Microbial consortia involved in fermented spelt sourdoughs: dynamics and characterization of yeasts and Lactic Acid Bacteria

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Running headline: Spelt sourdough microbiota

Significance and Impact of the Study: Nowadays, there is a renewed interest in products based on spelt. This “ancient grain" is a highly nutritional grain, however, its use is limited to bread making processes not yet standardized. The low baking and sensory quality of spelt can be overcome through fermentation processes. However, the autochthonous microbiota of spelt sourdough is poorly known. This study highlights the dynamics of microbial communities involved in sourdough fermentation of spelt and provides the basis for the selection of autochthonous cultures, with the aim of improving the nutritional potential of spelt and its rheology and breadmaking properties.

Abstract

This study aims to describe the native microbiota of fermented spelt, taking into consideration both lactic acid bacteria (LAB) and yeasts, for which little data are available. Five samples of commercial spelt flour were subjected to spontaneous fermentation to obtain a type I sourdough. A total of 186 LAB and 174 yeast strains were selected at different refreshment steps and subjected to further analyses. Within LAB, coccal isolates constituted 78.5% of the total LAB strains, with the dominance of *Pediococcus pentosaceus*. Although documented before as a component, this is the first report of a spelt sourdough fermentation dominated by this homofermentative LAB, characterized by a high acidification rate, ability to utilize a wide range of carbon sources and to grow in high osmolarity condition. Yeast communities resulted in four dominant species, *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus, Pichia fermentans* and *Clavispora lusitaniae*. This study highlights for the first time the biodiversity and dynamics of yeast communities involved in sourdough fermentation of spelt. Compared to commercial baker's yeast, autochthonous *W. anomalus, P. fermentans* and *S. cerevisiae* strains show a good performance, and their use could be an advantage for their acquired adaptation to the environment, providing stability to the fermentation process.

Keywords: Spelt, Sourdough microbiota, Lactic Acid Bacteria, Yeasts, Molecular identification, Characterization

Introduction

Cereals represent a staple component of the human diet, and grains such as wheat, rice and, in developing or emerging countries sorghum, maize and millets, provide more than half of the total world calorie intake (FAO 2012). The cultivation of these grains saw an important increase due to their high yield, but current trends are going towards the rediscovery of “ancient grains", such as spelt (*Triticum spelta*), mainly for the increased interest in locally grown crops with particular nutritional properties (Troccoli *et al.* 2005; Frakolaki *et al.* 2018). Particularly, spelt seem to be popular and largely cultivated in Germany, Belgium, Austria, Slovenia and Northern Italy. Despite a lower grain yield, spelt shows an increased resistance to harsh and unsuitable conditions. Indeed, this species can grow in marginal lands, with low soil and climatic requirements, and is suitable for growing without the use of pesticides, making it adequate for the expanding organic food market (Troccoli *et al.* 2005). From a nutritional point of view, spelt has a higher protein content than the standard cultivars of wheat, a higher content of soluble dietary fibre, a high vitamin, minerals and unsaturated fatty acid content, as well as a lower phytic acid content (Bonafaccia *et al.* 2000; Kohajdová and Karovičová 2008). However, its use is still limited in bread making processes, mainly due to the low baking and sensory quality. These limitations can be overcome through a sourdough fermentation process, where a specific microbiota, deriving from natural flour contamination and the environment, interacts for the processing of the matrix in a high quality product. During sourdough fermentation, a succession of naturally occurring microorganisms results in a population dominated by lactic acid bacteria (LAB) and yeasts. They play a key role for their multiple “functional” metabolic properties (Ganzle 2009).

The biodiversity of the natural microbial population in traditional sourdoughs represents an interesting way for the selection of novel types of cultures, well adapted to the specific fermentation process and able to give a satisfactory performance in the process. This search requires a screening

of a large number of isolates in small-scale fermentations, through which it is possible to highlight the dominant microbial strains characterizing the sourdough and responsible for the final product.

Despite the renewed interest in products based on spelt, sourdough fermentation process and the autochthonous microbiota of this 'ancient grain' are poorly known. The literature refers to a few works, mainly focusing on LAB (Van der Meulen *et al.* 2007; Coda *et al.* 2010). No data are available up to now on the autochthonous yeasts characterizing spelt flour and their interaction with autochthonous LAB species.

This study aims to describe the native microbiota of spontaneously fermented spelt, taking into consideration both lactic acid bacteria (LAB) and yeasts. The dominating strains were identified and characterized.

Results and Discussion

Microbial population of spelt sourdough

Fermentation was characterized by a rapid increase of LAB within the first days of propagation. The number of LAB increased from 10^4-10^5 CFU g^{-1} of the initial dough, going up to 10^9 CFU g^{-1} after two days of fermentation. This value persisted during all refreshment steps, with little variations. For yeast counts, more variation was observed. Yeasts generally started in a much lower concentration than LAB and reached high amounts (10^6-10^7 CFU g^{-1}), only after 4-5 days of back-slopping. Moulds reached between 3 and 4 logarithmic cycles; in the first steps, but they were completely absent starting from the 5^{th} refreshment, probably due to the antimould activity of LAB and/or yeast communities. In sample B, no mould growth was observed. Viable cell counts performed on different refreshment steps are reported in Table S1.

89 The pH values did not differ during fermentation for all sourdoughs. The initial pH value resulted to
90 be 6.1±0.27; after 2 days of fermentation an important decrease of the pH was observed, reaching a
91 value of 3.87±0.17, which was maintained until the end of the fermentation (3.64±0.06).

92 **Molecular identification**

93 Within LAB, coccal isolates constituted 78.5% of the total LAB strains, characterizing the spelt
94 sourdough analyzed. The identification approach allowed to classify the 146 coccal LAB into three
95 different genera, namely *Pediococcus*, *Leuconostoc* and *Weissella*. The first step, the RSA analysis,
96 highlighted 3 different clusters, one of which included the majority of strains (140). This major
97 cluster was characterized by an RSA profile associated to *Pediococcus* genus (310, 480, 500 bp),
98 the other two clusters showed an RSA profile typical of *Leuconostoc* (600 bp) and *Weissella* (450,
99 550, 650 bp) genus (Kabadjova et al., 2002). The results were confirmed by genus-specific PCR
100 experiments. The strains characterizing the major cluster were further identified at the species-level.
101 Species-specific PCR confirmed 136 strains to be *Ped. pentosaceus*, and 4 strains were ascribed to
102 *Ped. acidilactici* species.

103 The RSA profiles of the 40 lactobacilli strains resulted to be poorly discriminating, for this
104 reason a series of species-specific PCRs and *16S* rRNA gene sequencing were carried out. This
105 approach allowed the identification of 18 *Lactobacillus brevis*, 11 *Lact. curvatus* 1 *Lact. fermentum*,
106 1 *Lact. rhamnosus*. 6 *Lact. plantarum*, 1 *Lact. paraplantarum* and 2 strains of *Lact. pentosus*.

107 For the identification of yeast strains, the Internal Transcribed Spacer (ITS) was amplified,
108 and all isolates were grouped in 4 clusters, characterized by a PCR product of 380, 430, 630 and
109 850 base pairs respectively. An ITS of 850 bp is typical of the genus *Saccharomyces* (Valente et al,
110 1996), and a species-specific PCR confirmed the belonging of 69 strains to *S. cerevisiae* species.
111 For the remaining clusters the 26S rRNA gene was amplified and sequenced. The results obtained
112 allowed to identify the 35 strains of the first cluster as *Clavispora lusitaniae*, the 11 strains

belonging to the second one as *Pichia fermentans*, and the third cluster was comprised of 59 strains of *Wickerhamomyces anomalus*.

Microbial population dynamics

Microbial populations of the 5 sourdoughs are reported in Figure 1. The distribution of LAB species isolated from the 5 spelt flour samples shows the dominance of *Ped. pentosaceus* during the fermentation, representing 73% of the total LAB isolated. Particularly, strains of this species were the only isolated during all refreshment steps in sample E and after four days of back-slopping in sample B. In sample D, although initially the refreshment step was characterized by the presence of *Lact. curvatus*, from the second refreshment step, *Ped. pentosaceus* became dominant. In sample C, the final stage of the fermentation was characterized by a codominance of *Ped. pentosaceus* and *Lact. brevis.* The sample A presented a different succession of bacterial species; the composition resulted more complex, and rods dominated the fermentation. Although *Ped. pentosaceus* and *Lact. brevis* played a significant role, other species such as *Leuconostoc* spp., *Weissella* spp., *Lact. fermentum* initially, and *Lact. plantarum* and *Ped. acidilactici* in the final phases dominated the microbial community. These species have been previously reported in sourdoughs made from a range of cereals, including spelt (Van der Meulen *et al.* 2007; Coda *et al*. 2010; Manini *et al.* 2014; Decimo *et al.* 2017). However, our findings highlight the dominance of *Ped. pentosaceus* at various steeping times, suggesting that this LAB species is the most important influential bacterial species involved in the spelt fermentation and may therefore be exploited as potential starter/adjunct culture.

Yeast communities of the 5 samples resulted in four dominant species, namely *S. cerevisiae*, *W. anomalus*, *P. fermentans* and *C. lusitaniae*. While the first three species are frequently detected in sourdough fermentation of different cereals (Carbonetto *et al.* 2018), *C. lusitaniae* is less frequently isolated from sourdoughs and considered an emerging pathogen (Gargeya *et al.*1990;

Zhang *et al.* 2010). The presence of this yeast as a dominating species in sample A and especially in sample B could thus be correlated to a low quality of the flours and their appropriateness for consumption should be investigated. While samples C, D and E led to the isolation of only one yeast species, *W. anomalus* in sample C and *S. cerevisiae* in samples D and E, sample A was comprised of three species, among which, *P. fermentans* that replaced *W. anomalus* in the last stage of fermentation. *W. anomal*us has been frequently isolated from spontaneous laboratory fermentations and its presence can be related to the flour natural microbiota; *S. cerevisiae* has been mainly isolated from bakery sourdoughs as opposed to laboratory spontaneous fermentations; this fact could imply a contamination from the bakery's environment, so *S. cerevisiae* could not be considered a natural component of the flour (Vrancken *et al.* 2010). This could be the case of samples D and E, where the only presence of *S. cerevisiae* could be due to a contamination of the flour in the producing facility. *P. fermentans* is not reported as a dominant species in traditional sourdoughs, but it seems play an important role in wheat bran fermentation (Manini *et al.* 2014) and in Boza, a Turkish cereal-based beverage (Caputo *et al.* 2012).

Sourdough fermentation-related characterization

A subset of four strains of *S. cerevisiae*, *P. fermentans*, *W. anomalus*, in comparison with a commercial baker's *S. cerevisiae* strain, and eight *P. pentosaceus* strains, were studied for some characteristics related to sourdough fermentation. Regarding yeasts (Table 1; Fig. S1), *P. fermentans* seems the species with less adaptability to different carbon sources, being unable to ferment sucrose and maltose. However, the lack of maltose fermentation ability could involve a non-competitive interaction with maltose-positive LAB. *W. anomalus* shows the better osmoadaptation, being able to grow in presence of 30% of the four carbon sources tested. Moreover, together with *S. cerevisiae* strains, *W. anomalus* shows a high tolerance to low pH

values. The presence of acetic acid did not limit the growth of *P. fermentans*, and at a minor extent, that of *S. cerevisiae* strains.

Regarding *Ped. pentosaceus*, all tested strains were able to ferment glucose, fructose and maltose (Table 2, Fig. S1). Sucrose utilization resulted to be a strain-specific characteristic. This aspect is well-known for this species, as sucrose-utilization plasmids have been widely described for the genus *Pediococcus* (Naumoff and Livshits 2001). All strains showed a good osmoadaptation to high carbon source concentrations and a good acidification ability: the rate of acidification was high during the initial 10 h of incubation (ΔpH 1.5), and then stabilizes at a pH value of 4.0 within the 24 h of incubation (Fig. S2). The high acidification activity after the first hours of fermentation is desired as it acts on bread structure. On the contrary, the ability to grow at low pH values is poor. This implies that the growth of the strains decreases and/or ceases when dough reaches a pH value < 4. Another characteristic that could be exploited is the autolysis ability, with the release of a pool of enzymatic activities of interest. The *Ped. pentosaceus* strains tested show a good degree of autolysis at pH 6.5, that decreases with the lowering of the pH.

In conclusion, this study is, to our knowledge, the first report that highlights the biodiversity and dynamics of microbial communities involved in sourdough fermentation of spelt, with specific regards to yeast population. Four dominant yeast species were recovered, including *C. lusitaniae*, whose role and safety deserve further studies. Compared to commercial baker's yeast, autochthonous *W. anomalus*, *P. fermentans* and *S. cerevisiae* strains show a good performance, and their use could be an advantage for their acquired adaptation to the environment, providing the stability of the fermentation process. Moreover, although documented before as a component (Van der Meulen *et al.* 2007; Weckx *et al.*2010), the paper is, to our knowledge, the first report of a spelt sourdough fermentation dominated by *Ped. pentosaceus*, a homofermentative LAB species, characterized by a high acidification rate, ability to utilize a wide range of carbon sources and to grow in high osmolarity condition. Thus, the data obtained provides the basis for the selection of

autochthonous mixed cultures, with the aim of improving the nutritional potential of spelt and its rheological and breadmaking properties. This will allow the incorporation of spelt flour in bakery products formulations, with wheat flour or as a substitute to wheat flour.

Materials and methods

Sourdough preparation and microbial population

Five samples (A, B, C, D and E) of commercial wholemeal spelt flour of different brands, produced in different Italian regions (Marche, Umbria, Puglia, Toscana and Veneto respectively) were subjected to spontaneous fermentation to obtain a type I sourdough preparation (Manini *et al.* 2014). This type of sourdough is characterized by daily back-sloppings performed by using the previous sourdough to inoculate a fresh water-flour mixture, that helps to maintain the microorganisms in an active state. The fermentation was carried out at 30°C during a period of 7 days. The pH values were recorded daily. Samples at different refreshment steps (2, 4, 5 and 7) were diluted and plated for the determination of: non-lactic acid bacteria (NLAB) on Plate Count Agar (PCA) (Sigma, St Louis, MO, USA), LAB on MRS agar (Difco Lab., Augsburg, Germany) and M17 agar (Difco), yeasts and moulds on Yeast Extract Glucose Chloramphenicol Agar (YGC) (Sigma). The plates were incubated at 30°C for 48 h. To characterize the autochthonous LAB and yeast population, from each flour sample about 10 colonies of LAB and 10 colonies of yeasts were recovered at the refreshment steps 2, 4, 5 and 7 and purified by successive streaking. A total of 186 LAB and 174 yeast strains were selected and subjected to identification and further analyses. A commercial baker's yeast was used for phenotypic comparison. Strains were routinely grown in MRS broth for LAB and Yeast extract Peptone Dextrose broth (YPD) (Sigma) for yeasts.

Molecular identification

212 Total bacterial DNA was extracted from 100 µL of an overnight culture, using the Microlysis kit
213 (Labogen, Rho, Italy) following the manufacturer's instructions. For yeasts, DNA extraction was
214 performed in a PRECELLYS®24-DUAL lyser/homogeniser (Bertin-technologies, Saint Quentin en
215 Yvelines, France), as previously described (Decimo *et al.* 2017).
216 For LAB identification, a first clustering step was obtained by a PCR amplification of the *16S–23S*
217 rDNA spacer region (RSA). Molecular identification of LAB isolates with different RSA patterns
218 was carried out using species-specific probes and/or by *16S* rDNA gene sequencing. Molecular
219 identification of yeast strains was carried out by a PCR amplification of the Internal Transcribed
220 Spacer (ITS), species-specific probes and/or partial *26S* rDNA gene sequencing. The list of primers
221 and relative thermal cycles is reported in Table S2. Amplification was carried out in a Mastercycler
222 (Eppendorf, Hamburg, Germany). PCR reactions were performed as described before (Decimo *et*
223 *al.* 2017). Amplicons were purified using NucleoSpin® Extract II (Macherey-Nagel, Düren,
224 Germany) and sequenced at Eurofins Genomics (Ebersberg, Germany). Sequence alignment was
225 carried out with ClustalW software. The NCBI BLAST software was used for sequence similarity
226 search (www.ncbi.nlm.nih.gov/BLAST).
227
228 **Sourdough fermentation-related characterization**
229 To investigate the physiological adaptation of the yeast and LAB community to the sourdough
230 fermentation environment, several characteristics were determined for a subset of strains: utilization
231 of different carbon sources, osmoadaptation, low pH tolerance, and the ability to grow in the
232 presence of acetic acid. For LAB strains autolysis degree and acidification ability were also
233 evaluated. Tests were carried out in duplicate. For carbon source utilization test, YP and a
234 LAB-basal medium (containing g l^{-1}: peptone 15, yeast extract 6, Tween 80 1 ml l^{-1}, pH 6.4) or
235 basal MRS were used for yeasts and LAB respectively. The filter-sterilized carbohydrates were
236 added at a final concentration of 10 g l^{-1} and, for evaluating the osmoadaptation of the strains, at a

final concentration of 300 g l^{-1}. To test low pH tolerance, the growth media were adjusted to pH 2.5 and 3.5 with 1 m l^{-1} HCl. To test the ability to grow in the presence of acetic acid, growth media were supplemented with 10 g l^{-1} of acetic acid, after which the pH was corrected to 5.0. All tests were carried out using 1% inoculum of fresh cultures. Growth was determined by measurements of OD_{600} after 24h of incubation at 30°C. Autolytic phenotype was tested according to Mora *et al.* (2003). The strains were incubated in MRS medium and cells were harvested during the exponential growth phase (OD_{600} between 0.8 and 1), washed in potassium phosphate buffer (50 mM, pH 6.5) and resuspended in the same buffer at pH 6.5 or 4, to and OD_{600} of 0.6 to 0.8. The suspension was incubated at 30°C and the degree of autolysis was expressed as the percentage decrease of the OD_{600} after 48 h.

Conflict of interest

The authors declare no conflict of interest

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Supporting Information

Table S1 Microbial population in spelt flour samples at various refreshment steps

Table S2 Primer sequences and relative thermal cycles used for the identification of the isolates

Figure S1 Growth at different concentrations of carbon sources. S1A: *Pediococcus pentosaceus* isolates tested. ◇ MB33 ◆ MB31 ○ FB22 ● FB14 ▲ CM73 ◆ CR35 ● SE136 ■ PPM1; S1B: Yeast isolates tested. *Wickerhamomyces anomalus* isolates: ■ FL11 ○ FL12 ● FL15 ◆ FL35; *Pichia fermentans* isolates: ■ CRL42 ▲ CRL49 ● CRL22 ● FL10; *Saccharomyces cerevisiae* isolates: △ D8 □ F1L10 ◆ B8 ◇ F2L20 ▲ BS.

Figure S2. Changes in pH during growth of the *Pediococcus pentosaceus* isolates tested in MRS broth.

Table S1 Microbial population in spelt flour samples at various refreshment steps

Spelt flour samples	Refreshment step	Viable cell count (logCFU g^{-1}) LAB (M17)	LAB (MRS)	Yeasts (YGC)	Moulds (YGC)	NLAB (PCA)
A	start	4.6±0.2	4.1±0.1	<1	3.3±0.1	4.5±0.1
	2	9.3±0.1	9.4±0.2	3.0±0.1	3.2±0.2	9.0±0.9
	4	9.4±0.7	9.5±0.2	7.8±0.5	-	9.4±0.5
	5	9.5±0.2	9.5±0.2	7.7±0.5	-	9.5±0.1
	7	9.5±0.2	9.4±0.7	4.1±0.8	-	8.4±0.1
B	start	4.8±0.0	4.9±0.3	3.7±0.2	-	6.6±0.8
	2	9.7±0.9	9.7±0.1	4.6±0.1	-	6.6±0.8
	4	8.8±0.2	9.2±0.1	6.2±0.8	-	5.1±0.1
	5	10.5±0.6	11.6±0.8	5.7±0.2	-	5.8±0.5
	7	11.9±0.8	12.6±0.8	8.1±0.1	-	6.3±0.1
C	start	3.7±0.5	3.8±0.1	<1	-	3.8±0.2
	2	9.4±0.1	9.3±0.4	<1	3.8±0.2	8.9±0.8
	4	8.2±0.1	9.1±0.3	6.3±0.6	3.8±0.5	9.0±0.3
	5	8.8±0.1	8.7±0.5	6.9±0.5	-	8.9±0.5
	7	9.3±0.8	9.4±0.1	5.4±0.8	-	9.3±0.7
D	start	4.9±0.1	4.1±0.5	<1	2.7±0.0	5.1±0.5
	2	8.9±0.4	8.2±0.4	<1	3.1±0.6	7.7±1.1
	4	9.7±0.8	8.9±0.8	5.5±0.1	-	9.1±0.4
	5	9.5±0.2	9.6±0.1	7.1±0.1	-	9.0±0.2
	7	10.1±0.8	9.6±0.1	7.2±0.8	-	8.3±0.2
E	start	4.5±0.1	3.9±0.1	<1		4.7±0.1
	2	9.7±0.5	8.9±0.8	2.7±0.0	3.5±0.5	7.2±0.8
	4	9.9±0.1	9.2±0.5	6.8±0.1	4.1±0.3	8.1±0.8
	5	10.1±0.6	9.3±0.2	7.5±0.2	-	8.5±0.5
	7	10.5±0.8	9.3±0.1	7.7±0.7	-	8.5±0.3

Microbial counts are expressed as log CFU g^{-1}. Values are the means ±standard deviation from two independent experiments

Table S2 Primer sequences and relative thermal cycles used for the identification of the isolates

Target	Thermal cycle (× 35 cycles)	Sequence (5'-3')	Reference
16S–23S rDNA spacer region (RSA).	94 °C × 45 s; 54 °C × 1 min 72 °C × 1 min	F: GAAGTCGTAACAAGG R: CAAGGCATCCACCGT	Lane, D.J. (1991) 16S/23S rRNA sequencing. In *Nucleic acid techniques in bacterial systematics* ed. Stackebrandt, E.and Goodfellow, M. pp 115–175. New York:Wiley.
16S rDNA gene	94 °C × 45 s; 55 °C × 45 s 72 °C × 1 min	F: AGAGTTTGATCCTGGCTCAG R: CTACGGCTACCTTGTTACGA	Lane (1991)
Lact plantarum	94 °C × 2 min; 56 °C × 1 min 72 °C × 1 min	F: CCGTTTATGCGGAACACC R: TCGGGATTACCAAACATCAC	Torriani, S., Felis, G.E. and Dellaglio, F. (2001) Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA* gene-derived primers. *Appl Environ Microbiol* **67**, 3450–3454.
Pediococcus spp.	94 °C × 45 s; 64 °C × 1 min 72 °C × 1 min	F: GAACTCGTGTACGTTGAAAAGTGCTGA R: GCGTCCCTCCATTGTTCAAACAAG	Pfannebecker, J. and Fröhlich, J. (2008) Use of a species-specific multiplex PCR for the identification of pediococci. *Int J Food Microbiol* **128**, 288-296
Ped. pentosaceus	94 °C × 1 min; 67 °C × 1 min 72 °C × 1 min	F: CCAGGTTGAAGGTGCAGTAAAAT R: CTGTCTCGCAGTCAAGCTC	Pfannebecker and Fröhlich (2008)
Lact. brevis	94 °C × 45 s; 48 °C × 45 s 72 °C × 1 min	F: TGTACACACCGCCCGTC R: TAATGATGACCTTGCGGTC	Coton, M., Berthier, F. and Coton, E. (2008) Rapid identification of the three major species of dairy obligate heterofermenters *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus parabuchneri* by species-specific duplex PCR. *FEMS Microbiol Lett* **284**, 150-157.
Lact. fermentum	94 °C × 45 s; 48 °C × 45 s 72 °C × 1 min	F: TGTACACACCGCCCGTC R: TTTTCTTGATTTTATTAG	Coton *et al.* (2008)
Lact. paraplantarum	94 °C × 45 s; 56 °C × 1 min 72 °C × 1 min	F: GTCACAGGCATTACGAAAAC R: TCGGGATTACCAAACATCAC	Torriani *et al.* (2001)
Lact. pentosus	94 °C × 45 s; 56 °C × 1 min 72 °C × 1 min	F: CAGTGGCGCGGTTGATAT R: TCGGGATTACCAAACATCAC	Torriani *et al.* (2001)
Lact. rhamnosus	94 °C × 45 s; 54 °C × 45 s 72 °C × 1 min	F: CCCACTGCTGCCTCCCGTAGGAGT R: TGCATCTTGATTTAATTTTG	Ward, L.J.H.M. and Timmins, J. (1999) Differentiation of *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* by polymerase chain reaction. *Lett Appl Microbiol* **29**, 90–92.
Lact.curvatus	94 °C × 45 s; 56 °C × 1 min 72 °C × 1 min	F: GCTGGATCACCTCCTTTC R: TTGGTACTATTTAATTCTTAG	Berthier, F. and Ehrlich, SD. (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

Leuconostoc spp.	94°C x 45 s; 55°C x 45 s 72 °C × 1 min	F: CCACAGCGAAAGGTGCTTGCAC R: GATCCATCTCTAGGTGACGCCG	Yost, C.K. and Nattress, F.M. (2000) The use of multiplex PCR reactions to characterize populations of lactic acid bacteria associated with meat spoilage. *Lett Appl Microbiol* **31**, 129-133.
Weissella spp.	94 °C × 45 s; 54 °C × 1 min 72 °C × 1 min	F: CGTGGGAAACCTACCTCTTA R: CCCTCAAACATCTAGCAC	Jang, J., Kim, B., Lee, J., Kim, J., Jeong, G. and Han, H. (2002) Identification of *Weissella* species by the genus-specific amplified ribosomal DNA restriction analysis. *FEMS Microbiol Lett* **212**, 29-34.
ITS1-5.8S-ITS2 internal transcribed spacer (ITS)	94 °C × 45 s; 60 °C × 1 min 72 °C × 1 min	F: TCCGTAGGTGAACCTGCGG R: TCCTCCGCTTATTGATATGC	Jespersen, L., Nielsen, D.S., Hønholt, S. and Jakobsen, M. (2005) Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. *FEMS Yeast Res* **5**, 441–453.
26S rDNA gene	94 °C × 2 min;52 °C × 1 min 72 °C × 1 min	F: GCATATCAATAAGCGGAGGAAAAG R: GGTCCGTGTTTCAAGACGG	Kurtzman, C.P. and Robnett, C.J. (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Ant van Leeuwen* **73**, 331–371.
S. cerevisiae	94 °C × 45 s; 61 °C × 1 min 72 °C × 1 min	F: GTTAGATCCCAGGCGTAGAACAG R: GCGAGTACTGGACCAAATCTTATG	de Melo Pereira, G.V., Ramos, C.L., Galvão, C., Souza Dias, E. and Schwan, R.F. (2010) Use of specific PCR primers to identify three important industrial species of *Saccharomyces* genus: *Saccharomyces cerevisiae, Saccharomyces bayanus* and *Saccharomyces pastorianus. Lett Appl Microbiol* **51**, 131–137.

Figure S1 Growth at different concentrations of carbon sources. S1A: *Pediococcus pentosaceus* isolates tested. ◇ MB33 ◆ MB31 ○ FB22 ● FB14 ▲ CM73 ◈ CR35 ● SE136 ■ PPM1; S1B: Yeast isolates tested. *Wickerhamomyces anomalus* isolates: ■ FL11 ○ FL12 ● FL15 ◆ FL35; *Pichia fermentans* isolates: ■ CRL42 ▲ CRL49 ● CRL22 ● FL10; *Saccharomyces cerevisiae* isolates: △ D8 □ F1L10 ◈ B8 ◇ F2L20 ▲ BS (commercial baker's yeast strain.)

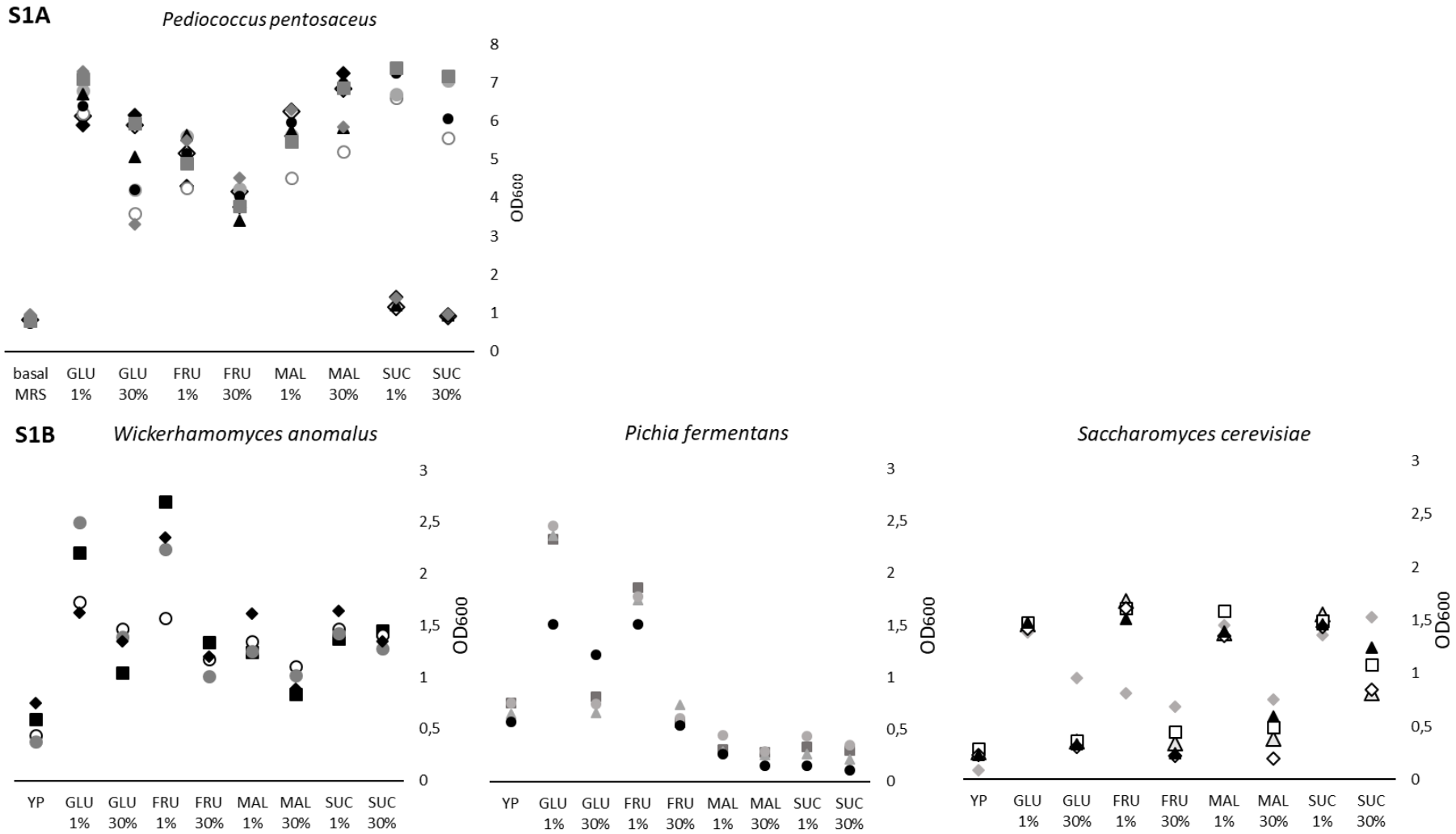


Figure S2 Changes in pH during growth of the *Pediococcus pentosaceus* isolates tested in MRS broth.

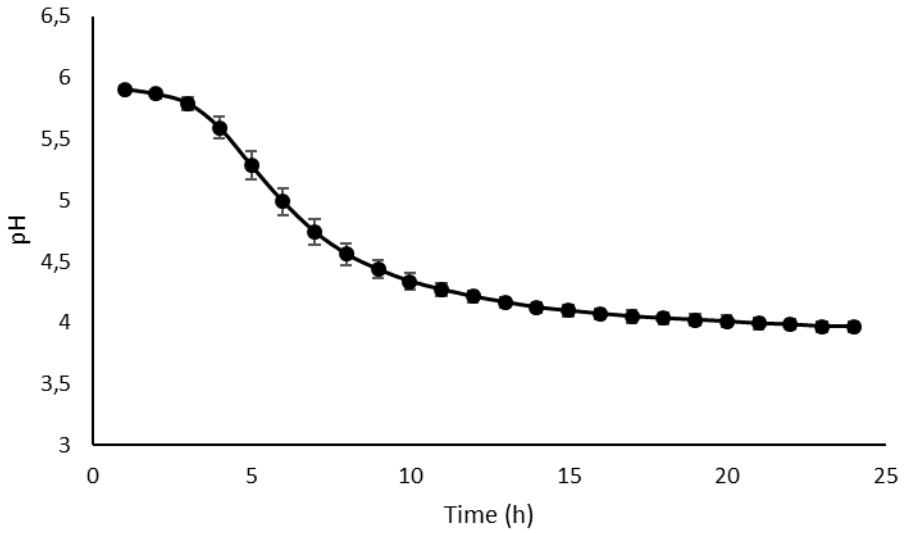


Table 1 Physiological profiles of the yeast strains tested

Species	Strain	Carbon source utilization (1%)				Osmoadaptation (30% carbon source)				Tolerance to low pH		Growth with acetic acid
		Glucose	Fructose	Sucrose	Maltose	Glucose	Fructose	Sucrose	Maltose	2.5	3.5	
Wickerhamomyces anomalus	FL 11	+	+	+	+	+	+	+	+	+	+	-
	FL 12	+	+	+	+	+	+	+	+	+	+	-
	FL 15	+	+	+	+	+	+	+	+	+	+	-
	FL 34	+	+	+	+	+	+	+	+	+	+	-
Pichia fermentans	CRL 42	+	+	-	-	+	+/-	-	-	-	+	+
	CRL 49	+	+	-	-	+	+/-	-	-	-	+	+
	CRL 22	+	+	-	-	+	+/-	-	-	-	+	+
	FL 10	+	+	-	-	+	+/-	-	-	-	+	+
Saccharomyces cerevisiae	D8	+	+	+	+	-/+	-	+/-	-/+	+	+	+
	F1L10	+	+	+	+	-/+	-	+	+/-	+	+	-/+
	B8	+	+/-	+	+	+	+/-	+	+/-	+	+	+
	F2L20	+	+	+	+	-/+	-	+	-	+	+	-
	BS*	+	+	+	+	-/+	-	+	+/-	-/+	+	-/+

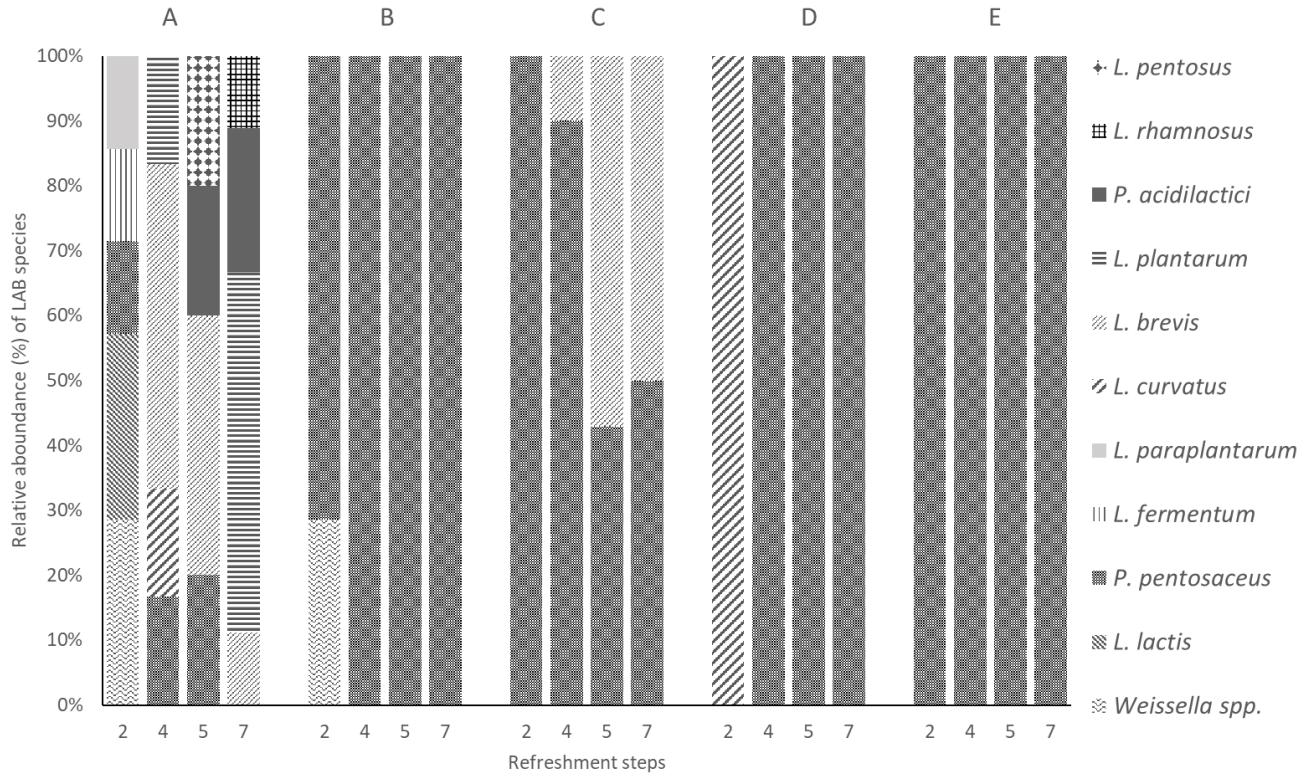
* BS strain: commercial baker's yeast

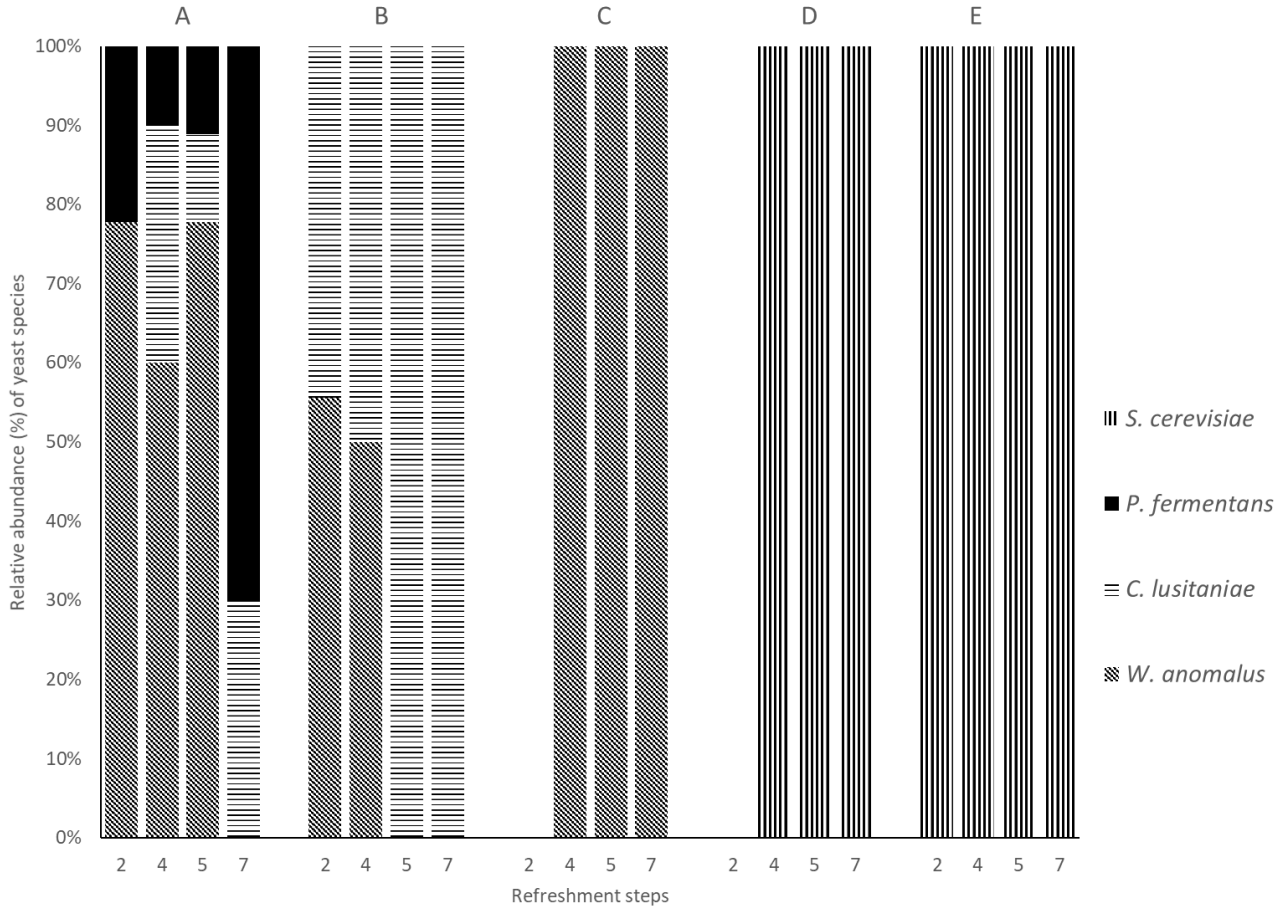
Table 2 Physiological profiles of the *Pediococcus pentosaceus* strains tested

Pediococcus pentosaceus strains	Carbon source utilization (1%)				Osmoadaptation (30% carbon source)				Tolerance to low pH		Growth with acetic acid	Autolysis (%)	
	Glucose	Fructose	Sucrose	Maltose	Glucose	Fructose	Sucrose	Maltose	2.5	3.5		pH 4	pH 6.5
PPM1	+	+	+	+	+	+	+	+	-	-	+	15.5	34.8
SSE136	+	+	+	+	+	+	+	+	-	-	+	0.0	36.2
CR35	+	+	-	+	+	+	-	+	-	-	+	1.9	28.6
CM73	+	+	-	+	+	+	-	+	-	+/-	+	14.8	29.8
FB14	+	+	+	+	+	+	+	+	-	-	+/-	16.4	34.9
FB22	+	+	+	+	+	+	+	+	-	+/-	+	2.9	27.1
MB31	+	+	-	+	+	+	-	+	-	-	+/-	10.0	36.9
MB33	+	+	-	+	+	+	-	+	-	-	+	17.0	38.2

Figure legend

Figure 1 Population dynamics of LAB (1A) and yeasts (1B) during sourdough-like fermentation of spelt flour. (1A) *Weissella* spp. *Leuconostoc* spp. *Pediococcus pentosaceus* *Lactobacillus fermentum* *Lactobacillus paraplantarum* *Lactobacillus curvatus* *Lactobacillus brevis* *Lactobacillus plantarum* *Pediococcus acidilactici* *Lactobacillus rhamnosus* *Lactobacillus pentosus* (1B) *Saccharomyces cerevisiae* *Pichia fermentans* *Clavispora lusitaniae* *Wickerhamomyces anomalus*


A
B
C
D
E
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance (%) of LAB species
2 4 5 7
Refreshment steps
L. pentosus
L. rhamnosus
P. acidilactici
L. plantarum
L. brevis
L. curvatus
L. paraplantarum
L. fermentum
P. pentosaceus
L. lactis
Weissella spp.


A
B
C
D
E
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative abundance (%) of yeast species
2 4 5 7
Refreshment steps
S. cerevisiae
P. fermentans
C. lusitaniae
W. anomalus

18

19