Screening sourdough samples for gliadin-degrading activity revealed *Lactobacillus casei* strains able to individually metabolize the coeliac disease-related 33-mer peptide.
Screening sourdough samples for gliadin-degrading activity revealed *Lactobacillus casei* strains able to individually metabolize the coeliac disease-related 33-mer peptide

Patricia Alvarez-Sieiro¹, Begoña Redruello¹,*, Victor Ladero, Maria Cruz Martín, María Fernández and Miguel A. Alvarez

*Dairy Research Institute (IPLA-CSIC), 33300 Villaviciosa, Asturias, Spain*

E-mail addresses:

P.A-S.: pas@ipla.csic.es
B.R.: bredruel@ipla.csic.es
V.L.: ladero@ipla.csic.es
M.C.M.: mcm@ipla.csic.es
M.F.: mfernandez@ipla.csic.es
M.A.A.: maag@ipla.csic.es

*Corresponding author: Begoña Redruello.
Dairy Research Institute (IPLA-CSIC), 33300 Villaviciosa, Asturias, Spain
Tel.: +34 985 89 21 31; Fax: +34 985 89 22 33. bredruel@ipla.csic.es

¹ These authors contributed equally to this work.
Abstract

A selective culture medium containing acid-hydrolyzed gliadins as the sole nitrogen source was used in the search for sourdough-indigenous lactic acid bacteria (LAB) with gliadin-metabolizing activity. Twenty gliadin-degrading LAB strains were isolated from 10 sourdoughs made in different ways and from different geographical regions. Fifteen of the 20 isolated strains were identified as Lactobacillus casei, a species usually reported as subdominant in sourdough populations. The other five gliadin-degrading strains belonged to the more commonly encountered sourdough species Leuconostoc mesenteroides and Lactobacillus plantarum. All these strains were shown to be safe in terms of their resistance to antimicrobial agents. When individually incubated with the α2-gliadin-derived immunotoxic 33-mer peptide (97.5 ppm), half of the L. casei strains metabolized at least 50% of it within 24 h. One strain metabolized 82% of the 33-mer peptide within 8 h, and fully made it disappear within 12 h. These results reveal for the first time the presence in sourdough of proteolytic L. casei strains with the capacity to individually metabolize the coeliac disease-related 33-mer peptide.

Keywords: Lactobacillus casei, gliadin proteolysis, lactic acid bacteria, 33-mer peptide, PFGE
Introduction

The sourdough ecosystem consists of a mixture of flour and water that is fermented by yeasts and lactic acid bacteria (LAB). Fermentation can follow either a traditional process involving indigenous and/or defined microorganisms as starters, or an industrial process based largely on the metabolism of baker’s yeasts (reviewed in Minervini et al. 2014 and De Vuyst et al. 2014). More than 70 species of LAB have been identified in traditional wheat and rye sourdoughs. *Lactobacillus* is the most common genus; *Leuconostoc*, *Pediococcus* and *Weissella* are found in much smaller numbers and almost exclusively in the early stages of fermentation. Obligate heterofermentative *Lactobacillus sanfranciscensis*, *Lactobacillus brevis* and *Lactobacillus fermentum* are the predominant lactobacilli, along with the facultative heterofermentative species *Lactobacillus plantarum* and *Lactobacillus paralimentarius*. The subdominant presence of *Lactobacillus curvatus*, *Lactobacillus casei* and *Lactobacillus sakei* has also been reported (Corsetti et al. 2003; Ladero et al. 2013; Minervini et al. 2014; De Vuyst et al. 2014).

The protein fraction of cereal flours comprises a mixture of water-soluble albumins and globulins, alcohol-soluble prolamin (called gliadins in wheat), and alcohol-insoluble glutelins (called glutenins in wheat). Wheat gluten is composed of roughly equal proportions of gliadins and glutenins (Wieser 2007). Substantial gluten hydrolysis occurs during sourdough fermentation, the result of the interplay between LAB-driven acidification of the dough and proteolytic activity of both cereal and microbial origin (Loponen et al. 2007; Rizzello et al. 2007; Gobetti et al. 1996; Di Cagno et al. 2002; De Angelis et al. 2006; M’hir et al. 2008; Gerez et al. 2012). Not all of the gluten is hydrolysed, however, and once ingested, the remainder undergoes proteolysis by the digestive enzymes. Some of the peptides produced are resistant to further breakdown.
and may accumulate in the intestinal lumen. In susceptible individuals this can trigger an inflammatory response leading to coeliac disease (Shan et al. 2002; Sollid and Khosla 2005). The 33-mer peptide of α2-gliadin (residues 57-89) is one of the most immunogenic of gluten breakdown products (Qiao et al. 2004); its degradation is the goal of any hydrolytic activity hoped to be of use in the detoxification of gliadins (Shan et al. 2004).

Over the last decade, the use of certain combinations of LAB strains (or their cell-free extracts) in sourdough fermentation have been shown to substantially reduce this food's toxic gluten content (Di Gagno et al. 2004; De Angelis et al. 2006; Rizello et al. 2007; Loponen et al. 2007; M’hir et al. 2008; Gerez et al. 2012). However, no strain has been found able to metabolize the 33-mer peptide on its own.

Previous papers have reported the isolation of proteolytic sourdough LAB in aqueous solutions of albumin and globulins - but not in gluten *per se*, although some strains were later found to degrade gliadin in experiments involving high performance liquid chromatography and/or two-dimensional protein electrophoresis analyses (Di Cagno et al. 2002; De Angelis et al. 2006; Gerez et al. 2006; Rizello et al. 2007). Recently, however, a culture medium that contains acid-solubilised gliadins as a sole nitrogen source was developed, allowing the selection of gliadin-degrading phenotypes (Alvarez-Sieiro et al. 2015). The present work reports the isolation of indigenous gliadin-degrading *L. casei* strains from different wheat and rye sourdough using an acid-solubilised gliadin mixture in a selective enrichment procedure. One of these strains - on its own - was able to fully metabolize the 33-mer peptide within 12 h, the shortest time ever reported for a sourdough LAB strain to perform this action.

**Materials and Methods**
Sourdoughs

Ten raw sourdough samples from different countries (Italy, Poland and Spain) involving wheat or rye flour, produced by traditional or yeast-leavening fermentation processes, and used for making bread, pizza or pasta, were collected in situ, immediately packed to preserve their original microbiota and sent to our laboratory (see Table 1). Five of these samples came from three household manufacturers, four from professional bakeries, and one was made in the laboratory according to the following traditional procedure: 25 g of milled wheat grains were mixed with 25 ml of sterile MilliQ water (Millipore, Bedford, MA, USA) and incubated at 20°C for 24 h. This process was repeated five times, substituting half of the fermented dough by fresh mixture at the beginning of each day.

All the Italian samples came from bakeries or household productions from Foggia, the Polish sourdough came from a bakery from Poznan and the Spanish industrial sourdough was collected in a bakery in Villaviciosa.

Culture media and growth conditions

de Man, Rogosa and Sharpe (MRS; Oxoid) was used as a selective culture medium for lactobacilli, while the capacity of the strains to grow on gliadins as a sole nitrogen source was tested on Acid Hydrolyzed Gliadin medium (AHG-M), prepared as described in Alvarez-Sieiro et al. (2015); briefly, a mixture of intact and partially hydrolyzed gliadins (nitrogen source) is added to a freshly autoclaved, chemically defined medium composed of a solution of salts (Na₂HPO₄, KH₂PO₄, (NH₄)₂SO₄, sodium acetate, NaCl, MgCl₂, CaCl₂, Na₂SO₄ and FeCl₃) plus glucose as carbon source.
Finally, a growth factors’ cocktail containing vitamins, nucleosides and enzymatic cofactors was also added after autoclaving.

Both MRS and AHG-M were supplemented with Tween 20 at 0.1 % (v/v; Sigma, Madrid, Spain) to enhance the growth of lactobacilli and adjusted to pH 5.5 to favour the growth of LAB. Moreover, both media were supplemented with 5 g/L of maltose (media then renamed as MRSm and AHG-Mm) to guarantee the growth of the obligate heterofermentative lactobacilli typically found in sourdoughs (Vera et al. 2009). Finally, 50 µg/ml cycloheximide (Sigma) were added to all media to inhibit the growth of yeasts and moulds.

**Sourdough sampling and bacteria counts: experimental design and statistics**

Ten grams of each sourdough were mixed with 90 mL of Ringer saline solution (Merck, Darmstadt, Germany) and homogenized for 5 min using a Lab-Blender 400 stomacher (Seward Ltd., London, UK). Bacterial colonies were enumerated (log cfu/g) after serial dilution in Ringer saline solution and plating on MRSm and AHG-Mm agar. For each medium, 48 h incubations were performed aerobically at 32ºC or 37ºC, or anaerobically at 37ºC, in a Mac 500 anaerobic workstation (Don Whitley Scientific, West Yorkshire, UK). Enrichment steps were performed by inoculating (1% v/v) the initial homogenized sourdough sample into 10 mL of AHG-Mm broth followed by incubation under the three sets of conditions described above. After 24 h, serial dilutions of each enrichment culture were prepared, plated on MRSm and AHG-Mm agar, and incubated under the appropriate conditions for bacterial counting. Two rounds of enrichment in AHG-Mm broth were performed, using the first enrichment step to provide a 1% inoculum for the second.
Each sourdough sampling was treated as an independent experiment. Within each experiment, mean and standard deviation log cfu/g values were calculated for each culture medium after each sampling step (i.e. at the beginning of the sampling, after the first round of enrichment and after the second round of enrichment) by computing the individual log cfu/g values obtained under the three tested environmental conditions. Within each experiment, statistical analysis of data was carried out using one-way ANOVA followed by Tukey’s multiple comparison tests (SigmaPlot (Systat Software, San Jose, CA, USA)). Significance was set at $p < 0.05$.

**Isolation of LAB**

Individual colonies of different morphologies were isolated from AHG-Mm plates and successively sub-cultured in this medium until a purified strain was obtained. Further selection was performed by isolating those colonies able to form a clearing halo when growing on AHG-Mm, indicating a gliadin-degrading phenotype (Alvarez-Sieiro et al. 2015).

The *Lactobacillus* and *Leuconostoc* strains isolated were routinely propagated for 24 h without aeration in MRS at 37°C or 32°C respectively. Bacterial stock cultures were prepared in a glycerol/MRS mixture (20%/80% v/v) and kept at -80°C.

**Molecular identification by 16S rRNA gene sequencing analysis**

Selected gliadin-degrading isolates were identified by partial amplification and sequencing of the 16S *rRNA* gene. Total DNA isolation, PCR amplification, DNA sequencing and bacterial species identification were performed as described in Herrero-Fresno et al. (2012).
Genetic typing by pulsed-field gel electrophoresis (PFGE)

The genomes of the selected LAB strains, plus that of the reference strain *L. casei* BL23 (Mazé et al. 2010), were analysed by PFGE as described by Herrero-Fresno et al. (2012). The agarose-embedded genomic DNA from *Lactobacillus* and *Leuconostoc* strains was digested with *Sfi*I or *Apa*I restriction enzymes respectively. The presence or absence of macrorestriction fragments in each strain was converted to binary scores for analysis by Genetools software (Syngene, Cambridge, UK). The number of shared fragments in the resulting digestion profiles was used to calculate the Dice similarity index (Dice 1945).

Antimicrobial susceptibility

Selected LAB strains were tested for antimicrobial susceptibility using a broth microdilution method (Huys et al. 2010). The battery of antimicrobial agents tested were those recommended by the EFSA (EFSA FEEDAP Panel 2012): ampicillin, clindamycin and penicillin G (dilution range 0.03 to 16 µg/ml), erythromycin (0.16 to 8 µg/ml), streptomycin and gentamicin (0.5 to 256 µg/ml), tetracycline and chloramphenicol (0.12 to 64 µg/ml) (all from Sigma). A strain was considered sensitive to a given antimicrobial agent when the minimal inhibitory concentration (MIC) was below or equal to the defined EFSA cut-off value for that bacterial group (EFSA 2012). A single dilution above the MIC value was accepted as within the normal variation around the mean; a strain returning such a result was therefore still considered sensitive to that antimicrobial agent (EFSA 2014).
Assessment of 33-mer peptide hydrolysis by reversed-phase high performance liquid chromatography (RP-HPLC)

The 33-mer peptide (LQLQPFPQPLPYQPQLPYPQLPYPQLPYPQPF; Immunosteps S.L., Salamanca, Spain) was dissolved in PBS, pH 7.4 (10 mM). Bacterial pellets harvested from exponential cultures in MRS broth (10⁹ cfu/ml) were washed twice with PBS buffer (pH 7.4) and resuspended in 1 mL of the same buffer for proteolysis reactions. These were performed at 37°C under static conditions and contained the peptide (50 µM = 195 ppm) and bacterial cell suspension (10¹⁰ cfu/ml). After different time intervals (from 0 to 24 h), 200 µl aliquots were removed, heated (95°C for 10 min) to inactivate enzymes, filtered through a 0.2 µm low binding protein filter (Pall, Madrid, Spain), and stored at -20°C. The presence of the 33-mer peptide was monitored by RP-HPLC according to Shan et al. (2002). Samples containing only the peptide were used to test for spontaneous degradation while samples carrying solely the strains examined served as controls of the synthesis of cellular endogenous peptides. L. casei BL23 was used as a negative control of hydrolysis (Alvarez-Sieiro et al. 2014).

Results and Discussion

Sourdough sampling, enrichment and bacterial counts

Mean bacterial numbers for given samples and culture media were calculated from the individual values obtained in each of the three culture conditions tested (32°C or 37°C under aerobiosis and 37°C under anaerobiosis) (Table 1). Results pertaining to individual culture conditions for each sample and medium can be found in Supplementary Table S1. Initial counts on MRSm were between 9.36 and 10.86 log cfu/g for all the traditional sourdough samples (Table 1). Compared to the initial MRSm
count results, those for AHG-Mm showed significant reductions of one to four orders of magnitude, except for the Spanish traditional bread sourdough sample and one of the Italian traditional pizza sourdoughs (no significant changes observed). These results indicate that, in the AHGm medium, a selection towards the growth of bacteria able to metabolize the AHG substrate was taking place (Table 1). MRSm is the medium of choice for the isolation of sourdough lactobacilli (Vera et al. 2009), but AHG-Mm contains partially hydrolyzed gliadins as the sole nitrogen source and does not contain free amino acids, thus obliging the microorganisms to possess enzymes that can metabolize gliadin (Alvarez-Sieiro et al. 2015). Furthermore, the putative liberation of ammonia from the inorganic ammonium sulphate salt present in the AHG-M medium would not result enough by itself for the growth and development of the sourdough LAB.

After two enrichment steps in AHG-Mm broth, a significative reduction in the bacterial count values was seen in MRSm medium compared to the initial MRSm counts, in all the sampled traditional sourdoughs. Bacterial counts for the AHG-Mm medium differed after the first enrichment step in comparison to the initial counts in this medium (Table 1): a significant decrease in the number of bacteria able to grow on AHG-Mm was seen in two samples while the rest of samples showed no significant changes. After the second round of enrichment in AHG-Mm broth, however, the bacterial count values recorded in this medium were significantly reduced in all samples with respect to the initial counts. Nonetheless, the overall reduction after the two enrichment steps was smaller in AHG-Mm (between 0.3 and 2.7 log cfu/g) than in MRSm (between 2.6 and 4.6 log cfu/g), probably due to the progressive loss of lactobacilli strains unable to use AHG as a sole nitrogen source.
Different results were obtained for the Spanish baker’s yeast-leavened sourdough sample (Table 1). Bacterial counts were the lowest detected, and remained constant across the different media and culture conditions (around 7.0 log cfu/g). The ability of yeasts to efficiently degrade the starch might lead to their rapid development and thus could explain the lesser participation of LAB in the fermentative process of this type of sourdough (Minervini et al. 2014; De Vuyst et al. 2014). The consistency in the bacterial count obtained in MRS-Mm after the two rounds of enrichment in AHG-Mm broth would seem to indicate the adaptation of the lactobacilli present in this sample to grow on the gliadin matrix (Table 1).

Identification and typification of gliadin-degrading LAB strains

Of the 267 colonies isolated from AHG-Mm plates, 48 (18%) formed degrading halos and were identified by 16s rRNA sequencing. Of these, 43 were found to be LAB, which were typified by PFGE into 20 different profiles: 15 *L. casei*, 1 *L. plantarum* and 4 *L. mesenteroides* (Fig. 1). As expected, the lactobacilli clustered into two PFGE groups, one containing *L. plantarum* IPLA88 alone, the other containing the *L. casei* strains (Fig. 1, panel A). For this latter group, no correlation was seen between the clustering of the strains and the type of final product, nor the type of processing involved. However, they did appear to cluster in a manner that reflected the geographic origin of the Polish and Italian strains (Fig. 1, panel A) (the two Spanish strains located randomly among the others). The existence of a correlation between geographic origin and the composition of sourdough microbiota is currently a matter of debate (De Vuyst et al. 2014; Minervini et al. 2014). Indeed, in contrast to the results for *L. casei*, the clustering of the *L. mesenteroides* strains (Fig. 1, panel B) suggests no such correlation.
exists, although this interpretation should be received with caution given the small number examined.

Gliadin-degrading LAB strains were detected in all the sourdough samples, although their diversity was greatest in the Italian wheat bread sourdough from a traditional bakery (5 strains), followed by the Italian bakery wheat pasta sourdough (3 strains). The rest of the sourdough samples had 1 or 2 gliadin-degrading strains (Fig. 1). Certainly, sourdoughs made from different cereals, that require different manufacturing processes (including technological variables such as the amount of water and salt added to the flour, the temperature used during fermentation, the number of back-slopping steps, or the pH), or that come from different geographical areas, might be expected to contain different microorganisms (Minervini et al. 2014).

*Leuconostoc* is usually found in the early stages of sourdough fermentation, while *L. plantarum* dominates mature sourdoughs together with *L. sanfranciscensis* and *L. fermentum* (Minervini et al. 2014). The abundance of *L. casei* strains isolated in the present work is noteworthy; this species is traditionally reported as subdominant within sourdough communities. The present selection procedure may therefore have promoted the growth of those strains able to utilize gliadins as a nitrogen source. Although it is traditionally assumed that LAB play a secondary role during sourdough proteolysis, acting after any cereal endogenous proteases, strain-specific proteolytic activities have also been detected in sourdough LAB (Gobetti et al. 1996; Di Gagno et al. 2004; De Angelis et al. 2006; Rizello et al. 2007; Loponen et al. 2007; M’hir et al. 2008; Gerez et al. 2012). Moreover, the proteolytic capacity of *L. casei* species during dairy fermentations is well-known, their activity degrading the casein matrix into oligopeptides, an essential step for the growth of the secondary LAB microbiota (Kojic et al. 1991).
The absence of obligate heterofermentative species within the isolates of the present work could be due to their incapacity to metabolize the gliadin substrate or to the absence of any metabolite within the culture media that would result essential for their growth and development; this could include additional carbon sources such as pentoses.

**Antimicrobial susceptibility of the isolated gliadin-degrading LAB strains**

The absence of acquired antimicrobial resistance is an important biosafety property of bacterial cultures intended for use as biologically active food additives, e.g. inside a yoghurt. Since they are generally consumed in large numbers, and may come into close contact with other bacteria in the human intestine, including pathogens, horizontal gene transfer is a concern (Teuber et al. 1999). Two of the four *L. mesenteroides* strains (*L. mesenteroides* IPLA12002 and IPLA12017) were clearly resistant to clindamycin, while *L. plantarum* 88 was sensitive to all the tested antimicrobial agents and just three of the *L. casei* strains exceeded the EFSA limit values for chloramphenicol (Table 2). However, the use of this antimicrobial agent is restricted in humans due to its adverse secondary effects (Shukla et al. 2011). These results give no reason for concern regarding the safety of most of the selected gliadin-degrading strains and support their possible use as biologically active additives. In addition, if the microorganisms are intended for use as food additives within any cereal-based dough which will suffer a baking step, their inactivation during the process would guarantee their safety to the potential consumers.

**Evaluation of the selected strains’ ability to hydrolyze the 33-mer peptide**
Incubations of each bacterial strain (at a cell density of $10^{10}$ cfu/ml) with the 33-mer peptide (at 50 µM = 195 ppm) were performed. Two of the strains were able to fully metabolize the 33-mer peptide after 24 h of incubation, five strains made disappear more than 50% of the peptide, and the remaining 13 strains left between 50% and 100% intact. The non-sourdough strain *L. casei* BL23 did not metabolize the peptide. No disappearance of the peptide occurred when incubated alone at any time (Fig. 2, panel A). The *L. casei* strains were more active than those of *Ln. mesenteroides* and *L. plantarum*. Indeed, *Ln. mesenteroides* IPLA12012 and *L. plantarum* 88 were unable to metabolize the 33-mer peptide, and the remaining *L. mesenteroides* strains managed no more than 15% peak area reduction. In contrast, between 90-100% reduction in peak area was seen when the peptide was individually incubated with *L. casei* IPLA12038 (from the Polish bread sourdough), IPLA12035 (from the Italian pizza sourdough) or IPLA12027 (from the Italian pasta sourdough).

The most active strain was *L. casei* IPLA12038, which reduced the peak area of the peptide by up to 82% within 8 h, and completely made it disappear within 12 h (Fig. 2, panel B). Interestingly, no intermediate hydrolysis products were observed when the chromatogram for the latter incubation was compared to that of a parallel incubation containing solely the strain (i.e., with no substrate). There are no previous reports of any individual LAB strain from sourdough harbouring the complete repertoire of enzymes and/or membrane transport systems needed to fully metabolize the 33-mer peptide. The ingestion of gliadin-degrading enzymes is currently being tested as an alternative to following a gluten-free diet, the only treatment currently available for patients with coeliac disease. The use of gliadin-degrading enzymes to eliminate flour toxicity during sourdough fermentation has been tested with selected LAB strains (Gerez et al. 2012), and via the cooperative action of lactobacilli and fungi (Rizzello et al. 2007; De Angelis...
et al. 2010). However, the apparently complete gliadin-degrading capacity of *L. casei* IPLA12038 demands it be further studied. It might be used as a starter in sourdough making and allow products to be made with far less gluten-based toxicity.

**Acknowledgments**

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susceptibility testing methods for bifidobacteria and nonenterococcal lactic acid


Figure captions

Fig. 1. Pulse-field gel electrophoresis patterns and derived dendrograms for the sourdough-isolated, gliadin-degrading lactobacilli (panel A) and \textit{Leuconostoc} (panel B) strains. Dendrograms were generated by Genetools software; distances were calculated using the Dice similarity index for endonuclease \textit{SfiI} (panel A) and \textit{ApaI} (panel B) restriction patterns. The strain names and sources of isolation are indicated.

Fig. 2. Incubation of the 33-mer peptide with selected gliadin-degrading LAB. (A) Percentage reduction of the 33-mer peptide peak area when incubated individually with the indicated strains for 8 h (grey stacked bars) or 24 h (black stacked bars). Reactions involving only the peptide, or the peptide in combination with the reference strain \textit{L. casei} BL23, were used as negative controls. Lm, \textit{Leuconostoc mesenteroides}. Lp, \textit{Lactobacillus plantarum}. Lc, \textit{Lactobacillus casei}. (B) Chromatogram obtained when the 33-mer peptide was incubated with \textit{L. casei} IPLA12038 for 0 h (top line) or 12 h (middle line). A reaction containing the strain alone was used to check the appearance of peptides derived from its normal metabolism (bottom line). The chromatogram section that contains the 33-mer peptide peak is shown enlarged in the inset.
Table 1
Sourdough samples and bacterial counts (mean ± standard deviation for the tested growing conditions) in the indicated culture media.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Processing Origin</th>
<th>Log · cfu/g without enrichment</th>
<th>Log · cfu/g 1st enrichment</th>
<th>Log · cfu/g 2nd enrichment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MRSm</td>
<td>AHG-Mm</td>
<td>MRSm</td>
</tr>
<tr>
<td>Wheat Bread</td>
<td>Traditional Laboratory Spain</td>
<td>9.36 ± 0.44&lt;sup&gt;a&lt;/sup&gt; 9.33 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.30 ± 0.02&lt;sup&gt;ab&lt;/sup&gt; 7.79 ± 1.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.84 ± 0.65&lt;sup&gt;b&lt;/sup&gt; 6.58 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wheat Bread</td>
<td>Yeast leavening Bakery Spain</td>
<td>7.80 ± 0.07&lt;sup&gt;a&lt;/sup&gt; 7.90 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.28 ± 0.35&lt;sup&gt;a&lt;/sup&gt; 6.00†</td>
<td>7.02 ± 0.58&lt;sup&gt;a&lt;/sup&gt; ND</td>
</tr>
<tr>
<td>Wheat Bread</td>
<td>Traditional Bakery Italy</td>
<td>9.68 ± 0.08&lt;sup&gt;a&lt;/sup&gt; 7.85 ± 0.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.96 ± 0.82&lt;sup&gt;ab&lt;/sup&gt; 7.85 ± 1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.90 ± 1.09&lt;sup&gt;ab&lt;/sup&gt; 7.15 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rye Bread</td>
<td>Traditional Bakery Poland</td>
<td>10.80 ± 0.18&lt;sup&gt;a&lt;/sup&gt; 6.88 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.59 ± 0.52&lt;sup&gt;a&lt;/sup&gt; 7.52 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.19 ± 0.41&lt;sup&gt;b&lt;/sup&gt; 5.91 ± 1.39&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Wheat Pasta</td>
<td>Traditional Bakery Italy</td>
<td>10.52 ± 0.14&lt;sup&gt;a&lt;/sup&gt; 9.10 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.74 ± 0.20&lt;sup&gt;b&lt;/sup&gt; 8.71 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.29 ± 0.32&lt;sup&gt;c&lt;/sup&gt; 7.40 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wheat Pasta</td>
<td>Traditional Household A&lt;sup&gt;*&lt;/sup&gt; Italy</td>
<td>10.86 ± 0.65&lt;sup&gt;a&lt;/sup&gt; 9.20 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.30 ± 0.09&lt;sup&gt;b&lt;/sup&gt; 9.18 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.96 ± 1.23&lt;sup&gt;c&lt;/sup&gt; 6.88 ± 0.91&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Wheat Pasta</td>
<td>Traditional Household B&lt;sup&gt;*&lt;/sup&gt; Italy</td>
<td>10.48 ± 0.17&lt;sup&gt;a&lt;/sup&gt; 7.61 ± 1.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.88 ± 0.16&lt;sup&gt;b&lt;/sup&gt; 9.05 ± 0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.46 ± 0.37&lt;sup&gt;b&lt;/sup&gt; 7.23 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wheat Pizza</td>
<td>Traditional Household A&lt;sup&gt;*&lt;/sup&gt; Italy</td>
<td>10.38 ± 0.15&lt;sup&gt;a&lt;/sup&gt; 8.80 ± 0.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.24 ± 0.21&lt;sup&gt;ab&lt;/sup&gt; 9.11 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.94 ± 0.47&lt;sup&gt;b&lt;/sup&gt; 7.49 ± 1.16&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
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<td>Traditional Household B&lt;sup&gt;*&lt;/sup&gt; Italy</td>
<td>10.38 ± 0.65&lt;sup&gt;a&lt;/sup&gt; 9.07 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.70 ± 0.35&lt;sup&gt;b&lt;/sup&gt; 8.76 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.50 ± 0.26&lt;sup&gt;c&lt;/sup&gt; 7.92 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>Traditional Household C&lt;sup&gt;*&lt;/sup&gt; Italy</td>
<td>10.72 ± 0.72&lt;sup&gt;a&lt;/sup&gt; 9.56 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.24 ± 0.04&lt;sup&gt;a&lt;/sup&gt; 9.08 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.77 ± 0.56&lt;sup&gt;b&lt;/sup&gt; 7.04 ± 1.17&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a</sup>B and C correspond to three different sources. †, data retrieved from the result of growth at 37°C. ND, not determined
<sup>a</sup><sup>c</sup>Values within a row with different superscript letters are significantly different (P < 0.05)
MRSm: de Man, Rogosa and Sharpe medium plus maltose
AHG-Mm: acid-hydrolyzed gliadin medium plus maltose
Table 2
MIC distribution for eight antimicrobial agents across 20 strains of gliadin-degrading LAB isolated from different sourdoughs.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Am</th>
<th>Pc</th>
<th>Cl</th>
<th>Gm</th>
<th>Sm</th>
<th>Tc</th>
<th>Cm</th>
<th>Em</th>
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<td>16</td>
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<td>2</td>
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</tr>
</tbody>
</table>

Shaded areas highlight MIC values above EFSA recommendations. Am, ampicillin; Pc, penicillin G; Cl, clindamycin, Gm, gentamicin, Sm, streptomycin, Tc, tetracycline; Cm, chloramphenicol; Em, erythromycin.