

Article

Identification of Lactic Acid Bacteria Isolated from Sourdoughs Produced with Flours of Different Cereals in North-Western Spain via MALDI-TOF MS and *pheS* Gene Analyses

Lorena Celador-Lera ¹, Rosana Chiva ² , Ana Jiménez-López ², Fernando Sánchez-Juanes ^{3,4} , José Antonio Uña ¹ , Raúl Rivas ^{1,5} , María Ángeles Santos ¹ , Encarna Velázquez ^{1,5,*} and Mercedes Tamame ² 

- ¹ Departamento de Microbiología y Genética, Universidad de Salamanca, 37007 Salamanca, Spain; lorenacelador@usal.es (L.C.-L.); jaua@usal.es (J.A.U.); raulrg@usal.es (R.R.); gemail@usal.es (M.Á.S.)
- ² Instituto de Biología Funcional y Genómica (IBFG), CSIC-Universidad de Salamanca, 37007 Salamanca, Spain; rosanachiva@usal.es (R.C.); anajim11@gmail.com (A.J.-L.); tamame@usal.es (M.T.)
- ³ Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, 37007 Salamanca, Spain; fsjuanes@usal.es
- ⁴ Instituto de Investigación Biomédica de Salamanca (IBSAL), Complejo Asistencial Universitario de Salamanca, CSIC-Universidad de Salamanca, 37007 Salamanca, Spain
- ⁵ Instituto Hispanoluso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, 37185 Salamanca, Spain
- * Correspondence: evp@usal.es

Abstract

Lactic acid bacteria (LAB) are Gram-positive cocci or rods from different genera and species that synergistically coexist with yeasts in cereal sourdoughs. Although the production and consumption of sourdough breads have increased in the last decade, there are still few studies that identify the LABs present in sourdoughs of different origins. In this study, we identified LAB strains isolated from wheat sourdoughs sourced from bakeries in north-western Spain and from experimental sourdoughs developed by a baker with four different cereal flours: wheat, rye, spelt, and tritordeum. Through a combination of MALDI-TOF and *pheS* gene sequence analyses, we identified *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* in all analysed sourdoughs. Moreover, *Lactiplantibacillus paraplantarum*, *Lactocaseibacillus paracasei*, *Lactococcus lactis*, *Pediococcus pentosaceus*, and *Weissella paramesenteroides* were identified in different sourdoughs. These species were also isolated from sourdoughs obtained in other European countries, although we did not identify the other LAB genera identified in some of them. Our results demonstrate the need to identify the LAB species from sourdoughs of different origins prior to designing suitable starters for inoculated sourdoughs, particularly those produced with innovative flours. To our knowledge, this is the first report on the identity of LABs present in tritordeum sourdoughs in Spain.



Academic Editor: Franco Van de Velde

Received: 28 May 2026

Revised: 30 July 2026

Accepted: 31 July 2026

Published: 6 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

Keywords: lactic acid bacteria; sourdough; cereals; MALDI-TOF MS; *pheS* gene; Spain

1. Introduction

The production and consumption of bread made with sourdoughs (called *levain* in French, *sauerteig* in German, *pasta madre* or *lievito madre* in Italian, and *masa madre* in Spanish) have increased considerably in the last decade. Sourdoughs can be developed with different cereals, and traditionally, wheat and rye are the most common cereals used in Europe [1]. More recently, spelt, an ancient variety of wheat [2], and tritordeum, a hybrid of wheat

and barley developed in Spain at the end of the last century [3], have also been employed. Currently, tritordeum is cultivated in several European countries. The highest producers are Spain, Italy, and Greece [4], where this cereal is already being used to produce bread as a reliable alternative to wheat in the development of low-gluten breads [5–7] to improve their digestibility [8].

Sourdoughs are characterized by very complex biological systems dominated by lactic acid bacteria (LABs), which synergistically coexist with yeasts that are well adapted to this acidic environment but are present at lower cell concentrations than LABs [9]. LABs isolated from sourdoughs are generally accepted as safe microorganisms that play important roles in food fermentation and preservation and therefore could be used to formulate novel starters for the bakery industry [10]. LABs are Gram-positive cocci or rods belonging to different families, genera, and species included in the order *Lactobacillales* [11]. They are ubiquitous bacteria in different systems, such as cereals in which flours contain LABs responsible for fermentation processes during food production [12]. The production and consumption of sourdough bread have increased considerably in the last decade, and studies on the identity and diversity of LABs in sourdoughs have been carried out in different countries, demonstrating that they belong to more than 100 species from 25 genera [13].

The genera *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Weissella* have been found in several European countries. They have been identified in wheat sourdoughs from Italy [14–23], France [24–26] and Belgium [27–29], *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Weissella* in Austria [30], Bulgaria [31], and Turkey [32–34], and *Lactobacillus* and *Weissella* in Greece [35]. In rye sourdoughs, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Weissella* have been found in Italy [17]; *Lactobacillus* in France [26]; *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella* in Austria [30] and Belgium [36]; *Lactobacillus*, *Pediococcus*, and *Weissella* in Estonia [37,38]; *Lactobacillus* and *Pediococcus* in Bulgaria [30,39]; *Lactobacillus*, *Leuconostoc*, and *Pediococcus* in Lithuania [40]; *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Weissella* in Turkey [33,41]; and *Leuconostoc* and *Weissella* in Spain [42]. In oat sourdoughs the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella* have been identified in Ireland [43]. In barley sourdoughs *Lactobacillus*, *Pediococcus*, and *Leuconostoc* have been found in Turkey [44], and *Lactobacillus*, *Leuconostoc*, and *Weissella* in Belgium [45]. In spelt sourdoughs *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* have been identified in Belgium [27], and *Pediococcus*, *Leuconostoc*, and *Weissella* have been found in Italy [46]. In *broa*, a traditional ancient bread from Portugal produced with maize and rye flours, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* have been identified [47,48].

Most of these studies were performed before the genus *Lactobacillus* was split into several new genera, as proposed by Zheng et al. [49]. Several studies were conducted afterwards identifying species from some of these new genera; particularly the genera *Companilactobacillus*, *Fructilactobacillus*, *Furfurilactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Lentiplantibacillus*, *Levilactobacillus*, *Limosilactobacillus*, *Loigolactobacillus*, and *Paucilactobacillus* were found in sourdoughs produced with different cereals in various European regions [50–52].

Studies published in the last decade enabled the identification of some of these new genera in wheat sourdoughs in Austria, Belgium, Bulgaria, France, Greece, Italy, Moldavia, and Turkey; in rye sourdoughs in Austria, Belgium, Estonia, France, Lithuania, and the United Kingdom; in spelt sourdoughs in Belgium and Italy; in triticale (hybrid of wheat and rye) sourdoughs in Belgium; in mixed wheat and rye and/or spelt sourdoughs in Spain; and in tritordeum sourdoughs in Italy [10,53–62]. A number of these new genera were also identified in sourdoughs composed of different cereals in Belgium, France, and the United Kingdom [63] and in traditional sourdoughs collected through a citizen science initiative in Belgium, France, the Netherlands, and Spain as part of the HealthFerm project [64]. The

identification of LABs in these European sourdoughs has been mainly achieved via the sequencing of the 16S rRNA gene and in some studies via MALDI-TOF MS [30,34,57,58] or *pheS* gene analyses [22,26–29,36]. Nevertheless, as far as we know, to date these methods have not been used in combination to assess LABs in sourdoughs.

Despite the range of studies conducted during last decade that focused on the identification of LAB species in sourdoughs worldwide, there is still a lack of data on the identity of LABs present in sourdoughs in many European countries. Therefore, more studies are necessary as the identification of LAB strains from sourdoughs produced in different regions with different types of flours would likely contribute to discovering a greater LAB biodiversity [39] and would enable the design of new starters to formulate new types of breads. Therefore, the aim of this study was to identify the LABs isolated from different cereal sourdoughs produced in north-western Spain via MALDI-TOF MS and *pheS* gene analyses, which have been combined in previous works to identify LAB in fermented foods [65,66].

2. Materials and Methods

2.1. LAB Isolation and Counting

LAB strains were isolated from wheat sourdoughs produced in different bakeries in three provinces of north-western Spain—Ávila, Salamanca, and Zamora—which were used to isolate yeasts in a previous study [67]. We also isolated LAB strains from experimental firm and liquid sourdoughs produced by an expert baker (E. Mateos) with flours of wheat, spelt, rye and tritordeum (Tables S1 and S2). These experimental sourdoughs were created through spontaneous fermentation of the cereal flours and propagated for about one month via daily back-slopping, as described by Chiva et al. [67].

To perform the isolation, 10 g samples from each sourdough were weighed, and 90 mL of sterile peptone water (1 g/L peptone, 8.5 g/L NaCl) was added in 250 mL flasks. The mixtures were incubated at 28 °C for 1 h with shaking at 200 rpm. Subsequently, 5 mL of each mixture were centrifuged at low speed ($3000\times g$) to remove the flour residue. Serial decimal dilutions from the resulting supernatants were inoculated onto MRS agar plates (Man, Rogosa and Sharpe medium, Sigma Co., Saint Louis, MO, USA). The plates were incubated at 28 °C for 4 days under aerobic conditions. At this time, the colonies were counted and transferred to new plates to obtain pure cultures for species identification.

2.2. MALDI-TOF MS and Data Analysis

Sample preparation and MALDI-TOF MS analysis were carried out as previously described [68]. Briefly, bacterial biomass (5–100 mg) was prepared using a matrix solution of saturated alpha-cyano-4-hydroxycinnamic acid (α -HCCA; Bruker Daltonics, Leipzig, Germany) in 50% acetonitrile and 2.5% trifluoroacetic acid. Mass spectrometry measurements were performed on an Autoflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Leipzig, Germany) equipped with a 200 Hz smart beam laser. Replicate analyses were performed in triplicate for each sample from independent target spots. Spectra were recorded in the linear, positive mode at a laser frequency of 200 Hz within a mass range of 2000 to 20,000 Da. The instrumental settings included an IS1 voltage of 20 kV, an IS2 voltage maintained at 18.6 kV, a lens voltage of 6 kV, and an extraction delay time of 40 ns. For each individual spectrum, a total of 500 laser shots were accumulated and analysed (10×50 laser shots from distinct positions of the target spot). External mass calibration was validated using the Bruker Bacterial Test Standard (BTS) (Bruker Daltonics, Leipzig, Germany) [69]. Microbial identification was performed according to the standard logarithmic score values and thresholds recommended by the manufacturer. For cluster analysis, a dendrogram was constructed based on the comparison of main strain-specific spectra using the statistical

toolbox in Matlab 7.1 (MathWorks Inc., Natick, MA, USA) integrated within the MALDI Biotyper 3.0 software (Bruker Daltonics, Leipzig, Germany), applying a correlation distance measure and average linkage hierarchical clustering.

2.3. Genomic DNA Extraction and Phylogenetic Analysis of *pheS* Gene

Genomic DNA of isolated strains was extracted with the Bacterial DNA Extraction Kit (Sigma-Aldrich, Saint Louis, MO, USA) following the manufacturer's instructions. The amplification and sequencing of *pheS* genes were carried out as indicated by Doan et al. [65]. The sequences obtained were compared with those from the GenBank using the Blastn programme [70]. The obtained sequences and those of related bacteria retrieved from GenBank were aligned using the Clustal W program [71]. The phylogenetic distances were calculated according to Kimura's two-parameter model [72], and phylogenetic trees were inferred using the neighbour joining model [73]. MEGA 7.0 [74] was used for all the phylogenetic analyses.

3. Results

3.1. LAB Cell Counting and Strain Isolation

The number of LABs in the bakery sourdoughs (mBAL) and experimental firm or liquid sourdoughs ranged from $2 (\pm 0.04)$ to $9 (\pm 0.1) \times 10^7$ CFU g⁻¹ and $1 (\pm 0.03)$ to $3 (\pm 0.07) \times 10^9$ CFU g⁻¹, respectively. A total of 213 and 166 strains were isolated from the bakery sourdoughs produced with wheat flours (mBAL) and experimental (firm and liquid) sourdoughs (one month old) formulated with wheat, rye, spelt, and tritordeum flours, respectively (Table 1). All of these strains were identified via MALDI-TOF MS.

Table 1. Results of the identification via MALDI-TOF and *pheS* gene sequence analyses of LAB strains isolated from fermented sourdoughs produced with different cereal flours.

Sourdough Codes (Number of Isolated Strains)	Cereal/Sourdough Type	Origin	Identified LAB Species via MALDI-TOF MS	Representative Strain/MALDI- TOF Group	<i>pheS</i> Gene Similarity Values (%) *
mBAL5 (1), mBAL11 (5), mBAL12 (9) mBAL13 (7), mBAL17 (1), mBAL18 (20)	wheat/firm	Ávila and Zamora bakeries	<i>Lactocaseibacillus paracasei</i>	mBAL11_1/III, mBAL12_21/V mBAL18_15/I, mBAL13_7/II, mBAL18_20/IV	99.7
mBAL4 (7), mBAL5 (4), mBAL11 (8), mBAL12 (3) mBAL1 (17), mBAL10 (2), mBAL19 (23) mBAL13 (1), mBAL14 (10), mBAL15 (14), mBAL17 (10)	wheat/firm	Ávila, Salamanca, and Zamora bakeries	<i>Lactiplantibacillus plantarum</i>	mBAL4_3/VI mBAL4_20/V mBAL1_4/IX, mBAL19_15/IV	100
BARI (15), BMOL (13) BPARi (14), BPMOL (13)	wheat/firm wheat/liquid	experimental	<i>Lactiplantibacillus plantarum</i>	BMOL_18/II BPMOL_17/VIII	100
BCEN (14) BPCEN (13)	rye/firm rye/liquid	experimental	<i>Lactiplantibacillus plantarum</i>	BCEN_10/VII BPCEN_14/XI	100
BESP (9) BPESP (12)	spelt/firm spelt/liquid	experimental	<i>Lactiplantibacillus plantarum</i>	BESP_5/III BPESP_5/X	100
BTRi (9) BPTRi (12)	tritordeum/firm tritordeum/liquid	experimental	<i>Lactiplantibacillus plantarum</i>	BTRi_13/I BPTRi_13/XII	100
mBAL4 (3)	wheat/firm	Ávila bakery	<i>Lactiplantibacillus paraplantarum</i>	mBAL4_6/II	100
BESP (4) BPESP (4)	spelt/firm spelt/liquid	experimental	<i>Lactiplantibacillus paraplantarum</i>	BESP_7/IV BPESP_2/III, BPESP_13/V	97.9
BTRi (1)	tritordeum/firm	experimental	<i>Lactiplantibacillus paraplantarum</i>	BTRi_2/I	97.9

Table 1. Cont.

Sourdough Codes (Number of Isolated Strains)	Cereal/Sourdough Type	Origin	Identified LAB Species via MALDI-TOF MS	Representative Strain/MALDI- TOF Group	<i>pheS</i> Gene Similarity Values (%) *
mBAL12 (2) mBAL1 (5) mBAL13 (16), mBAL17 (2)	wheat/firm	Ávila, Salamanca, and Zamora bakeries	<i>Levilactobacillus brevis</i>	mBAL12_11/IX mBAL1_3/IV mBAL13_8/VI	98.7–99.2
BMOL (3) BPARi (2), BPMOL (4)	wheat/firm wheat/liquid	experimental	<i>Levilactobacillus brevis</i>	BMOL_20/I BPARi_7/XI	98.7
BPCEN (2)	rye/liquid	experimental	<i>Levilactobacillus brevis</i>	BPCEN_1/X	99.2
BESP (5) BPESP (3)	spelt/firm spelt/liquid	experimental	<i>Levilactobacillus brevis</i>	BESP_10/VII, BPESP_11/III BPESP_14/V	98.7–99.2
BTRi (10) BPTRi (4)	tritordeum/firm tritordeum/liquid	experimental	<i>Levilactobacillus brevis</i>	BTRi_3/VIII BPTRi_5/II	99.2
mBAL4 (1), mBAL12 (5) mBAL9 (1), mBAL10 (5)	wheat/firm	Ávila and Salamanca bakeries	<i>Pediococcus pentosaceus</i>	mBAL4_8/III, mBAL12_24/II mBAL10_7/I	99.7–100
mBAL9 (22), mBAL10 (2)	wheat/firm	Salamanca bakeries	<i>Lactococcus lactis</i>	mBAL9_4/I, mBAL9_16/III, mBAL10_23/II	100
mBAL10 (7)	wheat/firm	Salamanca bakery	<i>Weissella paramesenteroides</i>	mBAL10_8/II mBAL10_18/I	100

* These values are calculated with respect to the strain type of each species.

3.2. MALDI-TOF MS Analysis

For sourdoughs baked in different provinces of north-western Spain with wheat flours, the results of the LAB analysis revealed that these strains matched with scores higher than 2.0 with respect to *Lactocaseibacillus* (Lc.) *paracasei*, *Lactiplantibacillus* (Lt.) *plantarum*, *Lactiplantibacillus* (Lt.) *paraplantarum*, *Levilactobacillus* (Lv.) *brevis*, *Lactococcus* (L.) *lactis*, *Pediococcus* (P.) *pentosaceus*, and *Weissella* (W.) *paramesenteroides* (Table 1 and Table S3). The most abundant species in the wheat fermented sourdoughs from Ávila bakeries was *Lt. plantarum*, with 46% of the isolated strains, followed by *Lc. paracasei* with 31%, *P. pentosaceus* with 13%, *Lt. paraplantarum*, and *Lv. brevis* with less than 10% for each one (Figure 1 and Table 2). In Salamanca bakeries, the most frequently isolated species was *Lt. plantarum*, with 50% of strains, followed by *L. lactis* with 29% of strains and *Lv. brevis*, *P. pentosaceus*, and *W. paramesenteroides* with less than 10% in each case. In Zamora bakeries, *Lt. plantarum* was the most frequently isolated species, with 43% of strains, followed by *Lc. paracasei* with 35% of strains and *Lv. brevis* with 22% (Figure 1 and Table 2).

Table 2. Percentages of the LAB species isolated in this study.

Sourdoughs	1	2	3	4	5	6	7
Fermented wheat sourdoughs from bakeries in different provinces							
Ávila mBAL4, mBAL5, mBAL11, mBAL12	31%	46%	6%		4%	13%	
Salamanca mBAL1, mBAL9, mBAL10		50%		29%	6%	7%	8%
Zamora mBAL13, mBAL14, mBAL15, mBAL17, mBAL18	35%	43%			22%		
Firm and liquid sourdoughs formulated with different cereals							
wheat		86%			14%		
rye		93%			7%		
spelt		56%	22%		22%		
tritordeum		58%	3%		39%		

1: *Lactocaseibacillus paracasei*, 2: *Lactiplantibacillus plantarum*, 3: *Lactiplantibacillus paraplantarum*, 4: *Lactococcus lactis*, 5: *Levilactobacillus brevis*, 6: *Pediococcus pentosaceus*, and 7: *Weissella paramesenteroides*.

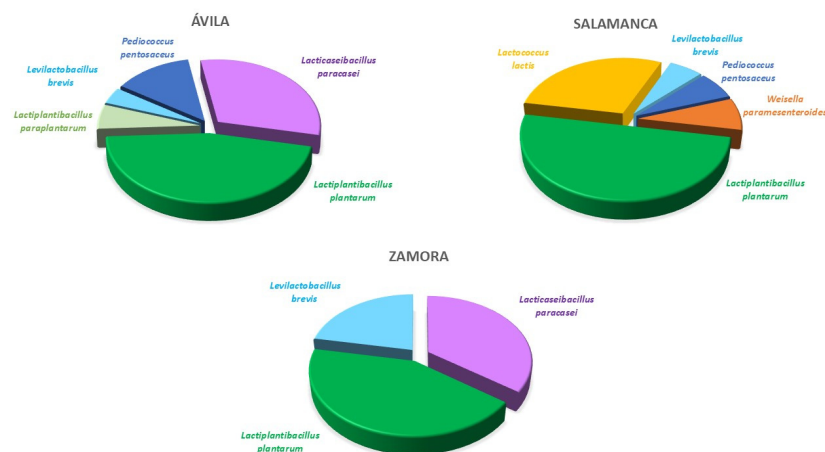


Figure 1. Pie charts showing the distribution of the different species of LAB strains isolated from wheat sourdoughs produced in bakeries from different provinces of north-western Spain.

The LAB strains isolated from liquid and firm sourdoughs formulated with different cereal flours matched with scores higher than 2.0 with respect to *Lt. plantarum*, *Lt. paraplantarum*, and *Lv. brevis* (Tables 1 and S3). The most abundant species (more than 50% of strains) in all these sourdoughs was *Lt. plantarum*—particularly in those elaborated with wheat and rye, where more than 85% of the isolated strains belonged to this species. *Lv. brevis* was also identified in all sourdoughs, although in smaller amounts with less than 40% of the isolated strains. In wheat and rye sourdoughs, *Lt. paraplantarum* was not found, representing its only proportion lower than 25% in sourdoughs baked with spelt and tritordeum (Figure 2 and Table 2). Although we found some differences between firm and liquid sourdoughs from the same cereal, this was the case for the minority of species, such as *Lv. brevis* in the case of rye and *Lt. paraplantarum* in the case of tritordeum (Figure 2 and Table 2).

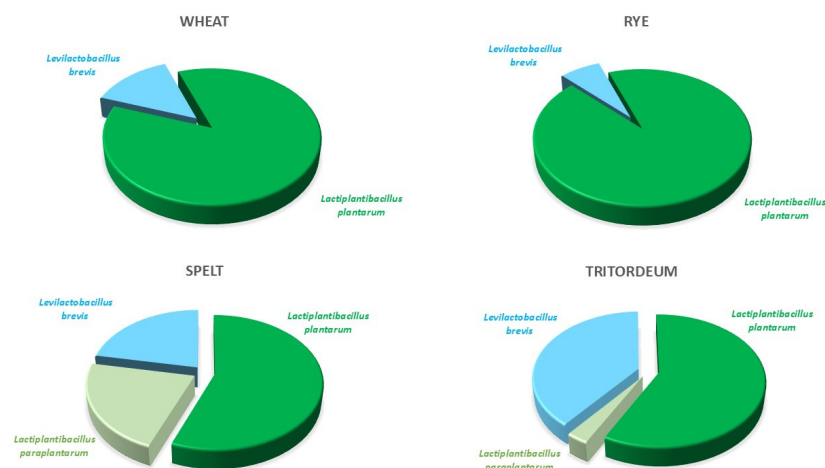


Figure 2. Pie charts showing the distribution of the different species of LAB strains isolated from one-month-old experimental sourdoughs formulated with different cereal flours.

3.3. *pheS* Gene Analysis

The analysis of partial sequences of *pheS* gene of strains isolated from different sourdoughs and representative of different MALDI-TOF MS groups revealed that they clustered with the type strains of the LAB species identified with this methodology (Figures S1–S7, Table 1). The results of the *pheS* analysis confirmed the identification obtained after MALDI-TOF MS analysis at genus and species levels for all strains isolated in this study, with similarity values of *pheS* gene sequences ranging from 97.9% to 100% (Figure 3 and Table 1).

All strains identified via MALDI-TOF MS as *Lt. plantarum*, *W. paramesenteroides*, or *L. lactis* exhibited 100% similarity with respect to the type strain of this species. In the case of *Lc. paracasei*, all strains demonstrated 99.7% similarity with respect to the type strain. In the case of *Lv. brevis*, the strains clustered into two separate groups, one of them with 98.7% similarity and other with 99.2% similarity with respect to the type strain. In the two remaining cases, the strains clustered into two separate groups, one of them with 100% similarity with respect to the type strain of the species identified via MALDI-TOF MS and the one exhibiting different similarity values with respect to those of the type strains. This was the case for *Lt. paraplantarum*, with 97.9% similarity, and *P. pentosaceus*, with 99.7% similarity, with respect to the type strains of these species (Table 1).

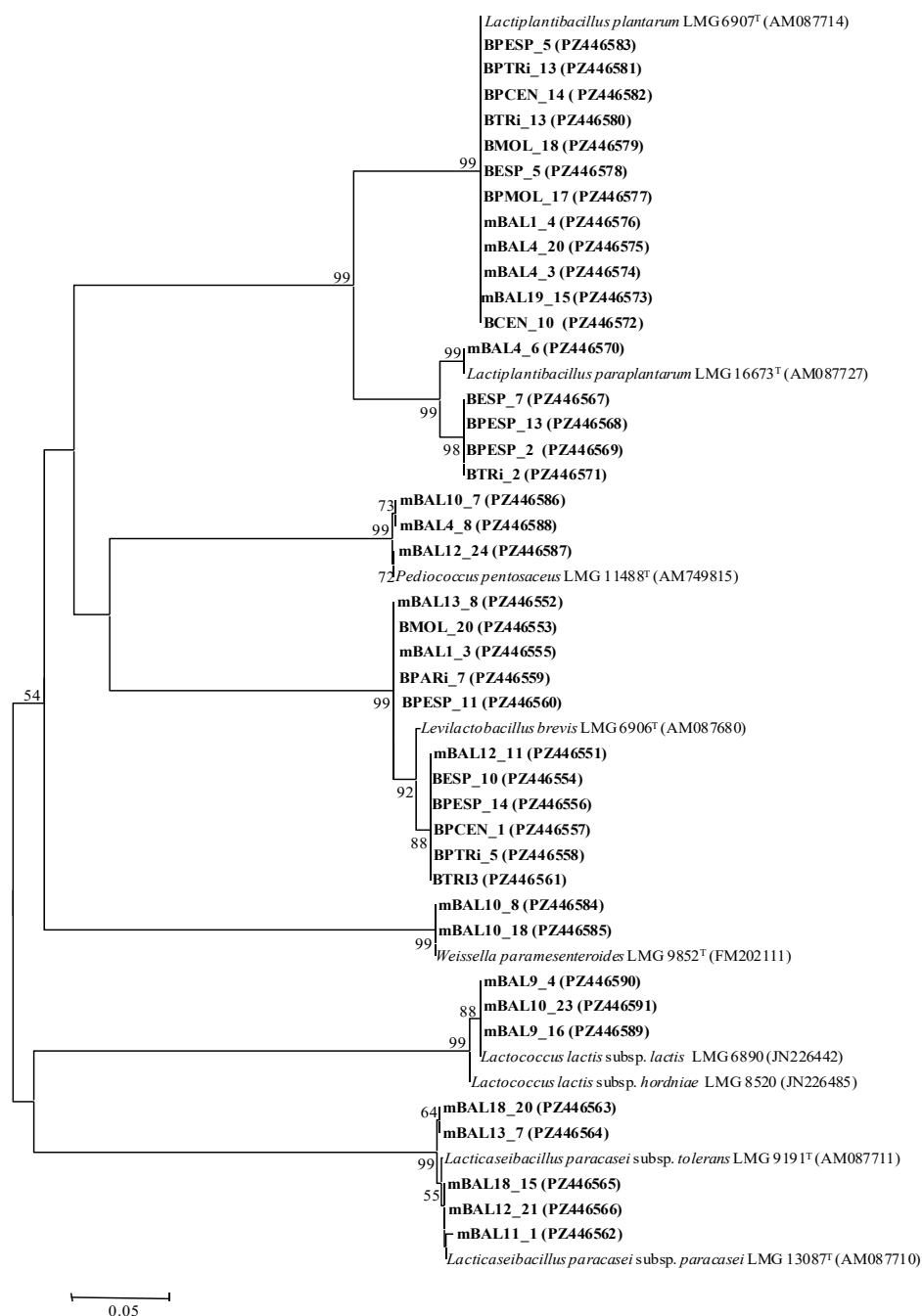


Figure 3. Neighbour joining phylogenetic unrooted tree based on *pheS* partial gene sequences (400 nt), showing the taxonomic location of representative strains from different groups of MALDI-TOF MS found in this study. Bootstrap values calculated for 1000 replications are indicated. Bar, 5 nt substitution per 100 nt. Accession numbers from Genbank are presented in brackets.

4. Discussion

LABs isolated from sourdoughs are generally considered safe microorganisms that play important roles in food fermentation and preservation and could be used in the formulation of novel starters for the bakery industry [10]. In Europe, the EFSA (European Food Safety Authority) has updated the list of “qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed”, including the current names of species of lactic acid bacteria [75]. To date more than 100 species belonging to more than 25 LAB genera have been isolated in sourdoughs [13], but few studies have been performed on the LAB species present in sourdoughs from specific countries. Therefore, the identification of LAB strains isolated from unstudied fermented matrices elaborated in different geographical locations would likely enhance our knowledge of their diversity and biotechnological potential [39]. LAB identification has been achieved using different approaches, but the MALDI-TOF MS methodology combined with *pheS* gene analysis has not been previously used for LAB identification in sourdoughs. However, these two methodologies have been used to identify LABs in some fermented foods [36,38] as the resolution of *pheS* gene analysis for the differentiation of LAB species is higher than that of the 16S rRNA gene and other housekeeping genes, such as *rpoA* [76]. Therefore, in the present study we used these two methods to identify LAB strains isolated from sourdoughs from 11 bakeries (Table S1) and in 10 one-month-old sourdoughs, experimentally formulated with four different cereal flours (Table S2). The results we obtained confirmed that MALDI-TOF MS and *pheS* gene analyses constitute a reliable combined approach for LAB identification, as has been demonstrated previously in other fermented foods [65,66].

We found differences in the LAB composition between the bakery and experimental wheat sourdoughs, since some species isolated in the bakery sourdough were not found in experimental ones, as was the case for *L. lactis*, *Lc. paracasei*, *P. pentosaceus*, *W. paramesenteroides*, and *Lt. paraplantarum* (Figures 1 and 2, Table 2). This may be attributed to different back-slopping practices (the use of fermented sourdough from previous batches) in the different Spanish bakeries and to the microorganisms naturally present at each bakery. Some researchers have already observed that higher numbers of reinoculation cycles in bakeries facilitates a high level of biodiversity and microbial stability in their sourdoughs compared to experimental breads, which are produced de novo or under restricted conditions that fail to replicate real-life environments [13,15,16].

In this study, the LAB species identified in sourdoughs from north-western Spain—*Lv. brevis*, *Lt. plantarum*, *Lt. paraplantarum*, *Lc. paracasei*, *L. lactis*, and *P. pentosaceus*—are included in the updated list of EFSA [75], and they have also been isolated in other European countries in sourdoughs formulated with the cereals analysed in this study: wheat, rye, spelt, and tritordeum [50–52]. Among these cereals, wheat is most traditionally used for bread-making in Europe [1], and rye is the second most used cereal after wheat [77]. Spelt is currently a trendy crop in some areas of Europe [78], and tritordeum is a promising cereal, which is already being used in some European countries' bakeries [79].

In Europe, studies on the LABs isolated from sourdoughs formulated with these cereals have demonstrated that *Lv. brevis* and *Lt. plantarum* are common in wheat sourdoughs in countries such as Belgium in northern Europe; Austria, France, and Moldavia in central Europe; and Bulgaria, Greece, Italy, Spain, and Turkey in southern Europe. To date, the remaining species identified in this work have been identified in fewer countries: *Lc. paracasei* was found in France, Italy, and Turkey; *L. lactis* was found in France, Greece, Italy, and Moldavia; *P. pentosaceus* was found in Bulgaria, France, Italy, and Turkey; *Lt. paraplantarum* was found in Bulgaria, France, Italy, and Turkey; and *W. paramesenteroides* was found in Italy and Turkey (Table 3).

Table 3. LABs identified in the cereal sourdoughs in this study in European countries where they have been found.

LAB Species	Country	Cereal	References
<i>Lactobacillus brevis</i> (currently <i>Levilactobacillus brevis</i>)	Austria	wheat and rye	[30]
	Belgium	wheat	[28]
	Belgium	spelt	[27]
	Belgium	rye	[36,63]
	Bulgaria	wheat	[31,60]
	Bulgaria	spelt	[60]
	Estonia	rye	[37,58]
	France	wheat	[24,25]
	Greece	wheat	[35,54]
	Italy	wheat	[14–16,20–23,56]
	Italy	spelt	[46]
	Lithuania	rye	[40]
	Moldavia	wheat	[61]
<i>Lactobacillus plantarum</i> (currently <i>Lactiplantibacillus plantarum</i>)	Spain	wheat, rye, spelt, and tritordeum	this study
	Turkey	wheat	[10,32,34,53,55]
	United Kingdom	rye	[63]
	Austria	wheat and rye	[30]
	Belgium	wheat	[27–29]
	Belgium	spelt	[27]
	Belgium	rye	[36]
	Bulgaria	wheat	[31,60]
	Bulgaria	spelt	[60]
	Estonia	rye	[37,38]
	France	wheat and rye	[24–26]
	Greece	wheat	[54]
	Italy	wheat	[14–18,20,21,23,56]
<i>Lactobacillus paraplantarum</i> (currently <i>Lactiplantibacillus paraplantarum</i>)	Italy	rye	[17]
	Italy	spelt	[46]
	Italy	tritordeum	[62]
	Lithuania	rye	[40]
	Moldavia	wheat	[61]
	Spain	wheat, rye, spelt, and tritordeum	this study
	Spain	wheat and rye	[59]
	Turkey	wheat	[10,32–34,53,55]
	Turkey	rye	[41]
	United Kingdom	rye	[63]
	Belgium	spelt	[27]
	Bulgaria	wheat and spelt	[60]
<i>Lactobacillus paracasei</i> (currently <i>Lactocaseibacillus paracasei</i>)	France	wheat	[25]
	Italy	wheat	[23]
	Italy	spelt	[46]
	Spain	wheat, spelt, and tritordeum	this study
	Turkey	wheat	[32,34]
	Austria	wheat and rye	[30]
	Belgium	wheat	[28,29]
	Belgium	rye	[63]
	Estonia	rye	[38,58]
	France	wheat	[24,25]
	Italy	wheat	[23]
	Spain	wheat and rye	[59]
	Spain	wheat	this study
<i>Lactococcus lactis</i>	Turkey	wheat	[10]
	United Kingdom	rye	[63]
	Belgium	wheat	[27]
	Belgium	spelt	[27]
	Belgium	rye	[36]
	France	wheat	[25]
	Greece	wheat	[54]
	Italy	wheat	[14–17]
	Lithuania	rye	[40]
	Spain	wheat	this study
	Turkey	rye	[41]

Table 3. Cont.

LAB Species	Country	Cereal	References
<i>Pediococcus pentosaceus</i>	Austria	wheat and rye	[30]
	Belgium	wheat	[27–29]
	Belgium	spelt	[27]
	Belgium	rye	[36]
	Bulgaria	wheat	[31]
	Estonia	rye	[37,58]
	France	wheat	[24,25]
	Italy	wheat	[15,16,18,20,21]
	Italy	rye	[17]
	Italy	spelt	[46]
	Moldavia	wheat	[61]
	Spain	wheat	this study
	Turkey	wheat	[33]
<i>Weissella paramesenteroides</i>	Italy	wheat	[15,16]
	Italy	tritordeum	[62]
	Spain	wheat	this study
	Turkey	wheat	[32]

In rye sourdoughs, *Lv. brevis* and *Lt. plantarum* were also found in more countries than the other species identified in this study. They were found in Belgium, Estonia, Lithuania, and the UK, whereas *Lc. paracasei* was isolated in Estonia and the UK; *L. lactis* was found in Estonia, Lithuania, and Turkey; and *P. pentosaceus* was identified in Estonia (Table 3).

Regarding spelt sourdoughs, *Lt. plantarum* and *Lv. brevis* were also the most commonly identified LABs in European countries, including Belgium, Bulgaria, and Italy. In Italy *Lt. paraplantarum* and *P. pentosaceus* were also isolated, whereas *L. lactis* was found in Belgium (Table 3).

Finally, the LAB species present in tritordeum sourdoughs have only been analysed in Italy to date [48]. In agreement with our results, *Lt. plantarum* and *Weissella paramesenteroides* were identified in Italian tritordeum sourdoughs, but *Lt. paraplantarum* and *Lv. brevis* were absent in these sourdoughs, which in turn contained strains of *Weissella confusa*, *Weissella cibaria*, and *Leuconostoc mesenteroides*, which were not found in this study (Table 3).

According to our results and those of studies performed in other European countries, the LAB species that are most widely distributed in this continent in wheat, rye, spelt, and tritordeum sourdoughs are *Lv. brevis* and *Lt. plantarum*, and *Lc. paracasei* and *P. pentosaceus* as well as *L. lactis*, *Lt. paraplantarum*, and *Weissella cibaria* are less common (Table 3).

5. Conclusions

Through the use of MALDI-TOF MS and *pheS* gene sequence analyses, we identified six genera of LABs—*Lactiplantibacillus*, *Levilactobacillus*, *Lacticaseibacillus* (all of them previously included into the genus *Lactobacillus*), *Lactococcus*, *Pediococcus*, and *Weissella*—in sourdoughs experimentally formulated with wheat, rye, spelt, or tritordeum by a baker in north-western Spain. *Lt. plantarum* and *Lv. brevis* were identified in all experimental sourdoughs (liquid and firm). These species have also been isolated in different cereal sourdoughs produced in European countries, confirming that they could be used in starters for bread production. It is remarkable that *Lt. paraplantarum* was identified in the sourdoughs formulated with tritordeum—a cereal developed in Spain and increasingly incorporated into bread production in other countries, such as Italy—in addition to *Lt. plantarum* and *Lv. brevis*. Our results agree with previous studies that have examined LABs in cereal sourdoughs from other European countries.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fermentation12080367/s1>: Table S1. Sourdoughs produced in bakeries from

different Spanish provinces. Table S2. Cereals used for sourdoughs created by the baker Enrique Mateos-Horganero from “Repostería Enrique Mateos” bakery in Entrala (Zamora province). Table S3. Results of the MALDI-TOF MS analysis. Figure S1. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Lacticaseibacillus paracasei* identified in this study. Distance is displayed in relative units. Figure S2. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Lactiplantibacillus plantarum* identified in this study. Distance is displayed in relative units. Figure S3. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Lactiplantibacillus paraplantarum* identified in this study. Distance is displayed in relative units. Figure S4. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Lactococcus lactis* identified in this study. Distance is displayed in relative units. Figure S5. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Levilactobacillus brevis* identified in this study. Distance is displayed in relative units. Figure S6. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Pediococcus pentosaceus* identified in this study. Distance is displayed in relative units. Figure S7. Cluster analysis of MALDI-TOF MS spectra of the strains belonging to the species *Weissella paramesenteroides* identified in this study. Distance is displayed in relative units.

Author Contributions: Conceptualization, M.T., E.V., M.Á.S. and R.R.; methodology, M.T., E.V., M.Á.S., R.R., R.C., L.C.-L., F.S.-J. and J.A.U.; validation, M.T., E.V., M.Á.S., R.R., R.C., L.C.-L., F.S.-J. and J.A.U.; investigation R.C., L.C.-L., J.A.U., F.S.-J. and A.J.-L.; resources, A.J.-L.; writing—original draft preparation, E.V. and M.T.; writing—review and editing, M.T., E.V., M.Á.S., R.R., F.S.-J., L.C.-L. and R.C.; visualisation, M.T., E.V., M.Á.S., R.R., R.C., F.S.-J. and L.C.-L.; supervision, M.T., E.V. and M.Á.S.; project administration, M.T.; funding acquisition, M.T., M.Á.S. and E.V. All authors have read and agreed to the published version of the manuscript.

Funding: This experimental work was supported in part by Projects Propan (funding number: IPT-2012-1321-06000) and InnoStarPan (funding number: RTC-2015-4391). Funders: Spanish Ministry of Science and Innovation (MICINN) and FEDER (European Regional Development Fund).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: We thank the baker E. Mateos-Horganero for the elaboration of the experimental sourdoughs analysed in this work (Repostería Mateos S.A.-Pan del Duero-Mahorpan, 49721, Zamora, Spain; mahorpan@hotmail.com). We also thank M.A. Carbajo for collecting some bakery sourdoughs from Zamora (Asezpan, Association of Bread Manufacturers, Zamora, Spain); flour factories for providing raw cereal materials and technical information; members of the Propan and Innostarpan R&D Consortia (companies Atrian Bakers, Apliena, Office S.L., and Agrasys S.L., at their headquarters in Barcelona, Spain; and AB Mauri Food, S.A., at their headquarters in Córdoba, Spain). This work is also based upon the work from European COST Action 18101, SOURDOMICS-Sourdough biotechnology network towards novel, healthier, and sustainable food, and bioprocesses, supported by COST, where R.C. and M.T. were members. R.C. and M.T. acknowledge the support of the Excellence Program of Junta de Castilla y León (Escalera de Excelencia CLU-2017-03 co-financed by the P.O. FEDER de Castilla y León 14-20; IES007P17). M.T. is grateful to T. Benítez (University of Sevilla, Spain) and A. Hinnebusch (NICH, NIH, USA) for their mentoring and continued support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

LAB	Lactic Acid Bacteria
MALDI-TOF MS	Matrix Assisted Laser Desorption/Ionisation Time-of-Flight
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid

References

- Suchintita Das, R.; Tiwari, B.K.; Garcia-Vaquero, M. The Fundamentals of Bread Making: The Science of Bread. In *Traditional European Breads: An Illustrative Compendium of Ancestral Knowledge and Cultural Heritage*; Garcia-Vaquero, M., Pastor, K., Orhun, G.E., McElhatton, A., Rocha, J.M.F., Eds.; Springer Nature: Cham, Switzerland, 2023; pp. 1–40.
- Lechterbeck, J.; Kerig, T. Inventions, innovations and the origins of spelt wheat. *Veget. Hist. Archaeobotany* **2024**, *33*, 547–557. [\[CrossRef\]](#)
- Martín, A.; Alvarez, J.B.; Martín, L.M.; Barro, F.; Ballesteros, J. The development of tritordeum: A novel cereal for food processing. *J. Cereal Sci.* **1999**, *30*, 85–95. [\[CrossRef\]](#)
- Sajid Mushtaq, B.; Nikoloudaki, O.; Gobetti, M.; Di Cagno, R. Exploring alternative cereal flours and their potential for sourdough fermentation: Insights from tritordeum and pseudocereals. *Adv. Food Nutr. Res.* **2025**, *117*, 155–192. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sánchez-León, S.; Haro, C.; Villatoro, M.; Vaquero, L.; Comino, I.; González-Amigo, A.B.; Vivas, S.; Pastor, J.; Sousa, C.; Landa, B.B.; et al. Tritordeum breads are well tolerated with preference over gluten-free breads in non-celiac wheat-sensitive patients and its consumption induce changes in gut bacteria. *J. Sci. Food Agric.* **2021**, *101*, 3508–3517. [\[PubMed\]](#)
- Vaquero, L.; Comino, I.; Vivas, S.; Rodríguez-Martín, L.; Giménez, M.J.; Pastor, J.; Sousa, C.; Barro, F. Tritordeum: A novel cereal for food processing with good acceptability and significant reduction in gluten immunogenic peptides in comparison with wheat. *J. Sci. Food Agric.* **2018**, *98*, 2201–2209. [\[PubMed\]](#)
- Haro, C.; Guzmán-López, M.H.; Marín-Sanz, M.; Sánchez-León, S.; Vaquero, L.; Pastor, J.; Comino, I.; Sousa, C.; Vivas, S.; Landa, B.B.; et al. Consumption of tritordeum bread reduces immunogenic gluten intake without altering the gut microbiota. *Foods* **2022**, *11*, 1439. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nitride, C.; D'Auria, G.; Dente, A.; Landolfi, V.; Picariello, G.; Mamone, G.; Blandino, M.; Romano, R.; Ferranti, P. Tritordeum as an Innovative Alternative to Wheat: A Comparative Digestion Study on Bread. *Molecules* **2022**, *27*, 1308. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lau, S.W.; Chong, A.Q.; Chin, N.L.; Talib, R.A.; Basha, R.K. Sourdough Microbiome Comparison and Benefits. *Microorganisms* **2021**, *9*, 1355. [\[CrossRef\]](#) [\[PubMed\]](#)
- Boyaci Gunduz, C.P.; Agirman, B.; Gaglio, R.; Franciosi, E.; Francesca, N.; Settanni, L.; Erten, H. Evaluation of the variations in chemical and microbiological properties of the sourdoughs produced with selected lactic acid bacteria strains during fermentation. *Food Chem.* **2022**, *14*, 100357. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ludwig, W.; Schleifer, K.H.; Whitman, W.B. *Lactobacillales* ord. nov. In *Association with Bergey's Manual Trust*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2015. [\[CrossRef\]](#)
- Ashaolu, T.J.; Reale, A. A holistic review on Euro-Asian lactic acid bacteria fermented cereals and vegetables. *Microorganisms* **2020**, *8*, 1176. [\[CrossRef\]](#) [\[PubMed\]](#)
- De Vuyst, L.; González-Alonso, V.; Raditya Wardhana, Y.; Pradal, I. Taxonomy and species diversity of sourdough lactic acid bacteria. In *Handbook on Sourdough Biotechnology*, 2nd ed.; Gobetti, M., Gänzle, M., Eds.; Springer Nature: Cham, Switzerland, 2023; pp. 97–160.
- Corsetti, A.; Lavermicocca, P.; Morea, M.; Baruzzi, F.; Tosti, N.; Gobetti, M. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *Int. J. Food Microbiol.* **2001**, *64*, 95–104. [\[CrossRef\]](#) [\[PubMed\]](#)
- Minervini, F.; Lattanzi, A.; De Angelis, M.; Di Cagno, R.; Gobetti, M. Influence of artisan bakery- or laboratory-propagated sourdoughs on the diversity of lactic acid bacterium and yeast microbiotas. *Appl. Environ. Microbiol.* **2012**, *78*, 5328–5340. [\[CrossRef\]](#)
- Minervini, F.; Di Cagno, R.; Lattanzi, A.; De Angelis, M.; Antonielli, L.; Cardinali, G.; Cappelle, S.; Gobetti, M. 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.* **2012**, *78*, 1251–1264. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ercolini, D.; Pontonio, E.; De Filippis, F.; Minervini, F.; La Storia, A.; Gobetti, M.; Di Cagno, R. Microbial ecology dynamics during rye and wheat sourdough preparation. *Appl. Environ. Microbiol.* **2013**, *79*, 7827–7836. [\[CrossRef\]](#) [\[PubMed\]](#)
- Corbo, M.R.; Bevilacqua, A.; Campaniello, D.; Speranza, B.; Sinigaglia, M. Selection of promising lactic acid bacteria as starter cultures for sourdough: Using a step-by-step approach through quantitative analyses and statistics. *J. Sci. Food Agric.* **2014**, *94*, 1772–1780. [\[PubMed\]](#)
- Di Cagno, R.; Pontonio, E.; Buchin, S.; De Angelis, M.; Lattanzi, A.; Valerio, F.; Gobetti, M.; Calasso, M. Diversity of the lactic acid bacterium and yeast microbiota in the switch from firm- to liquid-sourdough fermentation. *Appl. Environ. Microbiol.* **2014**, *80*, 3161–3172. [\[CrossRef\]](#) [\[PubMed\]](#)
- Manini, F.; Brasca, M.; Plumed-Ferrer, C.; Morandi, S.; Erba, D.; Casiraghi, M.C. Study of the Chemical Changes and Evolution of Microbiota During Sourdoughlike Fermentation of Wheat Bran. *Cereal Chem.* **2014**, *91*, 342–349. [\[CrossRef\]](#)
- Manini, F.; Casiraghi, M.C.; Poutanen, K.; Brasca, M.; Erba, D.; Plumed-Ferrer, C. Characterization of lactic acid bacteria isolated from wheat bran sourdough. *LWT Food Sci. Technol.* **2016**, *66*, 275–283. [\[CrossRef\]](#)

22. Martorana, A.; Giuffrè, A.M.; Capocasale, M.; Zappia, C.; Sidari, R. Sourdoughs as a source of lactic acid bacteria and yeasts with technological characteristics useful for improved bakery products. *Eur. Food Res. Technol.* **2018**, *244*, 1873–1885. [[CrossRef](#)]
23. Reale, A.; Di Renzo, T.; Boscaino, F.; Nazzaro, F.; Fratianni, F.; Aponte, M. Lactic acid bacteria biota and aroma profile of italian traditional sourdoughs from the irpinian area in Italy. *Front. Microbiol.* **2019**, *10*, 1621.
24. Michel, E.; Monfort, C.; Deffrasnes, M.; Guezenc, S.; Lhomme, E.; Barret, M.; Sicard, D.; Dousset, X.; Onno, B. Characterization of relative abundance of lactic acid bacteria species in French organic sourdough by cultural, qPCR and MiSeq high-throughput sequencing methods. *Int. J. Food Microbiol.* **2016**, *239*, 35–43. [[CrossRef](#)] [[PubMed](#)]
25. Robert, H.; Gabriel, V.; Fontagné-Faucher, C. Biodiversity of lactic acid bacteria in French wheat sourdough as determined by molecular characterization using species-specific PCR. *Int. J. Food Microbiol.* **2009**, *135*, 53–59. [[CrossRef](#)] [[PubMed](#)]
26. Lhomme, E.; Lattanzi, A.; Dousset, X.; Minervini, F.; De Angelis, M.; Lacaze, G.; Onno, B.; Gobbetti, A. Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. *Food Microbiol.* **2015**, *215*, 161–170.
27. Van der Meulen, R.; Scheirlinck, I.; Van Schoor, A.; Huys, G.; Vancanneyt, M.; Vandamme, P.; De Vuyst, L. Population dynamics and metabolite target analysis of lactic acid bacteria during laboratory fermentations of wheat and spelt sourdoughs. *Appl. Environ. Microbiol.* **2007**, *73*, 4741–4750. [[CrossRef](#)] [[PubMed](#)]
28. Scheirlinck, I.; Van der Meulen, R.; Van Schoor, A.; Vancanneyt, M.; De Vuyst, L.; Vandamme, P.; Huys, G. Influence of geographical origin and flour type on diversity of lactic acid bacteria in traditional Belgian sourdoughs. *Appl. Environ. Microbiol.* **2007**, *73*, 6262–6269. [[CrossRef](#)] [[PubMed](#)]
29. Scheirlinck, I.; Van der Meulen, R.; De Vuyst, L.; Vandamme, P.; Huys, G. Molecular source tracking of predominant lactic acid bacteria in traditional Belgian sourdoughs and their production environments. *J. Appl. Microbiol.* **2009**, *106*, 1081–1092. [[CrossRef](#)] [[PubMed](#)]
30. Fraberger, V.; Unger, C.; Kummer, C.; Domig, K.J. Insights into microbial diversity of traditional Austrian sourdough. *Food Sci. Technol.* **2020**, *127*, 109358. [[CrossRef](#)]
31. Petkova, M.; Stefanova, P.; Gotcheva, V.; Angelov, A. Isolation and characterization of lactic acid bacteria and yeasts from typical Bulgarian sourdoughs. *Microorganisms* **2021**, *9*, 1346. [[CrossRef](#)] [[PubMed](#)]
32. Dertli, E.; Mercan, E.; Arıcı, M.; Yılmaz, M.T.; Sagdıç, O. Characterisation of lactic acid bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics. *LWT-Food Sci. Technol.* **2016**, *71*, e116–e124. [[CrossRef](#)]
33. Yağmur, G.; Tanguer, H.; Leventdurur, S.; Bağder Elmacı, S.; Ünal Turhan, E.; Francesca, N.; Settanni, L.; Moschetti, G.; Erten, H. Identification of Predominant Lactic Acid Bacteria and Yeasts of Turkish Sourdoughs and Selection of Starter Cultures for Liquid Sourdough Production Using Different Flours and Dough Yields. *Pol. J. Food Nutr. Sci.* **2016**, *66*, 99–107. [[CrossRef](#)]
34. Sevgili, A.; Erkmen, O.; Koçaslan, S. Identification of lactic acid bacteria and yeasts from traditional sourdoughs and sourdough production by enrichment. *Czech J. Food Sci.* **2021**, *39*, 312–318. [[CrossRef](#)]
35. De Vuyst, L.; Schrijvers, V.; Paramithiotis, S.; Hoste, B.; Vancanneyt, M.; Swings, J.; Kalantzopoulos, G.; Tsakalidou, E.; Messens, W. The biodiversity of lactic acid bacteria in Greek traditional wheat sourdoughs is reflected in both composition and metabolite formation. *Appl. Environ. Microbiol.* **2002**, *68*, 6059–6069. [[CrossRef](#)] [[PubMed](#)]
36. Weckx, S.; Van der Meulen, R.; Maes, D.; Scheirlinck, I.; Huys, G.; Vandamme, P.; De Vuyst, L. Lactic acid bacteria community dynamics and metabolite production of rye sourdough fermentations share characteristics of wheat and spelt sourdough fermentations. *Food Microbiol.* **2010**, *27*, 1000–1008. [[CrossRef](#)] [[PubMed](#)]
37. Bessmeltseva, M.; Viard, E.; Simm, J.; Paalme, T.; Sarand, I. Evolution of bacterial consortia in spontaneously started rye sourdoughs during two months of daily propagation. *PLoS ONE* **2014**, *9*, e95449. [[CrossRef](#)] [[PubMed](#)]
38. Viard, E.; Bessmeltseva, M.; Sim, J.; Talve, T.; Aaspõllu, A.; Paalme, T.; Sarand, I. Diversity and Stability of Lactic Acid Bacteria in Rye Sourdoughs of Four Bakeries with Different Propagation Parameters. *PLoS ONE* **2016**, *11*, e0148325. [[CrossRef](#)] [[PubMed](#)]
39. Ganchev, I.; Koleva, Z.; Kizheva, Y.; Moncheva, P.; Hristova, P. Lactic acid bacteria from spontaneously fermented rye sourdough. *Bulg. J. Agric. Sci.* **2014**, *20*, 69–73.
40. Bartkiene, E.; Lele, V.; Ruzauskas, M.; Domig, K.J.; Starkute, V.; Zavistanaviciute, P.; Bartkevics, V.; Pugajeva, I.; Klupsaite, D.; Juodeikiene, G.; et al. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. *Microorganisms* **2019**, *8*, 64. [[CrossRef](#)] [[PubMed](#)]
41. Ispirli, H.; Demirbaş, F.; Yüzer, M.O.; Dertli, E. Identification of lactic acid bacteria from spontaneous rye sourdough and determination of their functional characteristics. *Food Biotech.* **2018**, *32*, 222–235. [[CrossRef](#)]
42. Llamas-Arriba, M.G.; Hernández-Alcántara, A.M.; Mohedano, M.L.; Chiva, R.; Celador-Lera, L.; Velázquez, E.; Prieto, A.; Dueñas, M.T.; Tamame, M.; López, P. Lactic acid bacteria isolated from fermented doughs in Spain produce dextrans and riboflavin. *Foods* **2021**, *10*, 2004. [[CrossRef](#)] [[PubMed](#)]
43. Hüttner, E.K.; Dal Bello, F.; Arendt, E.K. Identification of lactic acid bacteria isolated from oat sourdoughs and investigation into their potential for the improvement of oat bread quality. *Eur. Food Res. Technol.* **2010**, *230*, 849–857. [[CrossRef](#)]
44. Çakır, E.; Arıcı, M.; Durak, M.Z. Biodiversity and techno-functional properties of lactic acid bacteria in fermented hull-less barley sourdough. *J. Biosci. Bioeng.* **2020**, *130*, 450–456. [[CrossRef](#)] [[PubMed](#)]

45. Harth, H.; Van Kerrebroeck, S.; De Vuyst, L. Community dynamics and metabolite target analysis of spontaneous, backslopped barley sourdough fermentations under laboratory and bakery conditions. *Int. J. Food Microbiol.* **2016**, *228*, 22–32. [[CrossRef](#)] [[PubMed](#)]
46. Korcari, D.; Ricci, G.; Quattrini, M.; Fortina, M.G. Microbial consortia involved in fermented spelt sourdoughs: Dynamics and characterization of yeasts and lactic acid bacteria. *Lett. Appl. Microbiol.* **2020**, *70*, 48–54. [[CrossRef](#)] [[PubMed](#)]
47. Rocha, J.M.; Malcata, F.X. On the microbiological profile of traditional Portuguese sourdough. *J. Food Prot.* **1999**, *62*, 1416–1429. [[CrossRef](#)] [[PubMed](#)]
48. Rocha, J.M.; Malcata, F.X. Microbial ecology dynamics in Portuguese broa sourdough. *J. Food Qual.* **2016**, *39*, 634–648. [[CrossRef](#)]
49. Zheng, J.; Wittouck, S.; Salvetti, E.; Franz, C.M.A.P.; Harris, H.M.B.; Mattarelli, P.; O'Toole, P.W.; Pot, B.; Vandamme, P.; Walter, J.; et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 2782–2858. [[CrossRef](#)] [[PubMed](#)]
50. da Rocha, J.M.F.; Russo, P.; Capozzi, V.; Fragasso, M.; Malcata, F.X. Sourdough Microbiota Diversity in Southern Europe. In *Sourdough Microbiota and Starter Cultures for Industry*; Ceresino, E.B., Juodeikiene, G., Schwenninger, S.M., Rocha, J.M.F., Eds.; Springer Nature: Cham, Switzerland, 2024; pp. 21–60.
51. Dybka-Stepień, K.; Otlewska, A.; Diowski, A. Sourdough Microbiota Diversity in Central Europe. In *Sourdough Microbiota and Starter Cultures for Industry*; Ceresino, E.B., Juodeikiene, G., Schwenninger, S.M., Rocha, J.M.F., Eds.; Springer Nature: Cham, Switzerland, 2024; pp. 61–92.
52. Schwab, C.; Bambace, M.F. Traditional and Novel Applications of Sourdough Microbes in Northern Europe and the Baltic Countries. In *Sourdough Microbiota and Starter Cultures for Industry*; Ceresino, E.B., Juodeikiene, G., Schwenninger, S.M., Rocha, J.M.F., Eds.; Springer Nature: Cham, Switzerland, 2024; pp. 93–130.
53. Aktepe, Y.; Aydın, F.; Bozoğlu, T.; Özer, G.; Çakır, I. Molecular characterization and multifunctional evaluation of lactic acid bacteria isolated from traditional sourdough. *Int. J. Food Microbiol.* **2024**, *423*, 110845. [[CrossRef](#)] [[PubMed](#)]
54. Syrokou, M.K.; Themeli, C.; Paramithiotis, S.; Mataragas, M.; Bosnea, L.; Argyri, A.A.; Chorianopoulos, N.G.; Skandamis, P.N.; Drosinos, E.H. Microbial Ecology of Greek Wheat Sourdoughs, Identified by a Culture-Dependent and a Culture-Independent Approach. *Foods* **2020**, *9*, 1603. [[CrossRef](#)] [[PubMed](#)]
55. Arsoy, E.S.; Gül, L.B.; Çon, A.H. Characterization and Selection of Potential Antifungal Lactic Acid Bacteria Isolated from Turkish Spontaneous Sourdough. *Curr. Microbiol.* **2022**, *79*, 148. [[CrossRef](#)] [[PubMed](#)]
56. Pino, A.; Russo, N.; Solieri, L.; Sola, L.; Caggia, C.; Randazzo, C.L. Microbial Consortia Involved in Traditional Sicilian Sourdough: Characterization of Lactic Acid Bacteria and Yeast Populations. *Microorganisms* **2022**, *10*, 283. [[CrossRef](#)] [[PubMed](#)]
57. González-Alonso, V.; Pradal, I.; Raditya Wardhana, Y.; Cnockaert, M.; Wieme, A.D.; Vandamme, P.; De Vuyst, L. Microbial ecology and metabolite dynamics of backslopped triticale sourdough productions and the impact of scale. *Int. J. Food Microbiol.* **2024**, *408*, 110445. [[CrossRef](#)] [[PubMed](#)]
58. Lutter, L.; Kuzina, A.; Andreson, H. Genotypic Stability of Lactic Acid Bacteria in Industrial Rye Bread Sourdoughs Assessed by ITS-PCR Analysis. *Microorganisms* **2024**, *12*, 1872. [[CrossRef](#)] [[PubMed](#)]
59. Sanmartín, G.; Sánchez-Adriá, I.E.; Prieto, J.A.; Estruch, F.; Randez-Gil, F. Bioprospecting of sourdough microbial species from artisan bakeries in the city of Valencia. *Food Microbiol.* **2024**, *120*, 104474. [[CrossRef](#)] [[PubMed](#)]
60. Urshev, Z.; Doynova, D.; Prasev, I.; Denkova-Kostova, R.; Koleva, A.; Denkova, Z.; Goranov, B.; Kostov, G. Identification of Lactic Acid Bacteria Strains Isolated from Sourdoughs Prepared with Different Flour Types. *Appl. Sci.* **2024**, *14*, 2093. [[CrossRef](#)]
61. Vurro, F.; Anelli, P.; Zocchi, D.M.; De Bellis, P.; Pieroni, A.; Pasqualone, A. Bessarabian wild hop sourdough: Microbial characterization and effect on the physicochemical properties and flavor of the bread. *Int. J. Gastron. Food Sci.* **2025**, *42*, 101377. [[CrossRef](#)]
62. Arora, K.; Carafa, I.; Fava, F.; Tuohy, K.M.; Nikoloudaki, O.; Gobbetti, M.; Di Cagno, R. Sourdough performances of the golden cereal tritordeum: Dynamics of microbial ecology, biochemical and nutritional features. *Int. J. Food Microbiol.* **2022**, *374*, 109725. [[CrossRef](#)] [[PubMed](#)]
63. Comasio, A.; Verce, M.; Van Kerrebroeck, S.; De Vuyst, L. Diverse Microbial Composition of Sourdoughs from Different Origins. *Front. Microbiol.* **2020**, *11*, 1212. [[CrossRef](#)] [[PubMed](#)]
64. Gettemans, T.; Pradal, I.; Uyttendaele, M.; De Vuyst, L.; Weckx, S. Decoding artisan sourdoughs: The interplay of microorganisms, metabolites, and production parameters. *Food Res. Int.* **2026**, *238*, 119441. [[CrossRef](#)]
65. Doan, N.T.; Van Hoorde, K.; Cnockaert, M.; De Brandt, E.; Aerts, M.; Le Thanh, B.; Vandamme, P. Validation of MALDI-TOF MS for rapid classification and identification of lactic acid bacteria, with a focus on isolates from traditional fermented foods in Northern Vietnam. *Lett. Appl. Microbiol.* **2012**, *55*, 265–273. [[CrossRef](#)] [[PubMed](#)]
66. Sánchez-Juanes, F.; Teixeira-Martín, V.; González-Buitrago, J.M.; Velázquez, E.; Flores-Félix, J.D. Identification of species and sub-species of lactic acid bacteria present in Spanish cheeses type “Torta” by MALDI-TOF MS and *pheS* gene analyses. *Microorganisms* **2020**, *8*, 301. [[CrossRef](#)] [[PubMed](#)]

67. Chiva, R.; Celador-Lera, L.; Uña, J.A.; Jiménez-López, A.; Espinosa-Alcantud, M.; Mateos-Horganero, E.; Vega, S.; Santos, M.Á.; Velázquez, E.; Tamame, M. Yeast biodiversity in fermented doughs and raw cereal matrices and the study of technological traits of selected strains isolated in Spain. *Microorganisms* **2021**, *9*, 47. [[CrossRef](#)] [[PubMed](#)]
68. Ferreira, L.; Sánchez-Juanes, F.; García-Fraile, P.; Rivas, R.; Mateos, P.F.; Martínez-Molina, E.; González-Buitrago, J.M.; Velázquez, E. MALDI-TOF mass spectrometry is a fast and reliable platform for identification and ecological studies of species from family *Rhizobiaceae*. *PLoS ONE* **2011**, *6*, e20223. [[CrossRef](#)] [[PubMed](#)]
69. BTS 8255343; Bruker Bacterial Test Standard. Charles River Laboratories: Wilmington, MA, USA, 2012.
70. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [[CrossRef](#)] [[PubMed](#)]
71. Thompson, J.D.; Gibson, T.J.; Plewniak, F.; Jeanmougin, F.; Higgins, D.G. The clustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **1997**, *24*, 4876–4882. [[CrossRef](#)] [[PubMed](#)]
72. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [[CrossRef](#)] [[PubMed](#)]
73. Saitou, N.; Nei, M. A neighbour-joining method: A new method for reconstructing phylogenetics trees. *Mol. Biol. Evol.* **1987**, *44*, 406–425. [[CrossRef](#)] [[PubMed](#)]
74. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *3*, 1870–1874. [[CrossRef](#)] [[PubMed](#)]
75. EFSA Panel on Biological Hazards (BIOHAZ); Koutsoumanis, K.; Allende, A.; Alvarez-Ordóñez, A.; Bolton, D.; Bover-Cid, S.; Chemaly, M.; De Cesare, A.; Hilbert, F.; Lindqvist, R.; et al. Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 19: Suitability of taxonomic units notified to EFSA until September 2023. *EFSA J.* **2024**, *22*, e8517. [[CrossRef](#)] [[PubMed](#)]
76. Naser, S.M.; Dawyndt, P.; Hoste, B.; Gevers, D.; Vandemeulebroecke, K.; Cleenwerck, I.; Vancanneyt, M.; Swings, J. Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2777–2789. [[CrossRef](#)] [[PubMed](#)]
77. Deleu, L.J.; Lemmens, E.; Redant, L.; Delcour, J.A. The major constituents of rye (*Secale cereale* L.) flour and their role in the production of rye bread, a food product to which a multitude of health aspects are ascribed. *Cereal Chem.* **2020**, *97*, 739–754. [[CrossRef](#)]
78. Alvarez, J.B. Spanish Spelt Wheat: From an Endangered Genetic Resource to a Trendy Crop. *Plants* **2021**, *10*, 2748. [[CrossRef](#)] [[PubMed](#)]
79. Papadopoulos, G.; Mavroeidis, A.; Stavropoulos, P.; Kakabouki, I. Triticum: A versatile and resilient cereal for Mediterranean agriculture and sustainable food production. *Cereal Res. Commun.* **2024**, *52*, 323–331. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.