Canadian Journal of Microbiology
Revue canadienne de microbiologie

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

Journal:	<i>Canadian Journal of Microbiology</i>
Manuscript ID	cjm-2015-0796.R1
Manuscript Type:	Article
Date Submitted by the Author:	14-Jan-2016
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Keyword:	<i>Lactobacillus casei</i>, gliadin proteolysis, lactic acid bacteria, 33-mer peptide, PFGE

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

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24

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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 α 2-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

48 Introduction

49 The sourdough ecosystem consists of a mixture of flour and water that is fermented
50 by yeasts and lactic acid bacteria (LAB). Fermentation can follow either a traditional
51 process involving indigenous and/or defined microorganisms as starters, or an industrial
52 process based largely on the metabolism of baker's yeasts (reviewed in Minervini et al.
53 2014 and De Vuyst et al. 2014). More than 70 species of LAB have been identified in
54 traditional wheat and rye sourdoughs. *Lactobacillus* is the most common genus;
55 *Leuconostoc*, *Pediococcus* and *Weissella* are found in much smaller numbers and
56 almost exclusively in the early stages of fermentation. Obligate heterofermentative
57 *Lactobacillus sanfranciscensis*, *Lactobacillus brevis* and *Lactobacillus fermentum* are
58 the predominant lactobacilli, along with the facultative heterofermentative species
59 *Lactobacillus plantarum* and *Lactobacillus paralimentarius*. The subdominant presence
60 of *Lactobacillus curvatus*, *Lactobacillus casei* and *Lactobacillus sakei* has also been
61 reported (Corsetti et al. 2003; Ladero et al. 2013; Minervini et al. 2014; De Vuyst et al.
62 2014).

63 The protein fraction of cereal flours comprises a mixture of water-soluble albumins and
64 globulins, alcohol-soluble prolamins (called gliadins in wheat), and alcohol-insoluble
65 glutelins (called glutenins in wheat). Wheat gluten is composed of roughly equal
66 proportions of gliadins and glutenins (Wieser 2007). Substantial gluten hydrolysis
67 occurs during sourdough fermentation, the result of the interplay between LAB-driven
68 acidification of the dough and proteolytic activity of both cereal and microbial origin
69 (Loponen et al. 2007; Rizzello et al. 2007; Gobetti et al. 1996; Di Cagno et al. 2002; De
70 Angelis et al. 2006; M'hir et al. 2008; Gerez et al. 2012). Not all of the gluten is
71 hydrolysed, however, and once ingested, the remainder undergoes proteolysis by the
72 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 α 2-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 Gagno 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

98 **Sourdoughs**

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

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

112

113 **Culture media and growth conditions**

114 de Man, Rogosa and Sharpe (MRS; Oxoid) was used as a selective culture medium
115 for lactobacilli, while the capacity of the strains to grow on gliadins as a sole nitrogen
116 source was tested on Acid Hydrolyzed Gliadin medium (AHG-M), prepared as
117 described in Alvarez-Sieiro et al. (2015); briefly, a mixture of intact and partially
118 hydrolyzed gliadins (nitrogen source) is added to a freshly autoclaved, chemically
119 defined medium composed of a solution of salts (Na_2HPO_4 , KH_2PO_4 , $(\text{NH}_4)_2\text{SO}_4$,
120 sodium acetate, NaCl , MgCl_2 , CaCl_2 , Na_2SO_4 and FeCl_3) plus glucose as carbon source.

121 Finally, a growth factors' cocktail containing vitamins, nucleosides and enzymatic
122 cofactors was also added after autoclaving.

123 Both MRS and AHG-M were supplemented with Tween 20 at 0.1 % (v/v; Sigma,
124 Madrid, Spain) to enhance the growth of lactobacilli and adjusted to pH 5.5 to favour
125 the growth of LAB. Moreover, both media were supplemented with 5 g/L of maltose
126 (media then renamed as MRSm and AHG-Mm) to guarantee the growth of the obligate
127 heterofermentative lactobacilli typically found in sourdoughs (Vera et al. 2009). Finally,
128 50 µg/ml cycloheximide (Sigma) were added to all media to inhibit the growth of yeasts
129 and moulds.

130

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

132 Ten grams of each sourdough were mixed with 90 mL of Ringer saline solution
133 (Merck, Darmstadt, Germany) and homogenized for 5 min using a Lab-Blender 400
134 stomacher (Seward Ltd., London, UK). Bacterial colonies were enumerated (log cfu/g)
135 after serial dilution in Ringer saline solution and plating on MRSm and AHG-Mm agar.
136 For each medium, 48 h incubations were performed aerobically at 32°C or 37°C, or
137 anaerobically at 37°C, in a Mac 500 anaerobic workstation (Don Whitley Scientific,
138 West Yorkshire, UK). Enrichment steps were performed by inoculating (1% v/v) the
139 initial homogenized sourdough sample into 10 mL of AHG-Mm broth followed by
140 incubation under the three sets of conditions described above. After 24 h, serial
141 dilutions of each enrichment culture were prepared, plated on MRSm and AHG-Mm
142 agar, and incubated under the appropriate conditions for bacterial counting. Two rounds
143 of enrichment in AHG-Mm broth were performed, using the first enrichment step to
144 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).

169

170 Genetic typing by pulsed-field gel electrophoresis (PFGE)

171 The genomes of the selected LAB strains, plus that of the reference strain *L. casei*
172 BL23 (Mazé et al. 2010), were analysed by PFGE as described by Herrero-Fresno et al.
173 (2012). The agarose-embedded genomic DNA from *Lactobacillus* and *Leuconostoc*
174 strains was digested with *Sfi*I or *Apa*I restriction enzymes respectively. The presence or
175 absence of macrorestriction fragments in each strain was converted to binary scores for
176 analysis by Genetools software (Syngene, Cambridge, UK). The number of shared
177 fragments in the resulting digestion profiles was used to calculate the Dice similarity
178 index (Dice 1945).

179

180 Antimicrobial susceptibility

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

192

193 **Assessment of 33-mer peptide hydrolysis by reversed-phase high performance**
 194 **liquid chromatography (RP-HPLC)**

195 The 33-mer peptide (LQLQPFQPQLPYPQPQLPYPQPQLPYPQPQPF;
 196 Immunosteps S.L., Salamanca, Spain) was dissolved in PBS, pH 7.4 (10 mM). Bacterial
 197 pellets harvested from exponential cultures in MRS broth (10^9 cfu/ml) were washed
 198 twice with PBS buffer (pH 7.4) and resuspended in 1 mL of the same buffer for
 199 proteolysis reactions. These were performed at 37°C under static conditions and
 200 contained the peptide (50 μ M = 195 ppm) and bacterial cell suspension (10^{10} cfu/ml).
 201 After different time intervals (from 0 to 24 h), 200 μ l aliquots were removed, heated
 202 (95°C for 10 min) to inactivate enzymes, filtered through a 0.2 μ m low binding protein
 203 filter (Pall, Madrid, Spain), and stored at -20°C. The presence of the 33-mer peptide was
 204 monitored by RP-HPLC according to Shan et al. (2002). Samples containing only the
 205 peptide were used to test for spontaneous degradation while samples carrying solely the
 206 strains examined served as controls of the synthesis of cellular endogenous peptides. *L.*
 207 *casei* BL23 was used as a negative control of hydrolysis (Alvarez-Sieiro et al. 2014).

208

209 **Results and Discussion**

210 **Sourdough sampling, enrichment and bacterial counts**

211 Mean bacterial numbers for given samples and culture media were calculated from
 212 the individual values obtained in each of the three culture conditions tested (32°C or
 213 37°C under aerobiosis and 37°C under anaerobiosis) (Table 1). Results pertaining to
 214 individual culture conditions for each sample and medium can be found in
 215 Supplementary Table S1. Initial counts on MRSm were between 9.36 and 10.86 log
 216 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 significant 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.

241 Different results were obtained for the Spanish baker's yeast-leavened sourdough
242 sample (Table 1). Bacterial counts were the lowest detected, and remained constant
243 across the different media and culture conditions (around 7.0 log cfu/g). The ability of
244 yeasts to efficiently degrade the starch might lead to their rapid development and thus
245 could explain the lesser participation of LAB in the fermentative process of this type of
246 sourdough (Minervini et al. 2014; De Vuyst et al. 2014). The consistency in the
247 bacterial count obtained in MRS-Mm after the two rounds of enrichment in AHG-Mm
248 broth would seem to indicate the adaptation of the lactobacilli present in this sample to
249 grow on the gliadin matrix (Table 1).

250

251 Identification and typification of gliadin-degrading LAB strains

252 Of the 267 colonies isolated from AHG-Mm plates, 48 (18%) formed degrading
253 halos and were identified by 16s rRNA sequencing. Of these, 43 were found to be LAB,
254 which were typified by PFGE into 20 different profiles: 15 *L. casei*, 1 *L. plantarum* and
255 4 *L. mesenteroides* (Fig. 1). As expected, the lactobacilli clustered into two PFGE
256 groups, one containing *L. plantarum* IPLA88 alone, the other containing the *L. casei*
257 strains (Fig. 1, panel A). For this latter group, no correlation was seen between the
258 clustering of the strains and the type of final product, nor the type of processing
259 involved. However, they did appear to cluster in a manner that reflected the geographic
260 origin of the Polish and Italian strains (Fig. 1, panel A) (the two Spanish strains located
261 randomly among the others). The existence of a correlation between geographic origin
262 and the composition of sourdough microbiota is currently a matter of debate (De Vuyst
263 et al. 2014; Minervini et al. 2014). Indeed, in contrast to the results for *L. casei*, the
264 clustering of the *L. mesenteroides* strains (Fig. 1, panel B) suggests no such correlation

265 exists, although this interpretation should be received with caution given the small
266 number examined.

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

276 *Leuconostoc* is usually found in the early stages of sourdough fermentation, while *L.*
277 *plantarum* dominates mature sourdoughs together with *L. sanfranciscensis* and *L.*
278 *fermentum* (Minervini et al. 2014). The abundance of *L. casei* strains isolated in the
279 present work is noteworthy; this species is traditionally reported as subdominant within
280 sourdough communities. The present selection procedure may therefore have promoted
281 the growth of those strains able to utilize gliadins as a nitrogen source. Although it is
282 traditionally assumed that LAB play a secondary role during sourdough proteolysis,
283 acting after any cereal endogenous proteases, strain-specific proteolytic activities have
284 also been detected in sourdough LAB (Gobetti et al. 1996; Di Gagno et al. 2004; De
285 Angelis et al. 2006; Rizello et al. 2007; Loponen et al. 2007; M'hir et al. 2008; Gerez et
286 al. 2012). Moreover, the proteolytic capacity of *L. casei* species during dairy
287 fermentations is well-known, their activity degrading the casein matrix into
288 oligopeptides, an essential step for the growth of the secondary LAB microbiota (Kojic
289 et al. 1991).

290 The absence of obligate heterofermentative species within the isolates of the present
291 work could be due to their incapacity to metabolize the gliadin substrate or to the
292 absence of any metabolite within the culture media that would result essential for their
293 growth and development; this could include additional carbon sources such as pentoses.

294

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

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

311

312 **Evaluation of the selected strains' ability to hydrolyze the 33-mer peptide**

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

326 The most active strain was *L. casei* IPLA12038, which reduced the peak area of the
327 peptide by up to 82% within 8 h, and completely made it disappear within 12 h (Fig. 2,
328 panel B). Interestingly, no intermediate hydrolysis products were observed when the
329 chromatogram for the latter incubation was compared to that of a parallel incubation
330 containing solely the strain (i.e., with no substrate). There are no previous reports of any
331 individual LAB strain from sourdough harbouring the complete repertoire of enzymes
332 and/or membrane transport systems needed to fully metabolize the 33-mer peptide. The
333 ingestion of gliadin-degrading enzymes is currently being tested as an alternative to
334 following a gluten-free diet, the only treatment currently available for patients with
335 coeliac disease. The use of gliadin-degrading enzymes to eliminate flour toxicity during
336 sourdough fermentation has been tested with selected LAB strains (Gerez et al. 2012),
337 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.

341

342 Acknowledgments

343 We thank Prof. Chaitan Khosla for his encouragement regarding the initiation of this
344 work. We thank Pascuale Russo and Thomasz Rychlik for kindly providing the Italian
345 and Polish sourdough samples respectively. We also thank Adrian Burton for language
346 assistance. This work was funded by Ministry of Economy and Competitiveness, Spain
347 (RM2010-00017-00-00) and also by the GRUPIN14-137 project, which is co-financed
348 by the Plan for Science, Technology and Innovation of the Principality of Asturias
349 2014-2017 and the European Regional Development Funds. P.A.S. was supported by a
350 predoctoral grant from the FICYT (BP09093).

351

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447 **Figure captions**

448

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

454

455 **Fig. 2.** Incubation of the 33-mer peptide with selected gliadin-degrading LAB. (A)
456 Percentage reduction of the 33-mer peptide peak area when incubated individually with
457 the indicated strains for 8 h (grey stacked bars) or 24 h (black stacked bars). Reactions
458 involving only the peptide, or the peptide in combination with the reference strain *L.*
459 *casei* BL23, were used as negative controls. Lm, *Leuconostoc mesenteroides*. Lp,
460 *Lactobacillus plantarum*. Lc, *Lactobacillus casei*. (B) Chromatogram obtained when the
461 33-mer peptide was incubated with *L. casei* IPLA12038 for 0 h (top line) or 12 h
462 (middle line). A reaction containing the strain alone was used to check the appearance
463 of peptides derived from its normal metabolism (bottom line). The chromatogram
464 section that contains the 33-mer peptide peak is shown enlarged in the inset.

465

466

467

261x134mm (120 x 120 DPI)

A

B

152x211mm (150 x 150 DPI)

Table 1
Sourdough samples and bacterial counts (mean ± standard deviation for the tested growing conditions) in the indicated culture media.

Sample			Log · cfu/g					
Product	Processing	Origin	without enrichment		1st enrichment		2nd enrichment	
			MRSm	AHG-Mm	MRSm	AHG-Mm	MRSm	AHG-Mm
Wheat Bread	Traditional Laboratory	Spain	9.36 ± 0.44 ^a	9.33 ± 0.04 ^a	8.30 ± 0.02 ^{ab}	7.79 ± 1.18 ^{ab}	6.84 ± 0.65 ^b	6.58 ± 0.71 ^b
Wheat Bread	Yeast leavening Bakery	Spain	7.80 ± 0.07 ^a	7.90 ± 0.03 ^a	7.28 ± 0.35 ^a	6.00†	7.02 ± 0.58 ^a	ND
Wheat Bread	Traditional Bakery	Italy	9.68 ± 0.08 ^a	7.85 ± 0.75 ^{ab}	7.96 ± 0.82 ^{ab}	7.85 ± 1.22 ^{ab}	5.90 ± 1.09 ^{ab}	7.15 ± 0.25 ^b
Rye Bread	Traditional Bakery	Poland	10.80 ± 0.18 ^a	6.88 ± 1.15 ^b	8.59 ± 0.52 ^a	7.52 ± 0.99 ^b	6.19 ± 0.41 ^b	5.91 ± 1.39 ^b
Wheat Pasta	Traditional Bakery	Italy	10.52 ± 0.14 ^a	9.10 ± 0.26 ^b	8.74 ± 0.20 ^b	8.71 ± 0.29 ^b	7.29 ± 0.32 ^c	7.40 ± 0.28 ^c
Wheat Pasta	Traditional Household A*	Italy	10.86 ± 0.65 ^a	9.20 ± 0.18 ^b	9.30 ± 0.09 ^b	9.18 ± 0.50 ^b	6.96 ± 1.23 ^c	6.88 ± 0.91 ^c
Wheat Pasta	Traditional Household B*	Italy	10.48 ± 0.17 ^a	7.61 ± 1.29 ^{ab}	8.88 ± 0.16 ^{ab}	9.05 ± 0.32 ^{ab}	7.46 ± 0.37 ^b	7.23 ± 0.11 ^b
Wheat Pizza	Traditional Household A*	Italy	10.38 ± 0.15 ^a	8.80 ± 0.59 ^{ab}	9.24 ± 0.21 ^{ab}	9.11 ± 0.14 ^{ab}	6.94 ± 0.47 ^b	7.49 ± 1.16 ^{ab}
Wheat Pizza	Traditional Household B*	Italy	10.38 ± 0.65 ^a	9.07 ± 0.11 ^b	8.70 ± 0.35 ^b	8.76 ± 0.17 ^{bc}	7.50 ± 0.26 ^c	7.92 ± 0.43 ^{bc}
Wheat Pizza	Traditional Household C*	Italy	10.72 ± 0.72 ^a	9.56 ± 0.14 ^a	9.24 ± 0.04 ^a	9.08 ± 0.38 ^a	6.77 ± 0.56 ^b	7.04 ± 1.17 ^b

*A, B and C correspond to three different sources. †, data retrieved from the result of growth at 37°C. ND, not determined

^{a-c} 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.

Strain	Antibiotic MIC (mg/L)							
	Am	Pc	Cl	Gm	Sm	Tc	Cm	Em
<i>L. casei</i> IPLA12001	2	0.5	0.06	2	16	4	16	0.12
<i>L. casei</i> IPLA12003	4	0.5	0.12	2	16	2	8	0.12
<i>L. casei</i> IPLA12014	2	0.25	0.03	1	16	2	8	0.06
<i>L. casei</i> IPLA12019	2	0.25	0.12	1	16	4	16	0.12
<i>L. casei</i> IPLA12023	2	0.5	0.12	1	16	4	16	0.12
<i>L. casei</i> IPLA12024	2	0.5	0.25	2	8	1	8	0.12
<i>L. casei</i> IPLA12026	2	0.5	0.12	2	16	1	4	0.06
<i>L. casei</i> IPLA12027	2	0.5	0.12	2	16	2	8	0.06
<i>L. casei</i> IPLA12030	2	0.5	0.12	2	16	4	8	0.12
<i>L. casei</i> IPLA12032	4	0.5	0.12	2	8	2	8	0.12
<i>L. casei</i> IPLA12034	2	0.25	0.06	0.5	4	0.5	4	0.06
<i>L. casei</i> IPLA12035	2	0.5	0.12	1	16	2	8	0.12
<i>L. casei</i> IPLA12037	4	0.5	0.12	2	32	2	8	0.12
<i>L. casei</i> IPLA12038	2	0.5	0.06	2	16	1	8	0.06
<i>L. casei</i> IPLA12042	2	0.5	0.12	2	16	2	8	0.12
<i>L. plantarum</i> IPLA88	1	0.5	0.03	0.5	0.5	32	8	0.06
<i>Ln. mesenteroides</i> IPLA12002	2	0.25	4	0.5	8	4	4	0.12
<i>Ln. mesenteroides</i> IPLA12012	4	1	0.12	1	32	8	8	0.25
<i>Ln. mesenteroides</i> IPLA12017	2	0.5	8	4	32	8	8	0.12
<i>Ln. mesenteroides</i> IPLA12018	4	1	0.06	0.5	16	8	8	0.25

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.