± 0.4^{D}	4.6 ± 0.1^{C}
F15	4.19 ± 0.01^{E}	6.40 ± 0.00^{C}	3.59 ± 0.40^{C}	0.43 ± 0.14^{A}	5.57	8.5 ± 0.4^{C}	7.6 ± 0.3^{F}	8.6 ± 0.4^{D}	6.7 ± 0.4^{E}
F21	4.17 ± 0.02^{E}	8.10 ± 0.20^{F}	5.07 ± 0.35^{D}	0.61 ± 0.10^{A}	5.54	6.7 ± 0.2^{B}	6.6 ± 0.2^{ED}	7.0 ± 0.3^{C}	4.5 ± 0.4^{C}
F25	4.62 ± 0.06^{I}	6.50 ± 0.10^{C}	3.14 ± 0.17^{B}	0.68 ± 0.12^{A}	3.08	8.5 ± 0.5^{C}	7.6 ± 0.2^{F}	8.4 ± 0.2^{D}	6.6 ± 0.3^{E}
F27	4.60 ± 0.02^{I}	7.00 ± 0.10^{D}	6.92 ± 0.32^{E}	1.36 ± 0.26^{B}	3.39	8.2 ± 0.6^{C}	8.5 ± 0.2^{G}	8.3 ± 0.3^{D}	6.0 ± 0.2^{E}
F32	4.00 ± 0.03^{BC}	10.90 ± 0.20^{I}	7.59 ± 0.26^{F}	1.21 ± 0.41^{B}	4.18	6.5 ± 0.1^{B}	6.0 ± 0.7^{D}	8.2 ± 0.9^{D}	4.6 ± 0.2^{C}
F36	3.81 ± 0.01^{A}	14.70 ± 0.00^{M}	9.41 ± 0.48^{G}	0.99 ± 0.11^{B}	6.34	7.3 ± 0.3^{B}	5.8 ± 0.2^{BC}	9.0 ± 0.1^{E}	5.4 ± 0.1^{D}
F37	4.47 ± 0.07^{H}	9.50 ± 0.00^{G}	3.55 ± 0.18^{C}	0.55 ± 0.15^{A}	4.30	8.9 ± 0.4^{C}	4.0 ± 0.2^{A}	7.4 ± 0.3^{C}	6.8 ± 0.2^{E}
F38	4.77 ± 0.08^{L}	6.00 ± 0.00^{B}	2.29 ± 0.27^{B}	0.99 ± 0.11^{B}	1.54	8.8 ± 0.2^{C}	4.9 ± 0.4^{B}	8.7 ± 0.1^{D}	6.6 ± 0.1^{E}
F54	4.30 ± 0.02^{G}	9.00 ± 0.20^{G}	1.97 ± 0.05^{B}	0.36 ± 0.02^{A}	3.65	4.9 ± 0.3^{A}	4.6 ± 0.4^{AC}	4.5 ± 0.3^{A}	2.9 ± 0.5^{A}
F55	3.95 ± 0.08^{B}	10.10 ± 0.00^{H}	1.99 ± 0.03^{B}	1.46 ± 0.09^{B}	0.91	6.5 ± 0.3^{B}	4.8 ± 0.6^{AC}	8.2 ± 0.4^{D}	4.4 ± 0.1^{C}
F59	4.63 ± 0.04^{I}	11.30 ± 0.20^{I}	2.10 ± 0.21^{BC}	0.38 ± 0.02^{A}	3.68	7.0 ± 0.8^{B}	5.2 ± 0.3^{BC}	7.1 ± 0.2^{C}	5.1 ± 0.3^{CD}
Statistical significance[b]	***	***	***	***	n.d.	***	***	***	***

Abbreviations: FQ, fermentation quotient; MRS, de Man-Rogosa-Sharpe agar for mesophilic rod LAB; M17, medium 17 agar for mesophilic coccus LAB; SDB, sourdough bacteria agar for typical sourdough LAB; WL, Wallerstein Laboratory Nutrient agar for yeasts.
Data within a column followed by the same letter are not significantly different according to Tukey's test.
Results indicate mean values ± S.D. of four plate counts (carried out in duplicate for two independent productions).
n.d., not determined.
[a] Calculated as ml of 0.1 N NaOH.
[b] P value:***, $P \leq 0.001$.

which also the levels of LAB on MRS and M17 were in the same order of magnitude. Yeast loads were in the range 2.9–6.8 Log CFU g^{-1}. Yeast/LAB ratios ranged between 1:10 and 1:10,000; however, for many samples, it was almost 1:100.

3.2. Isolation, grouping and identification of LAB

Two-hundred and seven colonies were collected from the 15 sourdoughs. The colonies were subjected to microscopic inspection, and separated into 53 cocci and 154 rods.

Based on the growth at 15 °C and 45 °C and the ability to produce CO_2 from glucose, tested for all bacteria, and for growth at pH 9.6 and in the presence of 6.5% (w/v) NaCl, tested only for cocci, the LAB cultures were separated into 10 groups (Table S1). The highest number of isolates was included in Group III, that was characterized by a FHe metabolism. Within rod shaped LAB, three groups (I, II and IV) resulted OHe while group V included thermophilic OHo cultures. LAB cocci were mostly OHo, and only 12 isolates (Groups VI and VII) showed an OHe metabolism.

In order to classify mesophilic and FHe LAB, the 112 isolates of Group III were subjected to RAPD analysis. The unique patterns were used to construct a dendrogram (Fig. S1) revealing the presence of 33 strains which were identified by 16S rRNA gene sequencing. A BLAST search revealed that the sequences had an identity of at least 97% with those available within the NCBI database. The species within the *L. plantarum* group and the strains of *L. curvatus* were also analysed with species-specific PCR strategies. All strains were confirmed to be members of the LAB group (Table 3). The species encompassing the most FHe LAB was *L. plantarum* with 29 strains.

To better evaluate the codominance between FHe and OHe LAB, rod isolates representative for the phenotypic groups I, II and IV were also genetically identified by 16S rRNA gene sequence analysis and species-specific and multiplex PCRs. At the same levels of *L. plantarum*, *L. curvatus* and *Lactobacillus graminis*, it was found the presence of the species *L. brevis* (8 isolates, Acc. No KM822618–KM822625), *L. sanfranciscensis* (3 isolates, Acc. No KM822615–KM822617), *L. rossiae* (2 isolates, Acc. No KM822613–KM822614) and *W. cibaria* (6 isolates, Acc. No KM668160–KM668165).

3.3. Technological traits of FHe LAB

The kinetics of acidification of the 33 FHe strains of LAB evaluated in SFE for 72 h are reported in Fig. 1. Fourteen strains, including 12 *L. plantarum*, *L. curvatus* PON100490 and *L. graminis* PON100244 determined the decrease of the SFE pH below 4.7 after 8 h. At 24 h, *L. plantarum* PON1004 and PON100148 reached the lowest values. All pHs were registered below 3.65 after 72 h of fermentation.

The production of lactic and acetic acid by the 14 strains that dropped the pH of SFE quickly was evaluated after 8 h of fermentation. The results (Table 4) showed that lactic acid was produced in the range of 0.31–0.81 mg g^{-1}, while acetic acid was found from 0.10 to 0.17 mg g^{-1}. The highest production of organic acids was registered for *L. plantarum* PON100148 for which the FQ was 3.14.

The 33 strains were also tested for their extracellular protease activity (Table 5). Thirteen strains were positive for the hydrolysis of BSA and 14 for that of gelatine. Interestingly, 12 of these strains (11 *L. plantarum* and 1 *L. curvatus*) showed the protease activity with both substrates.

The antimicrobial activity of the 33 FHe LAB was evaluated against bacteria and moulds (Table 5). Except *L. plantarum* PON100282 and PON100498 and *L. curvatus* PON100250, all other strains produced active substances. In general, the antibacterial activity was mostly registered against *L. sakei* 2313, both in terms of positive strains and width of the inhibition zone. All excreted antibacterial compounds were hydrolysed by proteolytic enzymes (data not shown), thus showing a general characteristic of bacteriocins (Jack et al., 1995). However, because the amino acid and nucleotide sequences of these substances have not yet been characterised, they shall be referred to as bacteriocin-like inhibitory substances (BLIS) (Corsetti et al., 2008). The anti-mould activity was less pronounced, but 11 *L. plantarum* showed inhibitory power.

3.4. Evolution of microbiological parameters during experimental sourdough fermentation

Based on the technological screening, *L. plantarum* PON100148 was selected to act as starter for experimental sourdough production. On the contrary, *L. plantarum* PON100282 characterised by negligible technological performances was used for comparison.

Table 3
Identification of sourdough FHe LAB strains.

Strain	No. of isolates	Species	Sourdough sample	% Similarity (accession no. of closest relative) by:		Sequence length (bp)	Accession no.
				GenBank	EzTaxon		
PON10003	3	*Lactobacillus plantarum*	F1	99 (JX183220.1)	99.80 (ACGZ01000098)	1482	KJ921803
PON10004	2	*L. plantarum*	F1	99 (EU559596.1)	98.86 (ACGZ01000098)	1504	KJ921804
PON10076	5	*L. plantarum*	F8	99 (AB598974.1)	99.45 (ACGZ01000098)	1466	KJ921805
PON100126	2	*L. plantarum*	F13	99 (KC753461.1)	99.72 (ACGZ01000098)	1419	KJ921806
PON100142	2	*L. plantarum*	F15	99 (AB933633.1)	97.90 (ACGZ01000098)	1332	KJ921807
PON100144	4	*L. plantarum*	F15	99 (EU626010.1)	99.03 (ACGZ01000098)	1342	KJ921808
PON100146	3	*L. plantarum*	F15	99 (GU369759.1)	99.72 (ACGZ01000098)	1412	KJ921809
PON100147	3	*L. plantarum*	F15	97 (JX262241.1)	98.01 (ACGZ01000098)	1316	KJ921810
PON100148	1	*L. plantarum*	F15	98 (GU290217.1)	98.73 (ACGZ01000098)	1339	KJ921811
PON100156	2	*L. plantarum*	F15	99 (FJ844944.1)	99.36 (ACGZ01000098)	1412	KJ921812
PON100159	5	*L. plantarum*	F15	98 (GU369759.1)	97.05 (ACGZ01000098)	1328	KJ921813
PON100163	3	*L. plantarum*	F15	99 (EU074833.1)	99.57 (ACGZ01000098)	1417	KJ921814
PON100164	3	*L. plantarum*	F15	99 (JF965386.1)	99.77 (ACGZ01000098)	1311	KJ921815
PON100196	2	*L. plantarum*	F1	97 (EU626009.1)	97.78 (ACGZ01000098)	1324	KJ921816
PON100205	3	*L. plantarum*	F1	97 (JF965386.1)	97.53 (ACGZ01000098)	1257	KJ921817
PON100218	2	*L. plantarum*	F21	99 (AB933633.1)	99.93 (ACGZ01000098)	1348	KJ921818
PON100244	2	*Lactobacillus graminis*	F9	99 (NR_042438.1)	99.73 (AM113778)	1491	KJ921819
PON100250	2	*Lactobacillus curvatus*	F8	99 (NR_114915.1)	99.77 (AJ621550)	1319	KJ921820
PON100259	6	*L. plantarum*	F25	99 (KJ026699.1)	99.57 (ACGZ01000098)	1413	KJ921821
PON100265	5	*L. plantarum*	F25	99 (KF668239.1)	99.35 (ACGZ01000098)	1391	KJ921822
PON100282	9	*L. plantarum*	F27	99 (AB601179.1)	99.67 (ACGZ01000098)	1501	KJ921823
PON100346	2	*L. graminis*	F32	99 (NR_042438.1)	99.86 (AM113778)	1424	KJ921824
PON100394	2	*L. plantarum*	F38	98 (JX262241.1)	97.87(ACGZ01000098)	1315	KJ921825
PON100396	2	*L. plantarum*	F38	99 (KJ508871.1)	99.78 (ACGZ01000098)	1373	KJ921826
PON100412	3	*L. plantarum*	F36	99 (NR_042254.1)	99.79 (AJ640078)	1419	KJ921827
PON100414	2	*L. plantarum*	F36	99 (NR_042254.1)	99.86 (AJ640078)	1402	KJ921828
PON100415	4	*L. plantarum*	F36	98 (NR_042254.1)	99.93 (AJ640078)	1377	KJ921829
PON100418	2	*L. plantarum*	F37	99 (KF317898.1)	99.86 (AJ640078)	1439	KJ921830
PON100485	4	*L. plantarum*	F54	99 (HM753266.1)	99.85 (ACGZ01000098)	1309	KJ921831
PON100490	3	*L. curvatus*	F54	99 (AB600200.1)	99.64 (AJ621550)	1466	KJ921832
PON100491	7	*L. plantarum*	F54	100 (AB933633.1)	100 (ACGZ01000098)	1342	KJ921833
PON100498	4	*L. plantarum*	F55	98 (GU369759.1)	98.84 (ACGZ01000098)	1381	KJ921834
PON100536	8	*L. plantarum*	F59	99 (KJ095651.1)	99.93 (AJ640078)	1414	KJ921836

The comparison of the plate count results with the control (non-inoculated) dough confirmed the dominance of the added cultures (Table 6). The results on MRS showed a development of *L. plantarum* PON100148 more rapid than *L. plantarum* PON100282, with a cell number of about 1 Log CFU g^{-1} both at 8 and 21 h. The dual combinations with *L. sanfranciscensis* LMG 17498^T showed that the OHe strain developed more rapidly than *L. plantarum* PON100282, but slower than *L. plantarum* PON100148.

3.5. Evolution of chemical parameters during experimental sourdough fermentation

The pH values registered for the experimental sourdoughs are reported in Table 6. *L. plantarum* PON100148 alone reached the same final pH of that showed in combination with *L. sanfranciscensis* LMG 17498^T. The pH levels registered in presence of *L. plantarum* PON100282 alone and together with *L. sanfranciscensis* LMG 17498^T were higher than those found for the corresponding trials carried out with *L. plantarum* PON100148, even though the acidifying activity of *L. sanfranciscensis* LMG 17498^T decreased strongly the pH of the dough started with *L. plantarum* PON100282.

TTA data (Table 6) confirmed the trend showed by pH.

The concentrations of the organic acids and the resulting FQ of the experimental sourdoughs are reported in Table 7. Although control dough and CY did not contain detectable levels of lactic and acetic acids at T_0, both acids were found after 8 h of fermentation; however, their concentrations were lower than those registered for the doughs inoculated with LAB. The highest levels of acids were registered in presence of *L. plantarum* PON100148, alone and in combination with *L. sanfranciscensis* LMG 17498^T. The presence of the last bacterium did not greatly modify the concentrations of the single acids as well as the FQ of the doughs inoculated with LAB.

The chromatographic analysis of the headspace of experimental sourdoughs revealed the presence of 31 compounds (Table 8). VOC fraction was composed of 4 acids, 9 alcohols, 4 aldehydes, 9 esters, 1 hydrocarbon, 3 ketones and phenol. In control dough at T_0 and after 8 h of fermentation a lower number of chemicals was detected (10 and 15, respectively). Furthermore, their relative abundances was sensibly lower than those displayed by both *L. plantarum* strains with or without the presence of *L. sanfranciscensis* LMG 17498^T. Unlike the other doughs, CY was characterized by seven additional compounds: 1 aldehyde (2-decanal) and 6 esters (ethyl octanoate, γ-butyrolactone, ethyldecanoate, ethylbenzoate, ethyl salicylate and ethyldodecanoate). The major contribution of *L. sanfranciscensis* LMG 17498^T was registered for acetic and hexanoic acid.

3.6. Characterization of breads

After baking, different determinations were performed on the experimental breads (Table 9). In presence of LAB and baker's yeast the height of breads reached higher value than control bread, with the highest value found for the combination *L. plantarum* PON100148/*L. sanfranciscensis* LMG 17498^T. The colour of crust and crumb resulted slightly different among breads. The parameters L^* and b^* registered for the crust did not statistically differ among the experimental breads obtained with the different combinations of LAB. The parameter a^* for the bread obtained with *L. plantarum* PON100148/*L. sanfranciscensis* LMG 17498^T was different from the

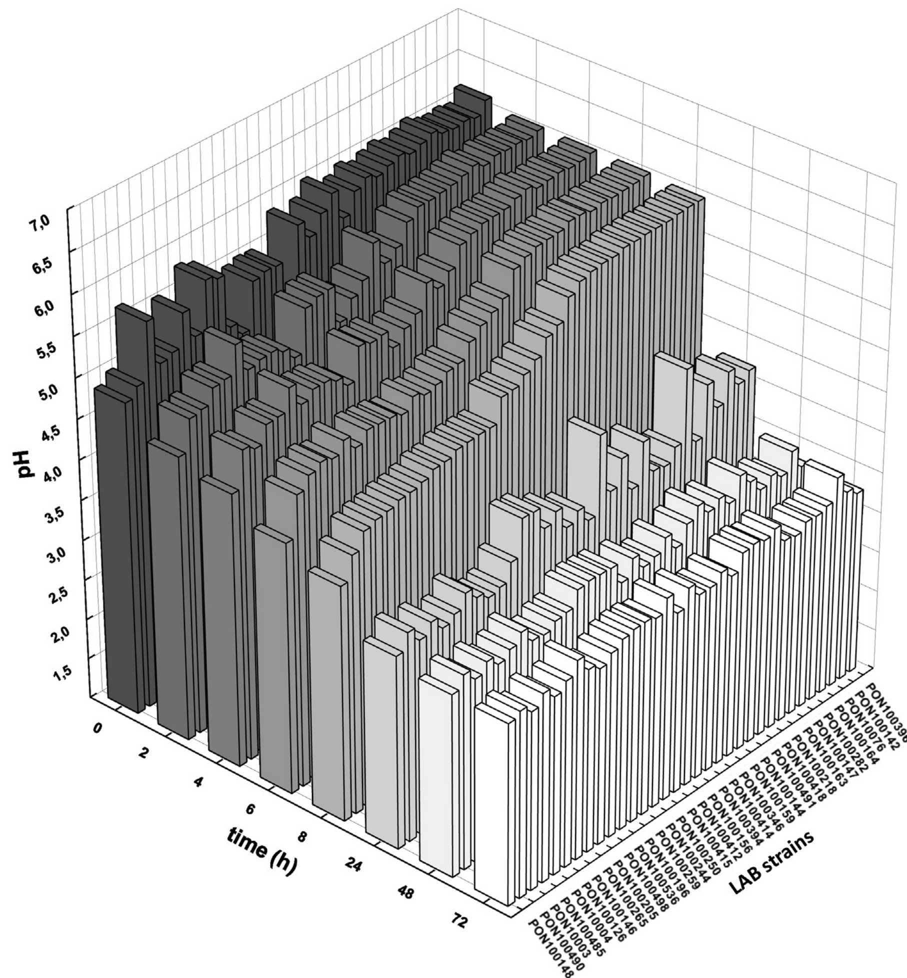

Fig. 1. Kinetics of acidification of FHe sourdough LAB evaluated in SFE. Histograms are in the order of increasing pH at 8 h.

Table 4
Organic acids produced by FHe LAB strains in sterile flour extract fermented for 8 h.

Strains	Lactic acid (mg g^{-1})	Acetic acid (mg g^{-1})	FQ
Control	0.02 ± 0.03^{A}	0.00^{A}	n.d.
CY	0.18 ± 0.03^{B}	0.11 ± 0.03^{B}	1.09
L. plantarum PON10003	0.53 ± 0.04^{C}	0.13 ± 0.03^{B}	2.77
L. plantarum PON10004	0.48 ± 0.05^{C}	0.13 ± 0.03^{B}	2.48
L. plantarum PON100126	0.51 ± 0.06^{C}	0.13 ± 0.04^{B}	2.55
L. plantarum PON100146	0.41 ± 0.02^{C}	0.14 ± 0.03^{B}	1.96
L. plantarum PON100148	0.81 ± 0.04^{D}	0.17 ± 0.03^{B}	3.14
L. plantarum PON100196	0.46 ± 0.04^{C}	0.10 ± 0.01^{B}	2.99
L. plantarum PON100205	0.42 ± 0.03^{C}	0.10 ± 0.01^{B}	2.72
L. graminis PON100244	0.41 ± 0.07^{C}	0.10 ± 0.02^{B}	2.71
L. plantarum PON100259	0.63 ± 0.05^{E}	0.10 ± 0.01^{B}	4.08
L. plantarum PON100265	0.54 ± 0.04^{C}	0.13 ± 0.03^{B}	2.81
L. plantarum PON100485	0.51 ± 0.04^{C}	0.13 ± 0.02^{B}	2.58
L. curvatus PON100490	0.31 ± 0.07^{F}	0.15 ± 0.02^{B}	1.43
L. plantarum PON100498	0.53 ± 0.03^{C}	0.10 ± 0.02^{B}	3.42
L. plantarum PON100536	0.46 ± 0.05^{C}	0.15 ± 0.02^{B}	2.01
Statistical significance[a]	***	*	n.d.

Abbreviations: CY, control yeast; FQ, fermentation quotient; *L.*, *Lactobacillus*; n.d., not determined.
Results indicate mean values ± SD of four measurements (carried out in duplicate for two independent fermentations).
Data within a column followed by the same letter are not significantly different according to Tukey's test.
n.d., not determined.
[a] P value: ***, $P \leq 0.001$; *, $P \leq 0.05$.

others. Regarding the colour of the crumb, small differences were recorded among breads except for the parameter b^*.

The firmness was strongly influenced by the LAB used. The lowest value was obtained in the sourdough inoculated with *L. plantarum* PON100148, followed by *L. plantarum* PON100282. The presence of *L. sanfranciscensis* LMG 17498^{T} determined higher values for firmness, that remained below the value displayed by the control bread.

The image analysis was performed to determine the qualitative characteristics of the crumb. The void fraction was not found to be statistically different in presence of the added LAB, but all breads obtained with the sourdoughs started with LAB differed from the control as well as CY bread. Contrarily to the mean cell area, the cell density was influenced by the LAB added.

4. Discussion

The role and the importance of OHe LAB in sourdough is well known (Gobbetti, 1998; De Vuyst et al., 2014; Minervini et al., 2014). The main species of this group are lactobacilli, weissellas and leuconostocs (Settanni and Moschetti, 2011), but the species considered to play a key role and to represent a stable element for the sourdoughs produced with the traditional processes is *L. sanfranciscensis* (Vogel et al., 2011). However, in the last few years, besides *L. sanfranciscensis*, another OHe *Lactobacillus* species,

Table 5
Proteolysis and inhibitory activity of sourdough LAB.

Strains	Extracellular protease activity[a]		Antibacterial activity[b]			Antifungal activity[b]	
	Substrate		Indicator strains			Indicator strains	
	BSA	Gelatine	*Ls. monocytogenes* ATCC 19114	*Ls. innocua* 4202	*L. sakei* 2313	*A. alternata Ra56*	*C. cladosporioides Sc71*
L. plantarum PON1003	+	−	−	1.51 ± 0.15	1.47 ± 0.12	−	−
L. plantarum PON1004	−	+	−	1.36 ± 0.10	1.68 ± 0.10	−	−
L. plantarum PON10076	−	−	−	1.44 ± 0.24	1.81 ± 0.20	−	−
L. plantarum PON100126	−	−	−	1.61 ± 0.10	1.98 ± 0.10	−	−
L. plantarum PON100142	+	+	−	1.02 ± 0.08	1.18 ± 0.10	−	−
L. plantarum PON100144	−	−	−	2.04 ± 0.10	2.12 ± 0.10	−	−
L. plantarum PON100146	+	+	1.48 ± 0.04	1.44 ± 0.08	1.70 ± 0.20	0.75 ± 0.05	−
L. plantarum PON100147	+	+	1.20 ± 0.08	1.34 ± 0.06	1.60 ± 0.10	−	−
L. plantarum PON100148	+	+	0.80 ± 0.00	0.84 ± 0.04	1.42 ± 0.08	0.85 ± 0.10	−
L. plantarum PON100156	−	+	−	−	0.24 ± 0.00	−	−
L. plantarum PON100159	+	+	1.66 ± 0.12	1.64 ± 0.20	1.92 ± 0.04	0.20 ± 0.00	−
L. plantarum PON100163	+	+	−	1.40 ± 0.00	1.42 ± 0.02	−	−
L. plantarum PON100164	+	+	−	−	1.80 ± 0.10	−	−
L. plantarum PON100196	+	+	−	−	−	0.60 ± 0.20	−
L. plantarum PON100205	+	+	1.44 ± 0.36	1.62 ± 0.00	2.10 ± 0.02	−	−
L. plantarum PON100218	−	−	−	1.10 ± 0.40	1.24 ± 0.62	0.85 ± 0.05	−
L. graminis PON100244	−	−	−	−	0.10 ± 0.00	−	−
L. curvatus PON100250	+	+	−	−	−	−	−
L. plantarum PON100259	−	−	−	0.64 ± 0.20	0.82 ± 0.04	0.70 ± 0.15	−
L. plantarum PON100265	−	−	−	0.48 ± 0.06	0.84 ± 0.10	−	−
L. plantarum PON100282	−	−	−	−	−	−	−
L. graminis PON100346	−	−	−	−	0.15 ± 0.14	−	−
L. plantarum PON100394	−	−	−	−	0.14 ± 0.10	−	−
L. plantarum PON100396	+	+	−	−	0.30 ± 0.04	−	−
L. plantarum PON100412	−	−	1.87 ± 0.32	1.91 ± 0.33	1.94 ± 0.86	0.60 ± 0.00	−
L. plantarum PON100414	−	−	1.74 ± 0.00	1.55 ± 0.42	1.98 ± 0.00	0.50 ± 0.20	0.90 ± 0.10
L. plantarum PON100415	−	−	1.72 ± 0.24	1.74 ± 0.02	2.01 ± 0.77	0.80 ± 0.00	−
L. plantarum PON100418	−	−	1.04 ± 0.14	1.04 ± 0.12	1.22 ± 0.06	0.60 ± 0.05	−
L. plantarum PON100485	−	−	−	0.77 ± 0.13	0.78 ± 0.28	−	−
L. curvatus PON100490	−	−	0.65 ± 0.15	0.34 ± 0.06	0.11 ± 0.25	0.60 ± 0.05	−
L. plantarum PON100491	+	+	1.75 ± 0.17	1.74 ± 0.16	1.65 ± 0.37	−	−
L. plantarum PON100498	−	−	−	−	−	−	−
L. plantarum PON100536	−	−	0.40 ± 0.20	1.58 ± 0.26	−	0.40 ± 0.00	−

Abbreviations: BSA, bovine serum albumin; *A.*, *Alternaria*; *C.*, *Cladosporium*; *Ls.*, *Listeria*; *L.*, *Lactobacillus*.
+, positive for protease production; −, negative for protease production or, in case of antibacterial tests, no inhibition found; +/−, little inhibition.
[a] The test was repeated twice.
[b] Width of the inhibition zone (mm). Results indicate mean ± S.D. of three independent experiments.

namely *L. rossiae*, is being frequently isolated at high numbers from sourdoughs produced in Italy (Valmorri et al., 2006; Di Cagno et al., 2007) and it has been also detected in Belgian sourdoughs (Scheirlinck et al., 2007, 2008). The majority of LAB screened and selected to act as starters in sourdough production belong to OHe species (Minervini et al., 2012; Settanni et al., 2013).

In general, FHe LAB are reported to be found at lower cell densities than the OHe species (Minervini et al., 2012). Despite this

Table 6
Microbial loads (Log CFU g^{-1}) of the experimental sourdoughs.

Strains	Media						pH	TTA
	MRS			SDB				
	T_0	8 h	21 h	T_0	8 h	21 h	21 h	21 h
Control	<1[A]	1.48 ± 0.05[A]	2.78 ± 0.01[A]	<2[A]	1.62 ± 0.01[A]	2.99 ± 0.00[A]	5.71 ± 0.02[C]	3.30 ± 0.00[A]
CY	<1[A]	7.85 ± 0.02[C]	8.05 ± 0.03[B]	<2[A]	6.54 ± 0.02[B]	7.40 ± 0.04[B]	5.24 ± 0.05[C]	8.60 ± 0.10[B]
L. plantarum PON100148	6.53 ± 0.04[C]	8.49 ± 0.06[D]	9.09 ± 0.00[C]	n.d.	n.d.	n.d.	3.48 ± 0.01[A]	12.50 ± 0.20[D]
L. plantarum PON100282	6.21 ± 0.00[B]	7.48 ± 0.00[B]	8.03 ± 0.01[B]	n.d.	n.d.	n.d.	4.09 ± 0.03[B]	10.10 ± 0.10[C]
L. plantarum PON100148 + *L. sanfranciscensis* LMG17498[T]	6.56 ± 0.05[C]	8.48 ± 0.02[D]	8.97 ± 0.01[C]	6.46 ± 0.11[C]	8.59 ± 0.03[D]	8.71 ± 0.03[D]	3.44 ± 0.04[A]	12.60 ± 0.00[D]
L. plantarum PON100282 + *L. sanfranciscensis* LMG17498[T]	6.24 ± 0.08[B]	8.01 ± 0.07[C]	8.12 ± 0.05[B]	6.28 ± 0.08[C]	8.03 ± 0.04[C]	8.32 ± 0.05[C]	3.95 ± 0.04[B]	11.40 ± 0.00[C]
Statistical significance	**	***	***	***	***	***	***	***

Abbreviations: CY, control yeast; MRS, de Man-Rogosa-Sharpe agar for mesophilic rod LAB; SDB, sourdough bacteria agar for typical sourdough LAB; TTA, total titratable acidity.
L., *Lactobacillus*.
n.d., not determined.
Results indicate mean values ± SD of four plate counts (carried out in duplicate for two independent productions).
Data within a column followed by the same letter are not significantly different according to Tukey's test.
P value: ***, P ≤ 0.001; **, P ≤ 0.01.

Table 7
Organic acids produced in experimental sourdoughs fermented for 8 h.

Strains	Lactic acid (mg g^{-1})	Acetic acid (mg g^{-1})	FQ
Control (T_0)	0.02 ± 0.02^{A}	0.00^{A}	n.d.
Control (T_8)	1.04 ± 0.13^{B}	0.13 ± 0.03^{A}	5.33
CY (T_0)	0.03 ± 0.01^{A}	0.00^{A}	n.d.
CY (T_8)	1.06 ± 0.13^{B}	0.31 ± 0.09^{B}	2.27
L. plantarum PON100148	4.03 ± 0.04^{C}	1.03 ± 0.03^{C}	2.61
L. plantarum PON100282	1.48 ± 0.05^{D}	0.33 ± 0.03^{B}	2.99
L. plantarum PON100148 + *L. sanfranciscensis* LMG17498^{T}	4.19 ± 0.09^{C}	1.05 ± 0.04^{C}	2.66
L. plantarum PON100282 + *L. sanfranciscensis* LMG17498^{T}	1.51 ± 0.02^{D}	0.34 ± 0.03^{B}	2.96
Statistical significance[a]	***	***	n.d.

Abbreviations: CY, control yeast; FQ, fermentation quotient; *L.*, *Lactobacillus*; n.d., not determined.
Results indicate mean values ± SD of four measurements (carried out in duplicate for two independent sourdough productions).
Data within a column followed by the same letter are not significantly different according to Tukey's test.
n.d., not determined.
[a] P value: ***, $P \leq 0.001$.

common trend, several FHe LAB, together with OHe LAB, are often isolated at dominant levels in sourdough, demonstrating an important role of these species during sourdough production. Among sourdough FHe species, *L. plantarum* is particularly encountered in sourdough ecosystems. Gobbetti (1998) reported on the strict association between *L. sanfranciscensis* and *L. plantarum* in Italian wheat sourdoughs and Minervini (2010) demonstrated the robustness of this species under daily back-slopping propagation using wheat flour. In this work, the LAB populations of 15 sourdoughs produced at industrial and artisanal level in Sicily have been investigated and the FHe species genetically characterized and evaluated *in vitro* and *in vivo* for their technological performances.

The general characteristics of the sourdoughs collected in western Sicily were determined by the measure of pH, TTA and the chromatographic analyses of lactic and acetic acid. The majority of samples showed an acidity level typical for sourdoughs produced in Italy (Minervini et al., 2012). However, the concentrations of lactic and acetic acid revealed that the FQ of only seven sourdoughs was in the range 1.5–4, considered to affect positively the aroma profile

Table 8
Analysis of volatile organic compounds emitted from experimental sourdoughs and fermented for 8 h.

Chemical compounds[a]	Sourdoughs[b]							
	Control (T_0)	Control (T_8)	CY (T_8)	PON100148	PON100282	PON100148 + LMG17498^{T}	PON100282 + LMG17498^{T}	Statistical significance
Ethanol	n.d	45.03 ± 2.01^{B}	74.79 ± 1.98^{E}	52.69 ± 2.98^{C}	72.51 ± 3.54^{E}	54.70 ± 2.54^{C}	60.26 ± 3.27^{D}	***
3-Methyl-1-butanol	9.68 ± 0.21^{E}	5.11 ± 0.08^{D}	5.15 ± 0.05^{D}	2.41 ± 0.21^{AB}	1.77 ± 0.09^{A}	3.35 ± 0.05^{C}	1.88 ± 0.06^{A}	***
Acetoin	n.d.	n.d.	n.d.	1.78 ± 0.14^{C}	1.33 ± 0.08^{B}	1.02 ± 0.05^{A}	1.99 ± 0.05^{D}	***
Tridecane	22.97 ± 0.81^{D}	9.14 ± 0.11^{C}	n.d.	5.82 ± 0.21^{B}	3.69 ± 0.12^{A}	5.18 ± 0.09^{B}	4.05 ± 0.02^{A}	***
6-Methyl-5-heptene-2-one	n.d.	n.d.	n.d.	1.22 ± 0.11^{D}	0.18 ± 0.02^{A}	0.79 ± 0.02^{C}	0.57 ± 0.02^{B}	***
1-Hexanol	31.07 ± 0.12^{G}	13.40 ± 0.12^{F}	1.50 ± 0.03^{A}	6.00 ± 0.08^{C}	5.33 ± 0.09^{B}	8.72 ± 0.17^{D}	5.16 ± 0.03^{B}	***
Ethyl octanoate	n.d.	n.d.	1.33 ± 0.03	n.d.	n.d.	n.d.	n.d.	N.S.
Acetic acid	n.d.	1.61 ± 0.04^{A}	n.d.	4.91 ± 0.09^{C}	1.48 ± 0.02^{A}	3.34 ± 0.05^{B}	8.41 ± 0.07^{D}	***
1-Octen-3-ol	21.22 ± 0.74^{G}	11.04 ± 0.11^{E}	1.14 ± 0.02^{A}	8.99 ± 0.08^{D}	4.77 ± 0.11^{B}	6.32 ± 0.09^{C}	4.98 ± 0.14^{B}	***
1-Heptanol	n.d.	0.90 ± 0.06^{C}	0.46 ± 0.02^{A}	0.81 ± 0.02^{C}	0.63 ± 0.03^{B}	0.96 ± 0.06^{C}	0.71 ± 0.06^{B}	***
Benzaldehyde	n.d.	1.11 ± 0.06^{D}	0.40 ± 0.01^{B}	0.20 ± 0.01^{A}	0.71 ± 0.09^{C}	0.32 ± 0.02^{B}	0.15 ± 0.01^{A}	***
2-Nonenal	n.d.	n.d.	0.17 ± 0.02^{A}	0.49 ± 0.02^{C}	0.45 ± 0.05^{C}	0.74 ± 0.01^{D}	0.27 ± 0.02^{B}	***
3,5-Octadien-2-one	1.27 ± 0.09^{C}	1.41 ± 0.08^{C}	n.d.	0.59 ± 0.02^{B}	0.36 ± 0.06^{A}	0.64 ± 0.04^{B}	0.38 ± 0.03^{A}	***
1-Octanol	1.91 ± 0.08^{E}	1.60 ± 0.07^{D}	0.32 ± 0.02^{A}	0.84 ± 0.03^{C}	0.73 ± 0.01^{C}	0.94 ± 0.07^{C}	0.57 ± 0.04^{B}	***
2-Decanal	n.d.	n.d.	0.65 ± 0.02^{A}	n.d.	n.d.	n.d.	n.d.	***
γ-Butyrolactone	n.d.	n.d.	0.20 ± 0.01	n.d.	n.d.	n.d.	n.d.	N.S.
Furfuryl alcohol	n.d.	n.d.	n.d.	1.81 ± 0.02^{C}	0.15 ± 0.01^{A}	2.53 ± 0.10^{D}	0.60 ± 0.05^{B}	***
Ethyldecanoate	n.d.	n.d.	1.05 ± 0.01	n.d.	n.d.	n.d.	n.d.	N.S.
Ethylbenzoate	n.d.	n.d.	0.10 ± 0.01	n.d.	n.d.	n.d.	n.d.	N.S.
3-Nonen-1-ol	n.d.	n.d.	1.69 ± 0.02^{D}	0.41 ± 0.02^{B}	0.22 ± 0.02^{A}	1.11 ± 0.02^{C}	0.33 ± 0.02^{B}	***
Pentanoic acid	n.d	n.d.	n.d.	0.35 ± 0.03^{B}	0.18 ± 0.01^{A}	0.37 ± 0.03^{B}	0.32 ± 0.03^{B}	***
Methyl salicylate	n.d.	n.d.	0.86 ± 0.01^{D}	0.13 ± 0.01^{A}	0.51 ± 0.01^{C}	0.29 ± 0.02^{B}	1.30 ± 0.11^{E}	***
Ethyl salicylate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.
Ethyldodecanoate	n.d.	n.d.	0.24 ± 0.01	n.d.	n.d.	n.d.	n.d.	N.S.
Tridecan-2-one	4.84 ± 0.11^{F}	3.62 ± 0.09^{E}	n.d.	1.39 ± 0.02^{C}	1.08 ± 0.02^{B}	1.77 ± 0.05^{D}	0.87 ± 0.08^{A}	***
Hexanoic acid	n.d.	n.d.	n.d.	6.80 ± 0.06^{D}	1.11 ± 0.03^{A}	4.24 ± 0.11^{B}	5.79 ± 0.24^{C}	***
Phenylethyl Alcohol	n.d.	0.72 ± 0.01^{A}	6.31 ± 2.31^{D}	0.37 ± 0.02^{A}	0.28 ± 0.01^{A}	0.32 ± 0.07^{A}	0.23 ± 0.01^{A}	***
Phenol	2.32 ± 0.13^{E}	1.50 ± 0.04^{D}	0.19 ± 0.01^{A}	0.36 ± 0.02^{B}	0.73 ± 0.01^{C}	0.67 ± 0.08^{C}	0.30 ± 0.01^{AB}	***
γ-Nonalactone	3.44 ± 0.08^{E}	3.11 ± 0.02^{D}	0.75 ± 0.01^{A}	1.01 ± 0.04^{A}	1.06 ± 0.02^{A}	1.27 ± 0.03^{B}	0.68 ± 0.05^{A}	***
Isophthalaldehyde	1.28 ± 0.02^{C}	0.70 ± 0.01^{B}	2.70 ± 0.02^{D}	0.23 ± 0.02^{A}	0.54 ± 0.16^{B}	0.26 ± 0.02^{A}	0.12 ± 0.01^{A}	***
Ethylhexadecanoate	n.d.	n.d.	n.d.	0.39 ± 0.03^{C}	0.20 ± 0.09^{B}	0.15 ± 0.02^{AB}	0.08 ± 0.00^{A}	***

LMG 17498^{T}; *L. plantarum* PON100282/*L. sanfranciscensis* LMG 17498^{T}.
Results indicate mean values of three measurements and are expressed as relative peak areas (peak area of each compound/total area) × l00.
Data within a line followed by the same letter are not significantly different according to Tukey's test (P value: ***, $P \leq 0.01$; NS, not significant).
[a] The chemicals are shown following their retention time.
[b] Sourdough fermented with: CY, control yeast; *L. plantarum* PON100148; *L. plantarum* PON100282; *L. plantarum* PON100148/*L. sanfranciscensis*.

Table 9
Characteristics of experimental breads.

Strain	Height (mm)	Crust dominance			Crumb colour			Firmness value (N)	Void fraction (%)	Cell density (n.cm^{-2})	Mean cell area (mm^2)
		L*	a*	b*	L*	a*	b*				
Control	25.00 ± 1.00^{A}	76.43 ± 1.76^{A}	1.31 ± 0.95^{A}	27.05 ± 1.65^{A}	64.74 ± 3.12^{A}	−1.49 ± 0.11^{A}	19.41 ± 0.46^{A}	67.30 ± 0.57^{A}	21.20 ± 0.99^{A}	21.00 ± 3.00^{A}	0.06 ± 0.01^{A}
CY	40.00 ± 0.00^{B}	71.00 ± 1.97^{B}	2.05 ± 1.06^{A}	29.44 ± 2.75^{B}	63.78 ± 3.47^{A}	−2.99 ± 0.15^{C}	23.65 ± 0.23^{B}	30.22 ± 0.14^{D}	26.35 ± 0.84^{B}	20.26 ± 5.14^{A}	0.32 ± 0.02^{B}
L. plantarum PON100148	40.00 ± 0.00^{B}	70.57 ± 1.73^{B}	5.41 ± 1.27^{B}	30.75 ± 1.31^{B}	70.75 ± 0.89^{B}	−2.19 ± 0.08^{B}	19.24 ± 0.45^{A}	11.58 ± 0.02^{B}	34.44 ± 4.33^{B}	35.00 ± 10.44^{B}	0.17 ± 0.06^{A}
L. plantarum PON100282	38.00 ± 0.00^{B}	68.48 ± 4.38^{B}	6.67 ± 2.26^{B}	32.18 ± 0.17^{B}	74.68 ± 0.67^{C}	−2.36 ± 0.10^{B}	20.09 ± 0.57^{A}	16.52 ± 0.38^{C}	30.33 ± 2.22^{B}	106.00 ± 12.52^{C}	0.17 ± 0.03^{A}
*L. plantarum*PON100148 + *L. sanfranciscensis* LMG17498^{T}	42.00 ± 1.00^{B}	72.68 ± 2.33^{B}	3.97 ± 1.42^{C}	29.13 ± 1.57^{B}	71.81 ± 0.89^{BC}	−2.15 ± 0.14^{B}	19.70 ± 0.04^{A}	30.38 ± 0.24^{D}	29.70 ± 0.97^{B}	49.33 ± 7.23^{D}	0.09 ± 0.03^{A}
L. plantarum PON100282 + *L. sanfranciscensis* LMG17498^{T}	40.00 ± 0.00^{B}	67.73 ± 5.97^{B}	6.71 ± 2.50^{B}	30.89 ± 0.74^{B}	72.81 ± 2.00^{BC}	−2.47 ± 0.02^{B}	20.64 ± 0.55^{A}	43.31 ± 0.12^{E}	27.64 ± 6.65^{B}	41.67 ± 17.01^{E}	0.31 ± 0.08^{B}
Statistical significance	***	***	***	***	***	***	N.S.	***	***	***	***

Abbreviations: CY, control yeast; *L.*, *Lactobacillus.*
Results indicate mean values of four measurements (carried out in duplicate for two independent productions).
Data within a column followed by the same letter are not significantly different according to Tukey's test (P value: ***, $P \le 0.01$; NS, not significant).

and the structure of the final products (Spicher, 1983). In particular, nine samples displayed very low levels of acetic acid. Thus, the high values of FQ for these sourdoughs are due to high productions of lactic acid and low generation of acetic acid. This observation could be due to high cell densities of OHo and FHe LAB species, which produce only lactic acid as main fermentation product in presence of hexose sugars (Axelsson, 1998).

Following the conventional approach applied to sourdough, the 15 samples were analysed for culturable LAB using MRS and M17 that are the media generally used for the plate counts of rod and coccus LAB, respectively, and SDB which is considered specific for sourdough LAB (Kline and Sugihara, 1971). Except one sourdough (F54), the other Sicilian samples harboured LAB at consistent levels, until 10^9 CFU g^{-1} in some cases. The levels of LAB estimated on SDB were above 10^8 CFU g^{-1} for the majority of samples indicating the dominance of this group of bacteria. The levels of LAB detected in this study were reported for other sourdough samples produced in Sicily (Minervini et al., 2012). Yeasts were also counted and, for many sourdoughs, yeast/LAB ratio was almost 1:100 which is in agreement with those reported in literature for type I sourdoughs (Gobbetti et al., 1994; Ottogalli et al., 1996; Valmorri et al., 2006).

Although a certain biodiversity of these LAB was found at strain level, only three species were identified. Twenty-nine FHe were *L. plantarum* which is an ubiquitous food related species reported to be typical also for sourdough environments (Corsetti and Settanni, 2007), followed by *L. curvatus* and *L. graminis* which are associated to unprocessed raw materials used in bread making (Corsetti et al., 2007). A consistent presence of FHe species has been also reported for sourdoughs produced in eastern Sicily (Randazzo et al., 2005). In order to better evaluate the codominance between FHe and OHe LAB in Sicilian sourdoughs, the representative OHe isolates from each sample, not characterized at strain level, were also identified. At this proposal, only the rod-shaped isolated were processed, because they are reported to reach the highest levels in mature sourdoughs (Corsetti and Settanni, 2007). *L. brevis*, *L. rossiae*, *L. sanfranciscensis* and *W. cibaria* were at the same levels of the FHe LAB. All these species are commonly associated to Italian sourdoughs (Minervini et al., 2014).

In this work, due to the high numbers of *L. plantarum* strains detected, they were better investigated for their technological traits that are useful for sourdough production and might explain the reasons for their codominance with OHe species.

L. plantarum might be competitive towards other wheat flour autochthonous species and the strains harbouring interesting metabolic traits could supersede other biotypes of the same species (Minervini et al., 2010). A similar behaviour for this species has been also reported during production of type II sourdoughs (Ravyts and De Vuyst, 2011) which are produced with rye flour and fermented at higher temperatures than those applied for traditional Italian sourdoughs. Bakery environment and flour may provide LAB that are found in sourdough (Scheirlink et al., 2009; Alfonzo et al., 2013). Alfonzo et al. (2013) reported that a consistent percentage of LAB present in several flour samples used for bread making in Sicily are *L. plantarum*. Several factors could have determined the robustness of *L. plantarum* strains in the Sicilian sourdoughs analysed in this study: competition for nutrients, fermentation of hexose and pentose carbohydrates, adaptation to environmental stresses, rapid acidification, synthesis of diacetyl and hydrogen peroxide, and production of bacteriocins (Gobbetti et al., 2005).

Twelve *L. plantarum* strains showed good acidifying abilities *in vitro* because they decreased rapidly the pH of SFE. The broths were subjected to the HPLC analysis to examine the concentrations of lactic and acetic acid. The highest production of organic acids was registered for *L. plantarum* PON100148 for which the FQ was 3.14. The extracellular protease activity, important for the rheology

and staleness of breads (Corsetti et al., 2000), was interesting for 11 *L. plantarum* strains. Several strains were producers of BLIS and some of them inhibited also fungi. The last aspect is important for the shelf life of the final products. Bacteriocins may act not only as inhibitors, but also as pheromones in the mechanism of inter-species cell–cell communication as demonstrated for plantaricin A produced by *L. plantarum* DC400 (Di Cagno et al., 2010). *L. plantarum* inhibits fungal indicators by the production of different molecules including benzoic acid, methylhydantoin, mevalonolactone, cyclo(Gly–Leu), cyclo(Phe–Pro), cyclo(Leu–Pro), Cyclo(Leu–Leu) and cyclo (Phe-OH-Pro), phenyllactic and 4-hydroxy-phenyllactic acids, 3-hydroxydecanoic, 3-hydroxy-5-cis-dodecenoic, 3-hydroxydodecanoic, 3-hydroxytetradecanoic acids (Niiku-Paavola et al., 1999; Lavermicocca et al., 2000; Ström et al., 2002; Magnusson et al., 2003; Sjögren et al., 2003; Del Bello et al., 2007; Yang et al., 2010), even though their contribution to bread preservation is quite limited due to their low concentrations.

L. plantarum PON100148 displayed characteristics technologically relevant to sourdough production and for this reason was selected to act as starter for experimental productions. The dual combination with *L. sanfranciscensis* LMG 17498^T did not greatly differ in terms of bacterial counts and pH drop from the sourdough produced with *L. plantarum* PON100148 alone. The role of *L. sanfranciscensis* LMG 17498^T in experimental sourdough production was previously evaluated singly by Settanni et al. (2013) and was confirmed to be negligible in this study when combined with other autochthonous LAB. This could be due to the fact that the type strain of the species *L. sanfranciscensis* was isolated from a sourdough environment different from that used in this study. The evolution of chemical parameters during fermentation showed that the highest levels of acids were registered in presence of *L. plantarum* PON100148, alone and in combination with *L. sanfranciscensis* LMG 17498^T. Furthermore, the analysis of the volatile fraction of the sourdoughs identified the presence of chemicals produced by the metabolism of LAB that are positive for the aroma and taste of the final bread (Hansen and Hansen, 1996), even though bread flavour consists of a wide range of chemical compounds obtained not only through dough fermentation by LAB, but also by yeasts, endogenous and microbial enzymatic activities in the dough, and lipid oxidations and thermal reactions during baking (Hansen and Schieberle, 2005; Pozo-Bayon et al., 2006). Ethanol was also detected in the presence of *L. plantarum* in rye sourdough (Ravyts and De Vuyst, 2011). *L. plantarum* contains set genes in its chromosome that encode enzymes catalysing the reactions of pyruvate metabolism which allow the production of different metabolites including acetone, ethanol and acetoin (Kleerebezem et al., 2002). High molecular weight alcohols, such as 1-hexanol and 1-octen-3-ol were synthesized during the fermentation process followed in this study. These compounds were produced most likely from the degradation of hydroperoxides which in turn are derived from unsaturated fatty acids (Rehman et al., 2006; Rizzello et al., 2010). The amyl alcohol, 3-methyl-1-butanol, can be formed by the fermentation process from isoleucine, leucine and valine, respectively, through deamination and decarboxylation reactions (Filannino et al., 2014). The characteristics of the breads, in particular softness, mean cell area and cell density indicated best the productions obtained in presence of *L. plantarum* PON100148.

In conclusions, this work demonstrated that *L. plantarum* is able to codominate with OHe LAB species in sourdough production and the strains found at the highest concentrations in mature sourdoughs are characterized by optimal technological traits. Works are being prepared to investigate the fitness provided by these characteristics and how they may ensure the persistence of the strains selected in this study over time, in order to develop starter cultures constituting stable elements for typical Sicilian sourdoughs.

Acknowledgements

This work was financially supported by the project for industrial research and training PON01_02249 “Application of molecular biotechnologies and pro-technological microorganisms for the characterisation and valorisation of dairy and bakery chains of typical products” of the Italian Ministry of Education, University and Research (CUP: B11C11000430005).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fm.2015.04.011.

References

Alfonzo, A., Lo Piccolo, S., Conigliaro, G., Ventorino, V., Burruano, S., Moschetti, G., 2012. Antifungal peptides produced by *Bacillus amyloliquefaciens* AG1 active against grapevine fungal pathogens. Ann. Microbiol. 62, 1593–1599.

Alfonzo, A., Ventimiglia, G., Corona, O., Di Gerlando, R., Gaglio, R., Francesca, N., Moschetti, G., Settanni, L., 2013. Diversity and technological potential of lactic acid bacteria of wheat flours. Food Microbiol. 36, 343–354.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., 1997. Grapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Axelsson, L., 1998. Lactic acid bacteria: classification and physiology. In: Salminen, S. (Ed.), Lactic Acid Bacteria Microbiology: and Functional Aspects. Marcel Dekker, New York, pp. 1–72.

Berthier, F., Ehrlich, S.D., 1998. Rapid species identification within two groups of closely related lactobacilli using PCR primers that target the 16S/23S rRNA spacer region. FEMS Microbiol. Lett. 161, 97–106.

Böcker, G., Stolz, P., Hammes, W.P., 1995. Neue Erkenntnisse zum Ökosystem Sauerteig und zur Physiologie des Sauerteig-Typischen Stämme *Lactobacillus sanfrancisco* und *Lactobacillus pontis*. Getreide Mehl Brot 49, 370–374.

Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B.K., Lim, Y.W., 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int. J. Syst. Evol. Microbiol. 57, 2259–2261.

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J., 2000. Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. J. Agric. Food Chem. 48, 3044–3051.

Corsetti, A., Settanni, L., 2007. Lactobacilli in sourdough fermentation: a review. Food Res. Int. 40, 539–558.

Corsetti, A., Settanni, L., Chaves-López, C., Felis, G.E., Mastrangelo, M., Suzzi, G., 2007. A taxonomic survey of lactic acid bacteria isolated from wheat (*Triticum durum*) kernels and non-conventional flours. Syst. Appl. Microbiol. 30, 561–571.

Corsetti, A., Settanni, L., Braga, T.M., De Fatima Silva Lopes, M., Suzzi, G., 2008. An investigation on the bacteriocinogenic potential of lactic acid bacteria associated with wheat (*Triticum durum*) kernels and non-conventional flours. LWT Food Sci. Technol. 41, 1173–1182.

Dal Bello, F., Clarke, C.I., Ryan, L.A.M., Ulmer, H., Schober, T.J., Ström, K., Sjögren, J., van Sinderen, D., Schnürer, J., Arendt, E.K., 2007. Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. J. Cereal Sci. 45, 309–318.

De Vuyst, L., Neysens, P., 2005. The sourdough microflora: biodiversity and metabolic interactions. Trends Food Sci. Technol. 16, 43–56.

De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, T., Weckx, S., 2009. Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. Food Microbiol. 26, 666–675.

De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H.M., Weckx, S., 2014. Microbial ecology of sourdough fermentations: diverse or uniform? Food Microbiol. 37, 11–29.

Di Cagno, R., De Angelis, M., Gallo, G., Settanni, L., Berloco, M.G., Siragusa, S., Parente, E., Corsetti, A., Gobbetti, M., 2007. Genotypic and phenotypic diversity of *Lactobacillus rossiae* strains isolated from sourdough. J. Appl. Microbiol. 103, 821–835.

Di Cagno, R., De Angelis, M., Calasso, M., Vicentini, O., Vernocchi, P., Ndagijimana, M., De Vincenzi, M., Dessì, M.R., Guerzoni, M.E., Gobbetti, M., 2010. Quorum sensing in sourdough *Lactobacillus plantarum* DC400: induction of plantaricin A (PlnA) under co-cultivation with other lactic acid bacteria and effect of PlnA on bacterial and Caco-2 cells. Proteomics. http://dx.doi.org/10.1002/pmic.200900565.

Filannino, P., Cardinali, G., Rizzello, G.C., Buchin, S., De Angelis, M., Gobbetti, M., Di Cagno, R., 2014. Metabolic responses of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices. Appl. Environ. Microbiol. 80, 2206–2215.

Fusco, V., Quero, G.M., Stea, G., Morea, M., Visconti, A., 2011. Novel PCR-based identification of *Weissella confusa* using an AFLP-derived marker. Int. J. Food Microbiol. 145, 437–443.

Gobbetti, M., 1998. The sourdough microflora: interactions of lactic acid bacteria and yeasts. Trends Food Sci. Technol. 9, 267–274.

Gobbetti, M., Corsetti, A., 1997. *Lactobacillus sanfrancisco* a key sourdough lactic acid bacterium: a review. Food Microbiol. 14, 175–187.

Gobbetti, M., Corsetti, A., Rossi, J., La Rosa, F., De Vincenzi, S., 1994. Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. Italian J. Food Sci. 6, 85–94.

Gobbetti, M., De Angelis, M., Corsetti, A., Di Cagno, R., 2005. Biochemistry and physiology of sourdough lactic acid bacteria. Trends Food Sci. Technol. 16, 57–69.

Hammes, W.P., Vogel, R.F., 1995. The genus *Lactobacillus*. In: Wood, B.J.B. (Ed.), The Genera of Lactic Acid Bacteria. Blackie Academic & Professional, London, pp. 19–54.

Hansen, Å., Hansen, B., 1996. Flavour of sourdough wheat bread crumb. Zeitchcrift Lebensm. Unterschung Forsch. 202, 244–249.

Hansen, Å., Schieberle, P., 2005. Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. Trends Food Sci. Technol. 16, 85–94.

Hartnett, D.J., Vaughan, A., Van Sinderen, D., 2002. Antimicrobial producing lactic acid bacteria isolated from raw barley and sorghum. J. Inst. Brew. 108, 169–177.

Iacumin, L., Cecchini, F., Manzano, M., Osualdini, M., Boscolo, D., Orlic, S., Comi, G., 2009. Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. Food Microbiol. 26, 128–135.

Jack, R.W., Tagg, J.R., Ray, B., 1995. Bacteriocins of Gram-positive bacteria. Microbiol. Rev. 59, 171–200.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O.P., Leer, R., Tarchini, R., Peters, S.A., Sandbrink, H.M., Fiers, M.E.J., Stiekema, W., Klein Lankhorst, R.M., Bron, P.A., Hoffer, S.M., Nierop Groot, M.N., Kerkhoven, R., de Vries, M., Ursing, B., de Vos, W.M., Siezen, R.J., 2002. Complete genome sequence of *Lactobacillus plantarum* WCFS1. Proc. Natl. Acad. Sci. 100, 1990–1995.

Kline, L., Sugihara, T.F., 1971. Microorganisms of the San Francisco sour dough bread process. II. Isolation and characterization of undescribed bacterial species responsible for the souring activity. Appl. Microbiol. 21, 459–465.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., Gobetti, M., 2000. Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl. Environ. Microbiol. 66, 4084–4090.

Luangsakul, N., Keeratipibul, S., Jindamorakot, S., Tanasupawat, S., 2009. Lactic acid bacteria and yeasts isolated from the starter doughs for Chinese steamed buns in Thailand. Food Sci. Technol. 42, 1404–1412.

Magnusson, J., Ström, K., Roos, S., Sjögren, J., Schnürer, J., 2003. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS Microbiol. Lett. 219, 129–135.

Menteş, Ö., Ercan, R., Akçelik, M., 2007. Inhibitor activities of two Lactobacillus strains, isolated from sourdough, against rope-forming Bacillus strains. Food Contr. 18, 359–363.

Minervini, F., De Angelis, M., Di Cagno, R., Pinto, D., Siragusa, S., Rizzello, C.G., Gobbetti, M., 2010. Robustness of *Lactobacillus plantarum* starters during daily propagation of wheat flour sourdough type I. Food Microbiol. 27 (7), 897–908.

Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappelle, S., Gobbetti, M., 2012. Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: interactions between ingredients and microbial species diversity. Appl. Environ. Microbiol. 78, 1251–1264.

Minervini, F., De Angelis, M., Di Cagno, R., Gobbetti, M., 2014. Ecological parameters influencing microbial diversity and stability of traditional sourdough. Int. J. food Microbiol. 171, 136–146.

Moore, M.M., Juga, B., Schober, T.J., Arendt, E.K., 2007. Effect of lactic acid bacteria on properties of gluten-free sourdoughs, batters, and quality and ultrastructure of gluten-free bread. Cereal Chem. 84, 357–364.

Niiku-Paavola, M.-L., Laitila, A., Mattila-Sandholm, T., Haikara, A., 1999. New types of antimicrobial compounds produced by Lactobacillus plantarum. J. Appl. Microbiol. 86, 29–35.

Ottogalli, G., Galli, A., Foschino, R., 1996. Italian bakery products obtained with sour dough: characterization of the typical microflora. Adv. Food Sci. 18, 131–144.

Pepe, O., Blaiotta, G., Moschetti, G., Greco, T., Villani, F., 2003. Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. Appl. Environ. Microbiol. 69, 2321–2329.

Pozo-Bayon, M.A., Guichard, E., Cayot, N., 2006. Flavor control in baked cereal products. Food Rev. Int. 22, 335–379.

Randazzo, C.L., Heilig, H., Restuccia, C., Giudici, P., Caggia, C., 2005. Bacterial population in traditional sourdough evaluated by molecular methods. J. Appl. Microbiol. 99, 251–258.

Ravyts, F., De Vuyst, L., 2011. Prevalence and impact of single-strain starter cultures of lactic acid bacteria on metabolite formation in sourdough. Food Microbiol. 28, 1129–1139.

Rehman, S., Paterson, A., Piggott, J.R., 2006. Flavour in sourdough breads: a review. Trends Food Sci. Technol. 17, 557–566.

Rizzello, C.G., Nionelli, L., Coda, R., De Angelis, M., Gobbetti, M., 2010. Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. Food Chem. 119, 1079–1089.

Robert, H., Gabriel, V., Fontagné-Faucher, C., 2009. Biodiversity of lactic acid bacteria in French wheat sourdough as determined by molecular characterization using species-specific PCR. Int. J. Food Microbiol. 135, 53–59.

Ryan, L.A.M., Dal Bello, F., Arendt, E.K., 2008. The use of sourdough fermented by antifungal LAB to reduce the amount of calcium propionate in bread. Int. J. Food Microbiol. 125, 274–278.

Scheirlinck, I., Van der Meulen, R., Van Schoor, A., Vancanneyt, M., De Vuyst, L., Vandamme, P., Huys, G., 2007. Influence of geographical origin and flour type on the diversity of lactic acid bacteria in traditional Belgian sourdoughs. Appl. Environ. Microbiol. 73, 6262–6269.

Scheirlinck, I., Van der Meulen, R., Van Schoor, A., Vancanneyt, M., De Vuyst, L.P.V., Huys, G., 2008. Taxonomic structure and stability of the bacterial community in Belgian sourdough ecosystems assessed by culturing and population fingerprinting. Appl. Environ. Microbiol. 74, 2414–2423.

Scheirlinck, I., Van der Meulen, R., De Vuyst, L., Vandamme, P., Huys, G., 2009. Molecular source tracking of predominant lactic acid bacteria in traditional Belgian sourdoughs and their production environments. J. Appl. Microbiol. 106, 1081–1092.

Schillinger, U., Lücke, F.K., 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55, 1901–1906.

Schober, T.J., Messerschmidt, M., Bean, S.R., Park, S.H., Arendt, E.K., 2005. Gluten free bread sorghum: quality difference among hybrids. Cereal Chem. 82, 394–404.

Settanni, L., Di Grigoli, A., Tornambé, G., Bellina, V., Francesca, N., Moschetti, G., Bonanno, A., 2012. Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a Caciocavallo type cheese. Int. J. Food Microbiol. 155, 73–81.

Settanni, L., Massitti, O., Van Sinderen, D., Corsetti, A., 2005b. *In situ* activity of a bacteriocin-producing *Lactococcus lactis* strain. Influence on the interactions between lactic acid bacteria during sourdough fermentation. J. Appl. Microbiol. 99, 670–681.

Settanni, L., Moschetti, G., 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiol. 27 (6), 691–697.

Settanni, L., Moschetti, G., 2011. Biodiversity of sourdough lactic acid bacteria. In: Rescigno, V., Maletta, S. (Eds.), Biodiversity Hotspots. Nova Science Publishers, New York, pp. 37–79.

Settanni, L., Van Sinderen, D., Rossi, J., Corsetti, A., 2005a. Rapid differentiation and *in situ* detection of 16 sourdough *Lactobacillus* species by multiplex PCR. Appl. Environ. Microbiol. 71, 3049–3059.

Settanni, L., Ventimiglia, G., Alfonzo, A., Corona, O., Miceli, A., Moschetti, G., 2013. An integrated technological approach to the selection of lactic acid bacteria of flour origin for sourdough production. Food Res. Int. 54, 1569–1578.

Sjögren, J., Magnusson, J., Broberg, A., Schnürer, J., Kenne, L., 2003. Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* MiLAB 14. Appl. Environ. Microbiol. 69, 7554–7557.

Spicher, G., 1983. Baked goods. In: Rehm, J.H., Reed, G. (Eds.), Biotechnology. Wiley-VCH, Weinheim, pp. 1–80.

Spicher, G., 1999. Zur Geschichte des Sauerteiges. In: Spicher, G., Stephan, H. (Eds.), Handbuch Sauerteig: Biologie, Biochemie, Technologie. Behr's Verlag, Hamburg, pp. 3–7.

Ström, K., Sjögren, J., Broberg, A., Schnürer, J., 2002. *Lactobacillus plantarum* MiLAB393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-L-Pro) and phenyl lactic acid. Appl. Environ. Microbiol. 68, 4322–4327.

Todorov, S., Onno, B., Sorokine, O., Chobert, J.M., Ivanova, I., Dousset, X., 1999. Detection and characterization of a novel antibacterial substance produced by *Lactobacillus plantarum* ST31 isolated from sourdough. Int. J. Food Microbiol. 48, 167–177.

Torriani, S., Felis, G.E., Dellaglio, F., 2001. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with *recA* gene-derived primers. Appl. Environ. Microbiol. 67, 3450–3454.

Valmorri, S., Settanni, L., Suzzi, G., Gardini, F., Vernocchi, P., Corsetti, A., 2006. Application of novel polyphasic approach to study the lactobacilli composition of sourdoughs from the Abruzzo region (Central Italy). Lett. Appl. Microbiol. 43, 343–349.

Vermelho, A.B., Meirelles, M.N.L., Lopes, A., Petinate, S.D.G., Chaia, A.A., Branquinha, M.H., 1996. Detection of extracellular proteases from microorganisms on agar plates. Mem. Ist. Oswaldo Cruz 91, 755–760.

Villani, F., Salzano, G., Sorrentino, E., Pepe, O., Marino, P., Coppola, S., 1993. Enterocin 226 NWC, a bacteriocin produced by *Enterococcus faecalis* 226, active against *Listeria monocytogenes*. J. Appl. Bacteriol. 74, 380–387.

Vogel, R.F., Pavlovic, M., Ehrmann, M.A., Wiezer, A., Liesegang, H., Offschanka, S., Voget, S., Angelov, A., Böcker, G., Liebl, W., 2011. Genomic analysis reveals *Lactobacillus sanfranciscensis* as stable element in traditional sourdoughs. Microb. Cell. Factories 10 (1). http://dx.doi.org/10.1186/1475-2859-10-S1-S6.

Währen, M., 1985. Die entwicklungsstationen vom korn zum brot in 5.und 4. jahrtausend. Neueste untersuchungsergebnisse von ausgrabungsfunden. Getreide Mehl Brot 39, 373–379.

Weisburg, W., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703.

Yang, E.J., Chang, H.C., 2010. Purification of a new antifungal compound produced by *Lactobacillus plantarum* AF1 isolated from kimchi. Int. J. Food Microbiol. 139, 56–63.

Zhang, J., Liu, W., Sun, Z., Bao, Q., Wang, F., Yu, J., Chen, W., Zhang, H., 2011. Diversity of lactic acid bacteria and yeasts in traditional sourdoughs collected from western region in Inner Mongolia of China. Food Control 22, 767–774.