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Title: Drivers for the establishment and composition of the sourdough lactic acid bacteria biota

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Abstract: The drivers for the establishment and composition of the sourdough microbiota, with particular emphasis on lactic acid bacteria, are reviewed and discussed. More than 60 different species of lactobacilli were identified from sourdoughs, showing the main overlapping between sourdough and human intestine ecosystems. The microbial kinetics during sourdough preparation was described by several studies using various methodological approaches, including culture-dependent and -independent (e.g., high throughput sequencing), and metabolite and meta-transcriptome analyses. Although the abundant microbial diversity harbored by flours, a succession of dominating and sub-dominating populations of lactic acid bacteria suddenly occurred during sourdough propagation, leading to the progressive assembly of the bacterial community. The contribution of all the potential sources (house microbiota, flour, types of flours and additional ingredients) for contaminating lactic acid bacteria was compared with the aim to find overlapping or specific routes that affect the sourdough microbiota. Once established and mature, pros and cons regarding the stability of the sourdough lactic acid bacteria biota were also reviewed, showing contradictory results, which were mainly dependent on the species/strains.

Probably, the future research efforts should be dedicated to decrease the sources/drivers of noticeable variation rather than to full standardization of the process for sourdough preparation and use.



UNIVERSITÀ  
DEGLI STUDI DI BARI  
ALDO MORO

DIPARTIMENTO DI SCIENZE  
DEL SUOLO, DELLA PIANTA E  
DEGLI ALIMENTI – Di.S.S.P.A.

*Bari, February 15<sup>th</sup>, 2016*

Dear Prof. L. Cocolin,

I would like to submit the manuscript “Drivers for the establishment and composition of the sourdough lactic acid bacteria biota” for publication on International Journal of Food Microbiology.

Best Regards

Marco Gobbetti

1      **Drivers for the establishment and composition of the sourdough lactic acid**  
2    **bacteria biota**

3  
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## 32 **1. Introduction**

33 Sourdough is one of the most ancient examples of natural starter, where contaminating lactic  
34 acid bacteria and yeasts coexist. Contrarily to other food and beverage fermentations, the pioneer  
35 studies describing the sourdough date back not later than about thirty years ago. Spicher and the  
36 Spanish group coordinated by Benedito de Barber were among the first researchers who studied the  
37 sourdough's world (Barber et al., 1989, 1991; Spicher, 1987). Later and continuously, an abundant  
38 literature has followed: 1122 published items dealing with sourdough were retrieved from the main  
39 literature databases in February 2016.

40 Undoubtedly, the knowledge about this natural starter has increased. A consensus on the  
41 capacity of sourdough to positively influence the sensory, nutritional, texture, and shelf-life features  
42 of baked goods was achieved (De Vuyst et al., 2009; Gobbetti et al., 2014). The main metabolic and  
43 functional traits of sourdough yeasts and, especially, lactic acid bacteria were described (for reviews  
44 see De Vuyst et al., 2009, 2014; Gobbetti et al., 2014; Minervini et al., 2014). Efforts to set up  
45 technology parameters (e.g., dough yield, percentage of sourdough inoculum, salt, pH, redox  
46 potential, time and temperature of fermentation, number and length of back slopping) and to  
47 establish their effects on the sourdough performance were also largely done (De Vuyst et al., 2009;  
48 Gänzle et al., 2007; Gänzle and Vogel, 2003; Gobbetti et al., 2005; Hammes et al., 2006; Minervini  
49 et al., 2014). The main applicative repercussion of this intensive work was an increased use of  
50 sourdough at industrial and, especially, artisanal levels. About 30 to 50% of the breads  
51 manufactured in European countries require the use of sourdough. In Italy, ca. 200 different types of  
52 traditional/typical sourdough breads are manufactured, especially by small or medium-size  
53 specialized bakeries (INSR 2000; Minervini et al., 2012a). Crackers, pizza, various sweet baked  
54 goods, and gluten-free products are also made using sourdough fermentation (De Vuyst et al., 2009;  
55 Gobbetti, 1998). Traditionally, type I sourdough, made from a firm dough fermented at 30-37°C  
56 and continuously back slopped, is the most largely used in the recipes of leavened baked goods.

57           Despite these evident progresses, the recent literature has markedly focused on the drivers  
58 that affect the establishment and composition of the sourdough biota. A more comprehensive  
59 interpretation of such factors is pivotal to standardize the sourdough performance and to allow the  
60 propagation and use of this natural starter more manageable and safe. Lactic acid bacteria, the  
61 microbial group showing both the highest influence on the sourdough performance and the highest  
62 sensitivity to ecological determinants, were subjected to an in depth study. Notwithstanding,  
63 species belonging to *Leuconostoc*, *Lactococcus*, *Enterococcus*, *Pediococcus* or *Weissella* genera,  
64 which had also been isolated from sourdoughs (De Vuyst and Neysens, 2005; Ehrmann and Vogel,  
65 2005; Hammes et al., 2005), the interest on most of the autochthonous sourdough species/strains  
66 was mainly confined to the *Lactobacillus* genus.

67           This review aims at describing and discussing the main recent and relevant data on diversity  
68 and, especially, factors (house microbiota, flour, flour varieties and ingredients) that drive the  
69 establishment and composition of the sourdough lactic acid bacteria biota. Once established, pros  
70 and cons regarding the stability of the sourdough lactic acid bacteria biota were also reviewed.

## 71 **2. Diversity of lactobacilli**

72           The diversity of the sourdough microbiota depends on a number of ecological (see below)  
73 and technology determinants (De Vuyst et al., 2009; Gänzle et al., 2007; Gänzle and Vogel, 2003;  
74 Gobbetti et al., 2005; Hammes et al., 2006; Minervini et al., 2014). Furthermore, the metabolic  
75 adaptability to sourdough stressing conditions, the nutritional interactions among microorganisms  
76 (e.g., between lactobacilli and yeasts), and the intrinsic robustness or weakness of microorganisms  
77 have an influence on the microbial diversity of sourdough (Minervini et al., 2014). Sourdoughs may  
78 harbor simple (few species) to very complex microbial consortia (Minervini et al., 2014) but, given  
79 the above numerous factors, the differences in taxonomy and metabolism shown by sourdough  
80 lactic acid bacteria are not surprising (De Vuyst and Neysens, 2005; De Vuyst et al., 2002).

81 Table 1 lists, not exhaustively, the species of lactobacilli that were identified from  
82 sourdoughs made from various flours and of different geographical origins. Differences between  
83 culture-dependent and -independent methods of analysis in some cases make difficult the data  
84 comparison. Nevertheless, more than 60 different species of lactobacilli were associated to  
85 sourdough during time, which is certainly representative of the very large diversity.

86 Selecting the species most frequently isolated from sourdoughs, and searching for other  
87 ecosystems wherein such species were also found (Figure 1), the major overlapping appears  
88 between sourdoughs and human intestine and feces. *Lactobacillus reuteri* inhabits gastro-intestinal  
89 tract of vertebrates and some sourdoughs. As recently suggested by comparative genomic analyses,  
90 vertebrate-symbiont strains of *L. reuteri* may have adapted to sourdough ecosystem, through  
91 positive selection exerted on several genes involved in energy conversion and carbohydrate  
92 metabolism (Zheng et al., 2015). Overall, the cross-contamination by lactobacilli of intestinal origin  
93 was postulated by several authors (De Angelis et al., 2006; Du Toit et al., 2003; Ehrmann and  
94 Vogel, 2005). On other hand, *Lactobacillus plantarum* is the most largely isolated species from  
95 other habitats. The ecological flexibility of *L. plantarum* is reflected by the observation that this  
96 species has one of the largest genomes known among lactic acid bacteria (Chevallier et al., 1994).  
97 Features of this huge environmental adaptation and potential of this bacterium were shown when  
98 the kinetics of growth, especially the steady state, of *L. plantarum* strains were compared using as  
99 substrate different vegetable juices and wheat flour hydrolysate used as the control. Juices, in  
100 particular cherry juice, induced specific metabolic traits. Although fermentable carbohydrates are  
101 available, decarboxylation of malic acid provides energy advantages due to the increased  
102 intracellular pH and the synthesis of reducing power. Conversion of branched chain amino acids  
103 into their corresponding 2-ketoacids leads to gain of ATP. Decarboxylation of histidine into  
104 histamine provides energy through the generation of proton motive force (Filannino et al., 2014).

### 105 **3. Microbial kinetics during sourdough preparation**

106           The procedure for preparing type I sourdough is extremely easy. Just mixing water and  
107 flour, leaving the dough at an appropriate temperature for a consistent number of hours, and then  
108 proceeding with a number of back-slopping steps, using a part of the previously fermented dough.  
109 But, what happens during back-slopping?

110           To the best of our knowledge, Onno and Russel (1994) were the first who attempted to  
111 describe the bacterial evolution during sourdough preparation. This was done in term of balance  
112 between decreasing Gram-negative towards increasing Gram-positive biota. A number of relevant  
113 papers succeeded to this first description. Some of the most representative studies (Van der Meulen  
114 et al., 2007; Weckx et al., 2010) combined the description of microbiological and metabolic  
115 features. The establishment of the sourdough microbiota occurred through a three-phase evolution,  
116 lasting 10 days, during which sourdough typical and less typical species alternated. This was proven  
117 under laboratory conditions, without addition of starter cultures, and using wheat, spelt or rye  
118 flours. All mature sourdoughs were dominated by a combination of obligate (e.g., *Lactobacillus*  
119 *fermentum*) and facultative (e.g., *L. plantarum*) hetero-fermentative lactobacilli (Van der Meulen et  
120 al., 2007). The codominance of obligate (*Lactobacillus brevis*, *Lactobacillus rossiae*, *Lactobacillus*  
121 *sanfranciscensis* and *Weissella cibaria*) hetero-fermentative lactic acid bacteria and *L. plantarum*  
122 was also shown in several traditional Sicilian (Southern Italy) sourdoughs (Ventimiglia et al.,  
123 2015). Homo-fermentative species were not usually retrieved in the stable ecosystem (Van der  
124 Meulen et al., 2007). Meta-transcriptome hybridization data, based on a lactic acid bacteria  
125 functional gene microarray, confirmed the previous results for wheat and spelt sourdoughs, also  
126 showing the functional role for *Lactococcus lactis* in the early stage of sourdough preparation and  
127 the dominance of *Pediococcus pentosaceus* in some fermentations (Weckx et al., 2010b). Other  
128 studies also emphasized the succession of lactic acid bacteria species. During back slopping (20 or  
129 30°C) of semi-solid (dough yield of 200) rye sourdoughs, the bacterial dynamics was described by  
130 DGGE coupled with pyrosequencing of bar-coded 16S rRNA gene amplicon and identification of  
131 isolates through partial sequencing of 16S rRNA gene (Bessmeltseva et al., 2014). Also in this case



132 facultative and obligate hetero-fermentative lactobacilli dominated, but their proportion varied  
133 depending on the temperature. The microbial kinetics was studied during preparation of an organic  
134 gluten-free sourdough (Lhomme et al., 2014). *L. sanfranciscensis*, *L. plantarum*, and *Lactobacillus*  
135 *spicheri* dominated the first phases of propagation but the 2 last species suddenly decreased, being  
136 *L. sanfranciscensis* and *Lactobacillus sakei* the predominant species at the latest stages.

137 The bacterial dynamics turning flours into mature rye or soft or durum wheat sourdoughs  
138 was investigated through high throughput sequencing (Ercolini et al., 2013). Permutation analysis  
139 based on biochemical and microbial features, and diversity indices all together demonstrated the  
140 maturity of the sourdoughs after 5 to 7 days of propagation. Flours showed a very large diversity,  
141 being mainly contaminated by metabolically active genera (*Acinetobacter*, *Pantoea*, *Pseudomonas*,  
142 *Comamonas*, *Enterobacter*, *Erwinia* and *Sphingomonas*) belonging to the phylum Proteobacteria or  
143 Bacteroidetes (genus *Chryseobacterium*). Soon after 1 day of propagation (just adding water to  
144 flour and waiting for fermentation), this population was almost completely inhibited, except for the  
145 *Enterobacteriaceae*. Although members of the phylum Firmicutes were present at very low or  
146 intermediate relative abundances in the flours, they became dominant after 1 day of propagation.  
147 Firmicutes were almost exclusively represented by lactic acid bacteria. A succession of dominating  
148 and sub-dominating populations of *L. sakei* group, *Leuconostoc* spp., *Weissella* spp., *Lactobacillus*  
149 spp. (mainly *L. plantarum*) and *Lc. lactis* occurred during propagation, leading to the progressive  
150 assembly of the bacterial community (Ercolini et al., 2013).

#### 151 **4. Sources for contaminating sourdough lactobacilli**

152 Overall, almost all the studies failed to link the species diversity of the sourdough biota with  
153 the geographical origin of the sourdough (De Vuyst et al., 2009). Therefore, being the bacteria not  
154 deliberately added to the sourdough, the house microbiota, flour and additional ingredients are  
155 certainly the most important sources for their contamination.

156           Although water, the other main ingredient of dough, has not to be considered as reservoir of  
157 bacterial inoculum, it did not receive the attention needed. Future studies should be carried out in  
158 order to ascertain the influence of water on sourdough baked good rheology and shelf life, and any  
159 microbial kinetics.

#### 160 4.1. House microbiota

161           In one of the first papers focusing on the house microbiota, bacterial isolates were collected  
162 from sourdoughs, flour, hands of the baker, air and equipment of several artisanal bakeries. At a  
163 considerable distance of time, key lactic acid bacteria, such as *L. sanfranciscensis* and *L. plantarum*,  
164 were found in bakery air and on baker's hands, as well as in the sourdoughs. The AFLP  
165 fingerprinting demonstrated that sourdough and bakery environment isolates were genetically  
166 indistinguishable. Specific strains of lactic acid bacteria persisted in artisan sourdoughs and  
167 circulated in the bakery environment, which acted as a potential carrier (Scheirlinck et al., 2008).

168           More recently and with an experimental approach similar to that adopted in the above paper,  
169 RNA was collected/extracted from flour, sourdough, dough mixer and storage box of four bakeries,  
170 and subjected to 16S metagenomics. A consistent presence of *L. sanfranciscensis* in storage box  
171 and dough mixer, as well as in the sourdough, was shown. For one bakery, the abundance of this  
172 species from equipment was markedly higher than that found in the flour. On the contrary, *W.*  
173 *cibaria* showed higher adaptability in sourdough than in bakery equipment, suggesting that its main  
174 origin was flour. The results of this study strengthened the hypothesis that dominant species of  
175 sourdough lactic acid bacteria highly contaminated the house microbiota (Minervini et al., 2015).

176           In another study (Minervini et al., 2012b), 7 mature type I sourdoughs from Southern Italy  
177 were comparatively back-slopped for 80 days at artisan bakery and laboratory levels under the same  
178 and constant technology parameters. Some of the bakeries considered were the same of the study  
179 previously cited (Minervini et al., 2015). Statistical permutation analysis based on culture-  
180 independent (DGGE bands) and -dependent analyses showed that, although starting from the same

181 sourdough, most of the sourdoughs propagated at laboratory (e.g., MT.A.I-V.L, MT.B.I-V.L)  
182 differed from those propagated at artisan bakeries (e.g., MT.A.I-V, MT.B.I-V) (Figure 2).  
183 Furthermore, some strains (e. g., belonging to *L. sanfranciscensis*) persisted during propagation of  
184 sourdough under both conditions, but most of the strains were identified only at either artisan  
185 bakery (*L. plantarum*) or laboratory (e.g., *Leuconostoc citreum*) levels. Occasional sourdough  
186 contamination by diverse species and strains of lactic acid bacteria was found depending on the  
187 environment of propagation. Therefore, the switch of the microbiota abundantly occurred  
188 depending on the environment of propagation. This finding was consistent with the decreased  
189 number of yeasts found in sourdough propagated at laboratory and with the persistence of  
190 *Saccharomyces cerevisiae*, which was shown for almost all the sourdoughs propagated at bakery  
191 but not in several sourdoughs propagated at laboratory (Minervini et al., 2012b; Vrancken et al.,  
192 2010). Within the same bakery, the switch from firm to liquid sourdough fermentation might be  
193 considered as another driver affecting the microbiota composition. Typical firm sourdoughs from  
194 Southern Italy were collected from bakeries, propagated for one week in laboratory under the  
195 conditions applied at the bakeries and then subjected to a comparative propagation under liquid and  
196 firm conditions for 28 days (Di Cagno et al., 2014). Compared to firm, liquid sourdoughs simplified  
197 the microbial diversity and harbored a lower number of strains, which were persistent. *L. plantarum*  
198 mainly dominated firm sourdoughs over time. *Leuconostoc lactis* and *L. brevis* dominated only  
199 some firm sourdoughs, and *L. sanfranciscensis* persisted for some time only in some firm  
200 sourdoughs. *Leuc. citreum* persisted in all firm and liquid sourdoughs, and it was the only species  
201 always detected in liquid sourdoughs. It was flanked by *Leuconostoc mesenteroides* in some  
202 sourdoughs.

203         The continuous introduction of flour into the bakery environment, as well as the almost daily  
204 propagation of type I sourdough, would help to build up a house microbiota that may serve as an  
205 important inoculum for each fermentation, likewise to what usually observed for instance in cheese  
206 and wine making (Scheirlinck et al., 2009).

## 207 4.2. Flour

208 If the house microbiota is certainly an important driver to establish the microbiota, lactic  
209 acid bacteria were also directly isolated from flour (De Vuyst et al., 2009). The flour microbiota  
210 may reflect the environmental contamination of the bakery, but most probably would be a reservoir  
211 of microbes that, directly or indirectly (via the house microbiota), contaminate sourdough. The total  
212 microbial population and the relative species proportion on cereal grains are affected by many  
213 factors, mainly climatic conditions (e.g., temperature and rainfall), physical damage due to insects  
214 or fungi attacks and use of insecticides and fungicides (Corsetti et al., 2007). Overall, microbes that  
215 contaminate cereals are concentrated in the outer layers of kernel, and they tend to stay in fractions  
216 rich in bran during milling. Consequently, flour obtained from milling should theoretically contain a  
217 lower bacterial load than caryopses, but the caryopsis conditioning may increase the microbial  
218 content of flour (Berghofer et al., 2003).

219 The correspondence between grains, bran and flour and mature sourdoughs was investigated  
220 through culture-dependent approach (Corsetti et al., 2007). The presence of a sub-dominant  
221 population of lactic acid bacteria, mainly consisting of *Enterococcus faecium*, *Enterococcus mundtii*  
222 and *Lactobacillus graminis*, was demonstrated. Lactic acid bacteria from wheat flours used in  
223 traditional bread making throughout Sicily were also characterized (Alfonso et al., 2013). Partial  
224 sequencing of 16S rRNA gene grouped the strains into six genera of lactic acid bacteria, being *W.*  
225 *cibaria*, *L. plantarum*, *Leuconostoc pseudomesenteroides* and *Leuc. citreum* the most prevalent  
226 species. The microbiotas of 19 Italian sourdoughs used for the manufacture of traditional/typical  
227 breads were investigated through a culture-dependent approach (Minervini et al., 2012a). As shown  
228 by principal component analysis (PCA) (Figure 3), a correlation was found between the type of  
229 flour and the microbial community of sourdoughs. *Triticum turgidum* ssp. *durum* flours were  
230 characterized by high levels of maltose, glucose, fructose, and free amino acids, correlated with the  
231 sole or main presence of obligate hetero-fermentative lactic acid bacteria, the lowest number of

232 facultative hetero-fermentative strains, and the low cell density of yeasts in the mature sourdoughs.  
233 On the same way, the more or less abundant presence of bran in flour may impact the resulting  
234 sourdough microbiota because of the relevant content of dietary fiber and bioactive compounds that  
235 may impact the microbial diversity (Katina et al., 2007, 2012; Liukkonen et al., 2003; Poutanen et  
236 al., 2009; Rizzello et al., 2010; Ross et al., 2004).

237         Since the presence of flour autochthonous strains was shown, it may be hypothesized that  
238 these strains are members of the plant endophytic microbiota. Epiphytic lactic acid bacteria were  
239 isolated from forage crops (Pang et al., 2012; Zhang et al., 2000), and lactobacilli inoculated into  
240 *Lolium perenne* were able to colonize roots at the endophytic level (Berlec, 2012). Considering 4  
241 plots of land for 2 durum wheat cultivars, a metagenomics study was carried out to describe the  
242 kinetic of the Firmicutes population through the main phenological stages of wheat plant (Minervini  
243 et al., 2015b). Endophytic and epiphytic populations were analyzed separately. Since the early  
244 stages of wheat growth, *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Enterococcus* were the main  
245 epiphytic and endophytic genera among lactic acid bacteria. *Paenibacillus*, *Bacillus*,  
246 *Exiguobacterium* and *Staphylococcus* completed the picture of the core genus microbiome. The  
247 relative abundance of each lactic acid bacterium genus was affected by cultivars, phenological  
248 stages, other Firmicutes genera, environmental temperature, and water activity of plant organs.  
249 Lactobacilli, showing the highest sensitivity to water activity, markedly decreased during milk  
250 development or physiological maturity. Nevertheless, strains belonging to the key sourdough  
251 species *L. plantarum* were typed and their presence as endophytes was shown through all the  
252 phenological stages of both the cultivars. Those strains persisted in the milled flour and bran too  
253 (Figure 4). Therefore, the presence of lactic acid bacteria as endophytes of wheat plant was proven  
254 (Minervini et al., 2015b).

#### 255 4.3. Flour species

256 A number of studies accumulated in the literature, dealing with sourdoughs based on either  
257 wheat flours coming from countries characterized by very diverse climates or obtained from milling  
258 grains of various species of non-wheat cereals and pseudo-cereals.

259 Six Albanian flours obtained by as many soft or durum wheat cultivars were used for  
260 making type I sourdoughs and as source of isolation of lactic acid bacteria (Nionelli et al., 2014).  
261 The predominant species were *L. plantarum* and *Leuc. mesenteroides*, some strains of which were  
262 suitable for making sourdoughs having high functional potential. Eight Iranian wheat flours were  
263 collected from industrial and artisanal mills, and also in this case used to prepare type I sourdough  
264 (Pontonio et al., 2015). No lactobacilli were detectable within the biota, and strains of *P.*  
265 *pentosaceus*, *W. cibaria*, *Weissella confusa* and *Leuc. citreum* were the most abundant. An in depth  
266 characterization of lactic acid bacteria was also carried out on sourdough samples collected from the  
267 Western region in Inner Mongolia of China and used for Chinese steamed breads (Zhang et al.,  
268 2011). Both the 16S rRNA gene sequencing and DGGE profile analysis indicated that *L. plantarum*  
269 group constituted the predominant biota. *Lactobacillus guizhouensis*, *L. rossiae* and *Lactobacillus*  
270 *zeae* were also found. Organic sourdoughs used for the manufacture of French bread were studied  
271 twice during time, using pyrosequencing of the 16S rRNA V1–V3 region (Lhomme et al., 2015,  
272 2016). Both culture-dependent and -independent methods identified *L. sanfranciscensis* as the  
273 dominant species in most of the sourdoughs. Sub-dominant species were *Lactobacillus curvatus*,  
274 *Lactobacillus hammesii*, *Lactobacillus paralimentarius*, *L. plantarum*, *Lactobacillus pentosus* and  
275 *L. sakei*. *T. turgidum* ssp. *durum* was grown according to conventional, organic (with cow or green  
276 manure) and without input farming systems to investigate the effect of the type of farming on the  
277 lactic acid bacteria biota (Rizzello et al., 2015). Before fermentation, the dough made with  
278 conventional flour showed the highest bacterial diversity. Flours were variously contaminated by  
279 genera belonging to the Proteobacteria, Firmicutes and Actinobacteria. The highest diversity of  
280 Firmicutes was found in mature type I sourdoughs made with organic and, especially, no input  
281 flours. The *L. plantarum* group was the only one commonly found in all the samples from doughs to

282 mature sourdoughs, with the highest dominance in the sourdoughs made from organic flour. *Leuc.*  
283 *citreum*, *Lc. lactis*, *P. pentosaceus*, *W. cibaria* and *Enterococcus lactis* were also identified  
284 depending on the farming system. Therefore, the farming system was another determinant affecting  
285 the sourdough microbiota. The organic cultivation of durum wheat was reflected along the flour-  
286 sourdough fermentation-bread axis (Rizzello et al., 2015).

287 The partial sequencing of the 16S rRNA, 16S/23S rRNA spacer region, *recA* and *pheS* genes  
288 was used to identify the lactic acid bacteria biota of spelt and emmer flours (Coda et al., 2010b).  
289 Spelt flour showed a large diversity, including *L. plantarum*, *L. brevis*, *L. curvatus*, *L.*  
290 *sanfranciscensis*, *L. fermentum*, *W. confusa* and *P. pentosaceus* strains. In part flanked by *L. rossiae*  
291 and *W. confusa*, *L. plantarum* dominated the biota of emmer flour. Acha, also known as white fonio  
292 or hungry rice (*Digitaria exiliis*), and Iburu, also known as black fonio or petit mil (*Digitaria*  
293 *iburua*), are some of the oldest African cereals (Jideani et al., 1994). The microbiological  
294 characterization of these flours revealed the dominance of *P. pentosaceus*, which was also suitable  
295 for making sourdoughs (Coda et al., 2010a). Ting is a fermented sorghum-based product of  
296 Botswana that is used to prepare porridges of different consistencies. The microbiota of several  
297 samples of ting consisted of 2/4 associated species, which mainly corresponded to *L. reuteri*, *L.*  
298 *fermentum*, *Lactobacillus harbinensis*, *L. plantarum*, *Lactobacillus parabuchneri*, *Lactobacillus*  
299 *casei* and *Lactobacillus coryneformis* (Monang and Ganzle, 2011). One of the most used way to  
300 increase the beneficial consumption of legumes is their incorporation in complex matrices with  
301 cereals also for making fermented baked goods (Gomez et al., 2008; Mohammed et al., 2012).  
302 Therefore, legume flours are further sources of contaminating lactic acid bacteria. Indeed, the biota  
303 of wheat-legume sourdough was characterized by a very large diversity, including *L. plantarum*, *L.*  
304 *sanfranciscensis*, *Leuc. mesenteroides*, *L. fermentum*, *W. cibaria*, *L. pentosus*, *L. coryneformis*, *L.*  
305 *rossiae*, *L. brevis*, *L. parabuchneri* and *Lactobacillus paraplantarum* (Rizzello et al., 2014).

306 Also gluten-free matrices were exploited for preparing sourdough, given the fact that they  
307 potentially harbor lactic acid bacteria. Four different laboratory scale gluten-free spontaneously

308 fermented sourdoughs were prepared from buckwheat or teff flours (Moroni et al., 2011). Overall, a  
309 broad spectrum of lactic acid bacteria was identified, belonging to *L. plantarum*, *Lactobacillus*  
310 *pontis*, *P. pentosaceus*, *Leuconostoc holzapfelii*, *Lactobacillus gallinarum*, *Lactobacillus vaginalis*,  
311 *L. sakei*, *L. graminis* and *W. cibaria*. Spontaneously fermented sourdoughs were prepared from 5  
312 amaranth flours to investigate the presence of autochthonous lactic acid bacteria suitable to be used  
313 as starter cultures (Sterr et al., 2009). Strains of *L. plantarum*, *L. sakei* and *P. pentosaceus* were  
314 abundantly found and their selection allowed to get stable amaranth-based sourdough. A large  
315 number of lactic acid bacteria isolates were identified from fermenting cassava in South Africa,  
316 Benin, Kenya and Germany (Kostinek et al., 2007). Most of the strains referred to *L. plantarum*, *L.*  
317 *pentosus*, *Leuconostoc fallax*, *Weissella paramesenteroides*, *L. fermentum*, *Leuc. mesenteroides* and  
318 *W. cibaria*.

319 Figure 5 summarizes all the above described results, also including lactic acid bacteria that  
320 were identified from other flours and countries. Without claiming any statistical significance, it  
321 emerges that most of wheat flours from other countries and/or from non-wheat matrices showed  
322 large diversity in some cases, and, in general, robust strains of *L. plantarum* are widely diffused and  
323 flanked by species less commonly associated to sourdough, such as *P. pentosaceus*, *Leuc.*  
324 *mesenteroides* and *Weissella* sp.

#### 325 4.4. Ingredients

326 Some traditional sourdoughs are prepared and propagated by incorporating into the dough  
327 additional ingredients such as salt, sugar, fruit and grape must. The main reason behind this choice  
328 is that such ingredients may drive spontaneous fermentation towards the dominance of pro-  
329 technological lactic acid bacteria and yeasts. Ingredients either influence intrinsic parameters (e.g.,  
330 concentration of mono- and di-saccharides, water activity) of dough or are carriers of naturally  
331 contaminating microbes (Minervini et al., 2014). The sourdoughs used for manufacturing Pane di  
332 Matera PGI (Official Journal of the European Union C128, Vol. 50, 9 June 2007) and Coppia



333 Ferrarese (Official Journal of the European Union C75/13, 14 March 2012) are prepared by adding  
334 to flour and water macerated ripe fruit or grape must, respectively. Apple, grape and sugarcane are  
335 commonly used in the preparation of Brazilian sourdoughs (Aplevicz et al., 2014).

336 The lactic acid bacteria biota of sourdoughs differed depending on the addition of apple,  
337 grape or yoghurt (typical Spanish protocols) as ingredients (Gordún et al., 2015). *P. pentosaceus*  
338 was the only species identified in the sourdough added with yoghurt. On the contrary, the  
339 sourdoughs added with either apple or grape harbored *L. plantarum*, *L. brevis*, *L. sakei*, and (only  
340 grape sourdough) *W. cibaria*. Five sourdoughs were manufactured using baker's yeast, macerated  
341 pears, grape must, honey and water from macerated pears as additional ingredients in the first step  
342 of propagation (Minervini et al., unpublished). 16S rRNA metagenomics analysis performed with  
343 Firmicutes-specific primers and using the Illumina MiSeq platform showed *Lactobacillus* sp. as the  
344 dominant species in macerated pears and related water, as well as in baker's yeast. *Lactococcus*  
345 (baker's yeast), *Leuconostoc* (macerated pears and related water) and *Streptococcus* (honey) were  
346 detected as subdominant OTUs. Compared to the control sourdough (prepared just with flour and  
347 water), lower values of alpha-diversity indices were found after the first fermentation and in mature  
348 sourdoughs when additional ingredients were used. Despite the use of the same flour, culture-  
349 dependent analysis showed that different strains of *L. plantarum* were detectable depending on the  
350 ingredient added. Compared to the control sourdough, other species were isolated from sourdough  
351 initially added with baker's yeast (*L. brevis*, *L. curvatus*, *L. fermentum*), grape must (*Leuc.*  
352 *mesenteroides*) or macerated pears (*L. pentosus*). Furthermore, the use of additional ingredients  
353 affected the concentration of some free amino acids (e.g., arg, glu, ile, leu and phe) and related  
354 metabolites (ornithine and  $\gamma$ -aminobutyric acid) in the mature sourdoughs (Minervini et al.,  
355 unpublished).

## 356 **5. Stability of the mature lactic acid bacteria biota**

357           Once mature, the sourdough lactic acid bacteria biota is affected by specific technology and  
358 other not fully controllable parameters (Minervini et al., 2014), which determine its performance  
359 and stability. Overall, while the technological and biochemical performances are considered mostly  
360 stable during time, a question mark has been addressed regarding the stability of the microbiota.  
361 Pros and cons alternately succeeded in the literature.

362           A number of reports showed the sourdough stability during long-time propagation and  
363 emphasized the persistence of several key species (Vogel et al., 2011). A total of 39 traditional  
364 sourdoughs were sampled at 11 bakeries located throughout Belgium and visited twice with a 1-  
365 year interval. The taxonomic structure and stability of the bacterial communities were assessed  
366 using both culture-dependent and -independent methods. Extensive metabolite target analysis was  
367 also carried out. *L. sanfranciscensis*, *L. paralimentarius*, *L. plantarum* and *L. pontis* dominated the  
368 biota of Belgian sourdoughs. This study reinforced the observation that the bakery environment  
369 largely determines the development of a stable lactic acid bacteria population in sourdoughs  
370 (Scheirlinck et al., 2008). Industrial French sourdoughs made from spontaneous fermentation were  
371 monitored during consecutive propagation (Vera et al., 2012). A stable lactobacilli biota, mainly  
372 consisting of 4 dominant species (*Lactobacillus panis*, *Lactobacillus frumenti*, *Lactobacillus*  
373 *amylolyticus* and *Lactobacillus acetotolerans*) was observed also in this study. A sourdough started  
374 from a 6-year-old freeze dried sourdough originating from the same bakery was stable during long-  
375 time propagation (Viiard et al., 2012). A unique microbial consortium, consisting of well adapted  
376 *Lactobacillus helveticus*, *L. panis* and *L. pontis*, was identified.

377           Some studies concluded that the stability of sourdough might be achieved depending on the  
378 strain. The stable persistence of *L. reuteri* in German rye sourdough, prepared for producing a  
379 commercially available baking aid, is due to the capacity of some strains to synthesize the antibiotic  
380 reutericyclin (Gänzle and Vogel, 2003). Likewise, the bacteriocin producer *Lactobacillus*  
381 *amylovorus* DCE 471 was shown to be a competitive starter culture for type II sourdough  
382 fermentations (Leroy et al., 2007). Sourdoughs consisting of complex associations between lactic

383 acid bacteria and yeasts were shown to be only in part stable. The dominance during long time back  
384 slopping propagation depended on the strain, coexisting microbes and technology factors  
385 (Vogelmann and Hertel, 2011).

386 Notwithstanding the remarkable stability of specific strains, some other studies were  
387 relatively less in favor of the long-time persistence of a stable sourdough lactic acid bacteria biota  
388 (Vogelmann and Hertel, 2011). The taxonomic architecture of sourdoughs singly started with  
389 different strains of *L. plantarum* and *L. sanfranciscensis* was monitored during propagation through  
390 culture-dependent and -independent approaches (Minervini et al., 2010; Siragusa et al., 2009)  
391 (Figure 6). As shown by RAPD-PCR analysis, five out of seven strains of *L. plantarum* maintained  
392 elevated cell numbers (ca. 9 log cfu/g) throughout 10 days of consecutive propagation. Together  
393 with other species of lactic acid bacteria originating from flour, at least one autochthonous strain of  
394 *L. plantarum* emerged. All emerging strains of *L. plantarum* showed different RAPD-PCR profiles  
395 compared to those used as starters (Minervini et al., 2010). When almost the same experiment was  
396 carried out with 9 strains of *L. sanfranciscensis*, only 3 starters dominated throughout 10 days of  
397 propagation. The others progressively decreased to less than 3 log cfu/g. An abundant and diverse  
398 biota emerged also in this case from the flour, consisting on *W. confusa*, *L. sanfranciscensis*, *L.*  
399 *plantarum*, *L. rossiae*, *L. brevis*, *Lc. lactis* and *P. pentosaceus*. The 3 persistent starters were further  
400 used for the production of sourdoughs and propagated by using another wheat flour whose lactic  
401 acid bacterium population in part differed from the previous one. Also in this case all the 3 starter  
402 strains persisted during propagation (Siragusa et al., 2009). Four sourdoughs were produced by  
403 using a starter mixture and propagated until the composition of the lactic acid bacteria biota  
404 remained stable (Meroth et al., 2003). Only a few *Lactobacillus* species were found to be  
405 competitive and became dominant. Regardless of the starter used, associations between *L.*  
406 *sanfranciscensis* and *Lactobacillus mindensis*, *Lactobacillus crispatus* and *L. pontis*, and *L.*  
407 *crispatus*, *L. panis* and *L. frumenti* were found (Meroth et al., 2003). Although using the same type  
408 of flour (e.g., *T. durum*) and technology, some sourdoughs change the dominant microbiota over a

409 few years of propagation. This is the case of Pane di Altamura with Protected Designation of Origin  
410 (PDO) that mainly harbored *L. plantarum* in the year 2004 (Ricciardi et al., 2005) and *W. cibaria*  
411 (Minervini et al., 2012a) in the year 2011. In agreement, the evolution of bacterial communities in  
412 sourdoughs fermented at the same temperature did not follow the same time course; furthermore  
413 changes in the composition of dominant and subdominant bacterial communities occurred even  
414 after six weeks of back slopping (Bessmeltseva et al., 2014). Recently, it was shown that the ratio of  
415 bacterial species (e.g., *Lactobacillus zymae* vs. other lactic acid bacterium species) in rye  
416 sourdoughs propagated without temperature control was affected by the season of sampling (Viiard  
417 et al., 2016). After having modified the protocol of propagation (applying controlled fermentation  
418 temperature, reduced inoculum rate, and longer fermentation time), the lactic acid bacteria biota  
419 became more stable and showed an increased proportion of *L. helveticus* and especially *L. pontis*.

## 420 **6. Conclusion**

421 Compared to other leavening agents, the use of sourdough has countless advantages that  
422 reflect on sensory, shelf-life, rheology, nutritional and functional features of baked goods.  
423 Nevertheless, the sourdough microbial consortia are frequently very diverse and not always stable,  
424 in particular the lactic acid bacteria biota. Consequently, the technology and functional  
425 performances may markedly vary between sourdoughs and during propagation and use. Numerous  
426 factors (more or less controllable) drive the establishment and composition of the sourdough lactic  
427 acid bacteria biota, which, although naturally moves towards a relative stability, needs of a daily  
428 care. Probably, the future research efforts should be dedicated to decrease sources of noticeable  
429 variation rather than to fully standardize the process .

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826 **Legends to Figures**

827 **Figure 1.** Pseudo heatmap depicting the presence (green) or absence (white) of the most frequently  
828 isolated sourdough *Lactobacillus* species, in other ecosystems wherein such species were also  
829 found. Bibliographic details are available from WFCC Global Catalogue of Microorganisms (GCM)  
830 (<http://gcm.wfcc.info/>), a system that provides a uniform interface for the scientific and industrial  
831 communities to access the comprehensive microbial resource information.

832 **Figure 2** (adapted from Minervini et al., 2012). Differences of Denaturing Gradient Gel  
833 Electrophoresis (DGGE) profiles, and species and number of bacterial strains identified through  
834 culture-dependent method, between the seven sourdoughs (AM.A, AM.B, MT.A, MT.B, MT.C,  
835 MT.D and VZ) propagated at artisan bakery and laboratory (L) levels for 1 (I), 20 (II), 40 (III), 60  
836 (IV) and 80 (V) days. Euclidean distance and McQuitty's criterion (weighted pair group method  
837 with averages) were used for clustering. Colors correspond to normalized mean data levels from  
838 zero (black), low (green) to high (red). The color scale, in terms of units of standard deviation, is  
839 also shown. DGGE LAB, total number of DGGE bands related to lactic acid bacteria; LAB strains,  
840 number of lactic acid bacteria strains; L. sf, *Lactobacillus sanfranciscensis*; L. pl, *Lactobacillus*  
841 *plantarum*; Ln. cit, *Leuconostoc citreum*; P. pent, *Pediococcus pentosaceus*; W. cib, *Weissella*  
842 *cibaria*; L. sak, *Lactobacillus sakei*; L. cur, *Lactobacillus curvatus*; L. cas, *Lactobacillus casei*; A.  
843 orl, *Acetobacter orleanensis*; L. parab, *Lactobacillus parabrevis*; L. bre, *Lactobacillus brevis*; Lc.  
844 Lac, *Lc. lactis* ssp. *lactis*.

845 **Figure 3** (adapted from Minervini et al., 2012). Score and loading plots of first and second principal  
846 components and clusters after Principal Component Analysis based on composition of flour  
847 (maltose, glucose, fructose, total and individual free amino acids) and microbial community (cell  
848 densities of lactic acid bacteria and yeasts, number of species and strains isolated from each  
849 sourdough, and percentage of obligate homo-fermentative, obligate and facultative hetero-  
850 fermentative lactic acid bacteria) data from sourdoughs used for the manufacture of  
851 traditional/typical Italian breads. Each sourdough is indicated by one letter and the number of

852 biological replicate follows the underscore. Sourdoughs based on *Triticum turgidum* ssp. *durum*  
853 flour are indicated by a black circle, whereas sourdoughs based on *Triticum aestivum* flour are  
854 indicated by a white circle. Total FAA, total free amino acids; Lactic acid bacteria, cell density of  
855 lactic acid bacteria; Yeasts, cell density of yeasts; LabSpp, number of lactic acid bacteria species;  
856 LabStr, number of lactic acid bacteria strains; YSpp, number of yeasts species; YStr, number of  
857 yeasts strains; HOF, obligately homo-fermentative lactic acid bacteria; HEFfa, facultative hetero-  
858 fermentative lactic acid bacteria; HEFob, obligate hetero-fermentative lactic acid bacteria.

859 **Figure 4.** (adapted from Minervini et al., 2015). Relative abundance (%) of endophytic  
860 *Lactobacillus plantarum* within the OTU belonging to lactic acid bacteria found on epigeous organs  
861 (leaves and spikes) and processed wheat (grain, bran and flour) of Odisseo (A) and Saragolla (B)  
862 durum wheat. Epigeous organs were analyzed at tillering, stem elongation, booting, flowering, milk  
863 development and physiological maturity stages.

864 **Figure 5.** Pseudo heatmap depicting the abundance of the main lactic acid bacteria isolated from  
865 Albanian (Nionelli et al., 2014), Iranian (Pontonio et al., 2015), Chinese (Monang and Ganzle,  
866 2011), Mongolian (Zhang et al., 2011), teff and buckwheat (Moroni et al., 2011), amaranth (Sterr et  
867 al., 2009), cassava (Kostinek et al., 2007), legume (Rizzello et al., 2014), quinoa (Rizzello et al.,  
868 2016), sorghum (Vieira-Dalodé, et al., 2007), French organic (Lhomme et al., 2015, 2016) and  
869 Italian organic (Rizzello et al., 2015) sourdoughs and Italian spelt and emmer (Coda et al., 2010b),  
870 African acha and iburu (Coda et al., 2010a), Italian wheat germ (Rizzello et al., 2010) and Tunisian  
871 (Mamhoud et al., 2015, submitted) flours. The abundance of each species is expressed as percentage  
872 ratio between the number of strains belonging to that species and the total number of strains found  
873 in a specific environment.

874 **Figure 6.** Persistence of *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* strains in  
875 sourdough after continuous 10 days-long propagation. In (a) the numbers refer to the different  
876 strains of *L. plantarum*. In (b) the LS numbers refer to the different strains of *L. sanfranciscensis*  
877 (adapted from Minervini et al., 2010 and from Siragusa et al., 2009).

878 **Table 1.** Non-exhaustive survey of the species diversity within the *Lactobacillus* genera in sourdoughs or flours and of different geographical origins. The  
 879 methods of identification are also reported.

Species	Flour	Country	Method of identification	Reference
<i>L. acetolerance</i>	Wheat	France	16s gene sequencing	Vera et al., 2012
<i>L. acidifarinae</i>	Wheat	Belgium	16s gene sequencing	Vancanneyt et al., 2005
<i>L. acidophilus</i>	Wheat	Italy	16s gene sequencing API50-CHL – ATB32	Corsetti et al., 2001 Gobbetti et al., 1994
		France	Phenotyping	Infantes and Tourner, 1991
	Rye	Germany	Phenotyping	Spicher and Schröder, 1978
		Finland	Phenotyping	Salovaara & Katunpää, 1984
	Rye-wheat Buckwheat Maize	Sweden Ireland Nigeria	Phenotyping DGGE Molecular	Spicher and Lönner, 1985 Moroni et al., 2011 Edema and Sanni, 2006
<i>L. alimentarius</i>	Wheat	Italy	DGGE API50-CHL – ATB32 16s gene sequencing	Randazzo et al., 2005 Gobbetti et al., 1994 Catzeddu et al., 2006
			16s-sequencing Multiplex-PCR	Corsetti et al., 2001 Valmorri et al., 2006a
	Maize (pozol)	Marocco	16s gene sequencing	Escalante et al., 2001
<i>L. amylolyticus</i>	Wheat	France	16s gene sequencing	Vera et al., 2012
			16s gene sequencing - SDS PAGE	Vera et al., 2009
<i>L. amylovorus</i>	Rye	France	16s gene sequencing	Muller et al., 2001
	Rye	Denmark	16s gene sequencing	Rosenquist and Hansen, 2000
	Sorghum	Sudan	Phenotyping	Hamad et al., 1992
	Teff Buckwheat	Ireland	DGGE	Moroni et al., 2011
<i>L. arizonensis</i>	Wheat	Italy	DGGE - phenotyping	Gatto and Torriani, 2004
<i>L. aviarius</i>	Wheat	China	Pyro-sequencing	Liu et al., 2016
<i>L. brevis</i>	Wheat	Italy	DGGE - phenotyping	Iacumin et al., 2009
			SDS-PAGE	Ricciardi et al., 2005
			16s gene sequencing	Catzeddu et al., 2006
			16s gene sequencing	Di Cagno et al., 2014
			16s gene sequencing -DGGE	Minervini et al., 2012b
			Pyrosequencing	Rizzello et al., 2015
			16s gene sequencing	Osimani et al., 2009
Phenotyping	Reale et al., 2005			
	Greece	16s gene sequencing	Corsetti et al., 2001 De Vuyst et al., 2002	



880 Table 1 (continue)

Species	Flour	Country	Method of identification	Reference
<i>L. brevis</i>	Wheat	China	16s-gene sequencing	Liu et al., 2016
		DGGE- (GTG) <sub>5</sub> -PCR - <i>phes</i>	Weckx et al., 2010b	
		Microarray	Weckx et al., 2010b	
	Rye	Belgium	rep-PCR - DGGE - DNA:DNA-SDS-PAGE - 16s gene sequencing	Weckx et al., 2010
	Spelt		DGGE Microarray	Weckx et al., 2010b
	Spelt	Italy	16s gene sequencing	Coda et al., 2010b
	Rye	Finland	Phenotyping	Salovaara & Katunpää, 1984
		Estonia	DGGE- pyro-sequencing	Bessmeltseva et al., 2014
		Russia	Phenotyping	Kazanskaya et al., 1983
	Wheat + Rye	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007c
		Sweden	Phenotyping	Spicher and Lönner, 1985
	Wheat + Legume	Italy	16s gene sequencing	Rizzello et al., 2014
Maize	Portugal	Biomerieux API galleries	Rocha and Malcata, 1999	
	Nigeria	Molecular	Edema and Sanni, 2006	
<i>L. buchneri</i>	Wheat	Marocco	Phenotyping	Faid et al., 1994
<i>L. casei</i>	Wheat	Italy	16s gene sequencing	Osimani et al., 2009
			DGGE	Randazzo et al., 2005
			SDS-PAGE	Ricciardi et al., 2005
			16s gene sequencing	Catzeddu et al., 2006
			16s gene sequencing	Minervini et al., 2012b
			16s gene sequencing	Palomba et al., 2011
			DGGE -16s gene sequencing	Reale et al., 2011
	France	16s gene sequencing	Robert et al., 2009	
		Phenotyping	Infantes and Tourner 1991	
		Phenotyping	Spicher and Schröder, 1978	
	Rye	Germany	16s-gene sequencing	Kitahara et al., 2005
			Phenotyping	Spicher 1984
	Wheat		Phenotyping	Faid et al., 1994
	Sourdough ferments	Marocco	Phenotyping	Boraam et al., 1993
Maize (pozol)		16s gene sequencing	Escalante et al., 2001	
Maize	Nigeria	Molecular	Edema and Sanni, 2006	
Wheat	Germany-Italy-Sweden-Switzerland	Phenotyping	Spicher, 1987	
Rye	Finland	Phenotyping	Salovaara & Katunpää, 1984	

Species	Flour	Country	Method of identification	Reference
<i>L. cellubiosus</i>	Sorghum	Arabia	Phenotyping	Gassem, 1999
<i>L. coryneformis</i>	Wheat	Italy	16s gene sequencing	Palomba et al., 2011
	Wheat-legume		16s gene sequencing	Rizzello et al., 2014
<i>L. crispatus</i>	Buckwheat	Ireland	DGGE	Moroni et al., 2011
	Cassava	Congo	DGGE	Miambi et al., 2003
<i>L. crustorum</i>	Wheat/rye	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007c
	Wheat	China	16s gene sequencing - pyrosequencing-	Liu et al., 2016
	Rye	Estonia	16s gene sequencing	Zhang et al., 2011
			DGGE- pyrosequencing	Bessmeltseva et al., 2014
<i>L. curvatus</i>	Wheat	Italy	Multiplex-PCR	Valmorri et al., 2006a
			16s gene sequencing - DGGE	Minervini et al., 2012b
			SDS-PAGE	Zotta et al., 2008
			16s gene sequencing	Osimani et al., 2009
	Wheat	Belgium	16s gene sequencing	Palomba et al., 2011
			Microarray	Weckx et al., 2010b
			16S rRNA-PCR-DGGE - rep-PCR - <i>phes</i>	Weckx et al., 2010b
	Rye	France	16s gene sequencing	Robert et al., 2009
		China	Phenotyping	Infantes and Tournier, 1991
			DGGE-16s gene sequencing	Zhang et al., 2011
		Spelt	Belgium	rep-PCR - DGGE - DNA:DNA- SDS-PAGE - 16s gene sequencing
<i>Phes</i> / 16s sequencing - DGGE- rep-PCR- DNA:DNA - Microarray	Weckx et al., 2010b			
Italy	16s gene sequencing			Coda et al., 2010b
<i>L. delbrueckii</i>	Maize	Portugal	Biomerieux API galleries	Rocha and Malcata, 1999
	Acha	Nigeria	16s gene sequencing	Coda et al., 2010a
	Wheat+Rye	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
	Rye bran	Finland	16s gene sequencing	Katina et al., 2007
	Maize (pozol)	Marocco	16s gene sequencing	Escalante et al., 2001
	Cassava	Congo	DGGE -16s-gene sequencing	Miambi et al., 2003
<i>L. delbrueckii</i>	Rye-wheat	Sweden	Phenotyping	Spicher and Lönner, 1985
	Wheat	Italy	16s gene sequencing	Corsetti et al., 2001
		Marocco	Phenotyping	Faid et al., 1994

Species	Flour	Country	Method of identification	Reference
<i>L. diolivorans</i>	Wheat	France	16S metagenetics	Lhomme et al., 2015
	Rye	Germany	Phenotyping	Spicher and Schröder 1978
	Rye-wheat	Sweden	Phenotyping	Spicher and Lönner, 1985
<i>L. farciminis</i>	Wheat	Italy	API50-CHL – ATB32	Gobbetti et al., 1994
			16s gene sequencing	Catzeddu et al., 2006
			SDS-PAGE	Corsetti et al., 2003
		China Germany-Italy-Sweden- Switzerland	16s gene- pyro-sequencing- Phenotyping	Liu et al., 2016 Spicher 1987
<i>L. farraginis</i>	Wheat	France	16S metagenetics	Lhomme et al., 2015
		Belgium	DGGE	Vrancken et al., 2011
<i>L. fermentum</i>	Wheat		Belgium	rep-PCR - <i>phes</i> sequence
		16S rRNA - PCR-DGGE - rep-PCR - <i>phes</i> - Microarray		Weckx et al., 2010b
		Italy	DGGE	Van der Meulen et al., 2007
			Phenotyping	Galli et al., 1988
			16s-sequencing	Corsetti et al., 2001
			Multiplex-PCR	Valmorri et al., 2006a
			SDS-PAGE	Corsetti et al., 2003
			Phenotyping	Reale et al., 2005
			Phenotyping	Spicher, 1959
			RFLP	Osimani et al., 2009
API50-CHL – ATB32	Gobbetti et al., 1994			
Germany	China	DGGE	Randazzo et al., 2005	
		16s-sequencing	Kitahara et al., 2005	
Cassava	Congo	DGGE-16s-sequencing	Zhang et al., 2011	
		16s gene sequencing - DGGE	Miambi et al., 2003	
Teff	Ireland	DGGE	Moroni et al., 2011	
		Ethiopia	DNA:DNA-RAPD	Nigatu, 2000
Sorghum	Africa	Phenotyping	Desiye and Abegaz, 2013	
		16s gene sequencing	Vieira-Dalodè et al., 2007	
		Phenotyping	Hamad et al., 1992	
		16s gene sequencing	Hamad et al., 1997	
Rye	Belgium	rep-PCR - DGGE - DNA:DNA-SDS-PAGE - 16s gene sequencing	Weckx et al., 2010	
		Phenotyping	Kazanskaya et al., 1983	
Wheat - Legume	Italy	16s gene sequencing	Rizzello et al., 2014	

Species	Flour	Country	Method of identification	Reference
<i>L. fermentum</i>	Wheat + Rye	Sweden	Phenotyping	Spicher and Lönner, 1985
		Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
	Buckwheat	Ireland	DGGE	Moroni et al., 2011
	Maize	Nigeria	Molecular	Edema and Sanni, 2006
	Spelt	Belgium	16s rRNA - PCR-DGGE - rep-PCR - <i>phes</i> sequencing	Weckx et al., 2010b
<i>L. fructivorans</i>	Wheat	Italy	Phenotyping-SDS-PAGE	Corsetti et al., 2003
			API50-CHL – ATB32	Gobbetti et al., 1994
<i>L. frumenti</i>	Wheat	France	RFLP-sequencing	Ferchichi et al., 2008
			TGGE	Ferchichi et al., 2007
			16s gene sequencing	Vera et al., 2012
<i>L. futsaii</i>	Rye		16s gene sequencing	Muller et al., 2001
	Wheat	China	Pyrosequencing	Liu et al., 2016
<i>L. gallinarum</i>	Buckwheat Teff	Ireland	DGGE	Moroni et al., 2011
	Wheat	Italy	16s gene sequencing	Minervini et al., 2012a Osimani et al., 2009
<i>L. graminis</i>	Buckwheat	Ireland	DGGE	Moroni et al., 2011
	Rye bran	Finland	16s gene sequencing	Katina et al., 2007
	Wheat	China	Pyrosequencing	Liu et al., 2016
		Italy	SDS-PAGE	Ricciardi et al., 2005
	Teff	Ethiopia	DNA:DNA - RAPD	Nigatu, 2000
<i>L. guizhouensis</i>	Wheat	China	Pyrosequencing	Liu et al., 2016
<i>L. hammesii</i>	Wheat	France	TGGE	Ferchichi et al., 2007
			16S metagenetics	Lhomme et al., 2015
			RFLP-sequencing	Ferchichi et al., 2008
		Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
<i>L. harbinensis</i>	Wheat+rye+spelt	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
	Rye	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
	Sorghum	Botswana	16s gene sequencing	Monang and Ganzle 2011
<i>L. heilongjiangensis</i>	Wheat	China	16s- pyro-sequencing	Liu et al., 2016
<i>L. helveticus</i>	Wheat	China	Pyrosequencing	Liu et al., 2016
			DGGE-16s-gene sequencing	Zhang et al., 2011
		Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
		Italy	16s gene sequencing	Osimani et al., 2009
	Sorghum	Sudan	16s gene sequencing	Hamad et al., 1997

Species	Flour	Country	Method of identification	Reference
<i>L. helveticus</i>	Buckwheat	Ireland	DGGE	Moroni et al., 2011
	Rye	Estonia	16s gene sequencing -DDGE-Rep(PCR)	Viiard et al., 2012
<i>L. hilgardii</i>	Wheat	France	16S metagenetics	Lhomme et al., 2015
		Germany	Phenotyping	Spicher, 1987
<i>L. johansonii</i>	Wheat	Belgium	DGGE	Vrancken et al., 2011
<i>L. kefirii</i>	Cassava	Congo	16s gene sequencing	Miambi et al., 2003
<i>L. kimchii</i>	Wheat	Italian	DGGE	Gatto and Torriani, 2004 Randazzo et al., 2005
	Rise	Germany	DGGE	Meroth et al., 2004
<i>L. letivazi</i>	Wheat	China	Pyrosequencing	Liu et al., 2016
<i>L. manihotivorans</i>	Cassava	Congo	DGGE	Miambi et al., 2003
<i>L. mindensis</i>	Wheat	China	16s gene sequencing pyrosequencing DGGE-16s-gene sequencing	Liu et al., 2016 Zhang et al., 2011
<i>L. mucosae</i>	Sorghum	Africa	16S gene-sequencing	Vieira-Dalodè et al., 2007
<i>L. namurensis</i>	Wheat+rye+spelt wheat	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
		Italy	16s-gene sequencing/phenotyping	Minervini et al., 2012
<i>L. nantensis</i>	Wheat	France	TGGE RFLP-sequencing Isolation	Ferchichi et al., 2007 Ferchichi et al., 2008 Valcheva et al., 2006
		China	16s- pyrosequencing-	Liu et al., 2016
	Spelt	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
	Rye		rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
<i>L. panis</i>	Wheat	France	Tgge RFLP-sequencing 16s gene sequencing SDS-PAGE 16s gene sequencing	Ferchichi et al., 2007 Ferchichi et al., 2008 Vera et al., 2009 Vera et al., 2012
			Estonia	16s gene sequencing -DGGE- PCR
	Rye	Germany	Isolation	Wiese et al., 1996
		Denmark	16s gene sequencing	Rosenquist and Hansen, 2000
<i>L. parabrevis</i>	Wheat	Italy	DGGE	Minervini et al., 2012b
<i>L. parabuchneri</i>	Teff	Ethiopia	DNA:DNA-RAPD	Nigatu, 2000
	Wheat-legume	Italy	16s gene sequencing	Rizzello et al., 2014
	Sorghum	Botswana	16s gene sequencing	Monang and Ganzle 2011
	Wheat	Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
<i>L. paracasei</i>	Wheat	Italy	16s-gene sequencing DGGE	Osimani et al., 2009 Randazzo et al., 2005

Species	Flour	Country	Method of identification	Reference	
<i>L. paracasei</i>	Wheat	Italy	DGGE -16s gene sequencing	Reale et al., 2011	
	Wheat + Rye	Belgium	SDS-PAGE	Ricciardi et al., 2005	
			rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
	<i>L. paralimentarius</i>	Wheat	Italy	DGGE	Gatto and Torriani, 2004
				DGGE	Iacumin et al., 2009
16s gene sequencing				Osimani et al., 2009	
16s gene sequencing -DGGE				Siragursa et al., 2009	
Multiplex-PCR				Valmorri et al., 2006a	
Wheat		France	16s gene sequencing	Minervini et al., 2012	
			RFLP-sequencing	Ferchichi et al., 2008	
			TGGE	Ferchichi et al., 2007	
			16s gene sequencing	Liu et al., 2016	
			DGGE-16s gene sequencing	Zhang et al., 2011	
<i>L. paraplantarum</i>	Wheat + Rye Wheat + Rye + Spelt	Belgium	16s gene sequencing /phenotyping	De Vuyst et al., 2002	
			USA	16s gene sequencing	Kitahara et al., 2005
	Spelt	Estonia	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
			rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
			rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
			rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
			DGGE- pyrosequencing	Bessmeltseva et al., 2014	
	Rise	Germany	DGGE	Meroth et al., 2004	
	<i>L. paraplantarum</i>	Wheat	Italy	DGGE - phenotyping	Gatto and Torriani, 2004
				SDS-PAGE	Zotta et al., 2008
Multiplex-PCR				Valmorri et al., 2006a	
Spelt		Belgium	France	16s gene sequencing	Robert et al., 2009
			DGGE	Van der Meulen et al., 2007	
<i>L. pentosus</i>	Wheat-legume	Italy	16S rRNA-PCR-DGGE+ rep-PCR - <i>phes</i>	Weckx et al., 2010b	
			16s gene sequencing	Rizzello et al., 2014	
	Wheat	Italy	Multiplex-PCR	Valmorri et al., 2006a	
			SDS-PAGE	Zotta et al., 2008	
			16s gene sequencing	Catteddu et al., 2006	
Wheat-legume	Italy	France	16s gene sequencing	Siragusa et al., 2009	
		16s gene sequencing	Robert et al., 2009		
<i>L. perolens</i>	Rise	Germany	16s gene sequencing	Rizzello et al., 2014	
	Cassava	Congo	DGGE	Meroth et al., 2004	
			16s gene sequencing	Miambi et al., 2003	

Species	Flour	Country	Method of identification	Reference
<i>L. plantarum</i>	Wheat	Italy	16s gene sequencing	Alfonso et al., 2013
			Pyrosequencing	Ercolini et al., 2013
			16s gene sequencing	Di Cagno et al., 2014
			pyrosequencing	Minervini et al., 2015
			16s gene sequencing -DGGE	Minervini et al., 2012b
			16s gene sequencing	Siragusa et al., 2009
			DGGE-phenotyping	Iacumin et al., 2009
			SDS-PAGE	Ricciardi et al., 2005
			Dependent	Galli et al., 1988
			Phenotyping	Reale et al., 2005
			API50-CHL – ATB32	Gobbetti et al., 1994
			DGGE -16s gene sequencing	Reale et al., 2011
			16s gene sequencing	Corsetti et al., 2001
			Phenotyping-SDS-PAGE	Corsetti et al., 2003
			16s gene sequencing	Catzeddu et al., 2006
			Multiplex-PCR	Valmorri et al., 2006a
			SDS-PAGE	Zotta et al., 2008
		16s gene sequencing	Osimani et al., 2009	
		DGGE - phenotyping	Gatto and Torriani, 2004	
		DGGE	Randazzo et al., 2005	
		DGGE	Garofalo et al., 2008	
		16s-gene sequencing	Palomba et al., 2011	
China	16s gene sequencing	Liu et al., 2016		
Greece	16s gene sequencing	Paramithiotis et al., 2010		
Belgium	DGGE	Vrancken et al., 2011		
	DGGE	Van der Meulen et al., 2007		
	Microarray rep-PCR - <i>phes</i> sequence	Weckx et al., 2010b Scheirlinck et al., 2007a		
USA	16s gene sequencing	Kitahara et al., 2005		
Albania	16s-gene sequencing	Nionelli et al., 2014		
France	PCR-TGGE	Lhomme et al., 2014		
	16s gene sequencing	Robert et al., 2009		
Germany	16s gene sequencing	Kitahara et al., 2005		
Marocco	Phenotyping	Spicher, 1959		
	Phenotyping	Faid et al., 1994		
Iran	Phenotyping	Azar et al., 1977		
Spelt	Italy	16s gene sequencing	Coda et al., 2010b	

Species	Flour	Country	Method of identification	Reference		
<i>L. plantarum</i>	Spelt	Belgium	16S rRNA -PCR-DGGE+ rep-PCR	Weckx et al., 2010b		
			- <i>phes</i>	Weckx et al., 2010b		
			Microarray	Scheirlinck et al., 2007a		
	Rye			rep-PCR - <i>phes</i> sequence	Van der Meulen et al., 2007	
				DGGE		
				Italy	Pyrosequencing	Ercolini et al., 2013
				Estonia	DGGE- Pyrosequencing	Bessmeltseva et al., 2014
				Finland	Api	Salovaara & Katunpää, 1984
				Belgium	rep-PCR - DGGE - DNA:DNA- SDS-PAGE - 16s gene sequencing	Weckx et al., 2010
	Wheat + Rye		Russia	Phenotyping	Kazanskaya et al., 1983	
			Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
	Teff		Sweden	Phenotyping	Spicher and Lönner, 1985	
			Ireland	DGGE	Moroni et al., 2011	
			Ethiopia	DNA:DNA-RAPD	Nigatu, 2000	
			Ethiopia	Phenotyping	Desiye and Abegaz, 2013	
	Buckwheat		Ireland	DGGE	Moroni et al., 2011	
	Wheat-legume Emmer		Italy	16s gene sequencing	Rizzello et al., 2014	
16s gene sequencing				Coda et al., 2010b		
Sorghum		Botswana	16s gene sequencing	Monang and Ganzle 2011		
Acha		Nigeria	16s gene sequencing	Coda et al., 2010a		
Amaranth		India-Peru-Mexico-Germany	16s gene sequencing	Sterr et al., 2009		
Maize		Nigeria	Molecular	Edema and Sanni, 2006		
		Marocco	16s gene sequencing	Escalante et al., 2001		
Quinoa		Argentina	16s gene sequencing	Rizzello et al., 2016		
Wheat		China	DGGE-16s-gene sequencing	Zhang et al., 2011		
Rye bran		Finland	Ribotyping -16s-gene sequencing	Katina et al., 2007		
		Thailand	16s gene sequencing	Luangsakul et al., 2009		
		Italy	16s gene sequencing			
		Italy	Pyrosequencing	Rizzello et al., 2015		
		France	TGGE	Ferchichi et al., 2007		
		France	RFLP-sequencing	Ferchichi et al., 2008		
<i>L. plantarum</i> group	Wheat	Italy	Pyrosequencing	Minervini et al., 2015		
		Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a		
		Belgium	DGGE	Vrancken et al., 2011		
		Belgium	Microarray	Weckx et al., 2010b		



Species	Flour	Country	Method of identification	Reference	
<i>L. pontis</i>	Wheat	France	TGGE	Ferchichi et al., 2007	
			RFLP-sequencing	Ferchichi et al., 2008	
		Italy	pyrosequencing	Minervini et al., 2015	
		Belgium	rep-PCR - <i>pbes</i> sequence	Scheirlinck et al., 2007a	
			DGGE	Vrancken et al., 2011	
			Microarray	Weckx et al., 2010b	
	Wheat + Rye	Belgium	rep-PCR - <i>pbes</i> sequence	Scheirlinck et al., 2007a	
	Spelt				
		Rye	Estonia	16s-gene sequencing-DDGE- rep-PCR	Viiard et al., 2012
			Germany	16s gene sequencing	Muller et al., 2001
					Kitahara et al., 2005
			Denmark	16s gene sequencing	Rosenquist and Hansen, 2000
		Sorghum	Sudan	16s gene sequencing	Hamad et al., 1997
				Phenotyping	Hamad et al., 1992
		Botswana	16s gene sequencing	Monang and Ganzle 2011	
	Spelt	Belgium	Microarray	Weckx et al., 2010b	
	Rye	Germany	16s gene sequencing	Muller et al., 2001	
		Italy	pyrosequencing	Minervini et al., 2015	
	Wheat		Microarray	Weckx et al., 2010b	
		Belgium	DGGE	Vrancken et al., 2011	
<i>L. reuteri</i>	Sorghum	Sudan	16s gene sequencing	Hamad et al., 1997	
			Phenotyping	Hamad et al., 1992	
			Botswana	16s gene sequencing	Monang and Ganzle 2011
	Rye	Germany	16s gene sequencing	Muller et al., 2001	
		Denmark	16s gene sequencing	Rosenquist and Hansen, 2000	
	Spelt	Belgium	Microarray	Weckx et al., 2010b	
<i>L. rhamnosus</i>	Wheat	Italy	16s gene sequencing	Osimani et al., 2009	
			DGGE	Randazzo et al., 2005	
	Rye-wheat	Sweden	Phenotyping	Spicher and Lönner, 1985	
<i>L. rossiae</i>	Wheat	China	16s-gene sequencing	Liu et al., 2016	
			16s-gene sequencing	Zhang et al., 2011	
			16s gene sequencing	Minervini et al., 2012ab	
		Italy	16s gene sequencing	Minervini et al., 2010	
			16s gene sequencing	Siragusa et al., 2009	

Species	Flour	Country	Method of identification	Reference	
<i>L. rossiae</i>	Wheat	Italy	Multiplex-PCR	Valmorri et al., 2006a	
	Emmer		16s gene sequencing	Coda et al., 2010b	
	Spelt	Italy	16s gene sequencing	Coda et al., 2010b	
		Belgium	DGGE	Van der Meulen et al., 2007	
	Spelt	Belgium	Microarray	Weckx et al., 2010b	
			rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
<i>L. sakei</i>	Rye bran	Finland	16s gene sequencing	Katina et al., 2007	
	Buckwheat	Ireland	DGGE	Moroni et al., 2011	
	Amaranth	India-Peru-Mexico-Germany	16s gene sequencing	Sterr et al., 2009	
				Minervini et al., 2012	
				16s gene sequencing	Minervini et al., 2012b
					Palomba et al., 2011
					Catzeddu et al., 2006
	Wheat	Italy	16s gene sequencing	Osimani et al., 2009	
				DGGE -16s gene sequencing	Reale et al., 2011
				Multiplex-PCR	Valmorri et al., 2006a
				16s gene sequencing	Di Cagno et al., 2014
	Belgium		Microarray	Weckx et al., 2010b	
	France		16s gene sequencing	Robert et al., 2009	
			PCR-TGGE	Lhomme et al., 2014	
<i>L. sanfranciscensis</i>			DGGE - phenotyping	Gatto and Torriani, 2004	
			Pyrosequencing	Minervini et al., 2015	
			Phenotyping	Galli et al., 1988	
			DGGE	Randazzo et al., 2005	
			16s gene sequencing	Di Cagno et al., 2014	
			16s gene sequencing	Minervini et al., 2012	
			16s gene sequencing	Siragusa et al., 2009	
	Wheat	Italy	Phenotyping-SDS-PAGE	Corsetti et al., 2003	
			16s gene sequencing -DGGE-	Minervini et al., 2012b	
			Api50-RAPD-PCR	Reale et al., 2005	
			16s gene sequencing	Corsetti et al., 2001	
		DGGE - phenotyping	Iacumin et al., 2009		
		16s gene sequencing	Palomba et al., 2011		
		16s gene sequencing	Osimani et al., 2009		

Species	Flour	Country	Method of identification	Reference	
<i>L. sanfranciscensis</i>	Wheat	China	16s- pyro- sequencing	Liu et al., 2016	
			DGGE- 16s gene sequencing	Zhang et al., 2011	
		France	PCR-TGGE	Lhomme et al., 2014	
			16S metagenetics - qPCR	Lhomme et al., 2015	
			RFLP-sequencing	Ferchichi et al., 2008	
			16s gene sequencing	Robert et al., 2009	
			TGGE	Ferchichi et al., 2007	
		Greece	16s gene sequencing - phenotyping	De Vuyst et al., 2002	
		USA	16s gene sequencing	Kitahara et al., 2005	
		Germany	16s gene sequencing	Kitahara et al., 2005	
		Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a	
		Marocco	Phenotyping	Faid et al., 1994	
		Wheat + Rye	France	16S metagenetics	Lhomme et al., 2015
			Belgium	rep-PCR - <i>phes</i> sequence	Scheirlinck et al., 2007a
Wheat + Legume	Sweden	Phenotyping	Spicher and Lönner, 1985		
	Italy	16s gene sequencing	Rizzello et al., 2014		
Rye	Finland	Phenotyping	Salovaara & Katunpää, 1984		
	Germany	16s gene sequencing	Kitahara et al., 2005		
	France	16S metagenetics	Lhomme et al., 2015		
Spelt	Italy	16s gene sequencing	Coda et al., 2010b		
		TGGE	Ferchichi et al., 2007		
<i>L. spicheri</i>	Wheat	France	PCR-TGGE	Lhomme et al., 2014	
			Italy	16s gene sequencing	Minervini et al., 2012
<i>L. tuccei</i>	Wheat	China	Pyrosequencing	Liu et al., 2016	
			Teff	DGGE	Moroni et al., 2011
<i>L. vaginalis</i>	Buckwheat	Ireland	DGGE	Moroni et al., 2011	
			Sorghum	16s gene sequencing	Hamad et al., 1997
	<i>L. zeaea</i>	Wheat	China	DGGE	Zhang et al., 2011
Italy			16s gene sequencing	Catzeddu et al., 2006	
<i>L. zymae</i>	Wheat	Greece	16s gene sequencing	De Vuyst et al., 2002	

894 *L. Lactobacillus*; DNA:DNA, DNA hybridation



Figure 2

The colors scale:

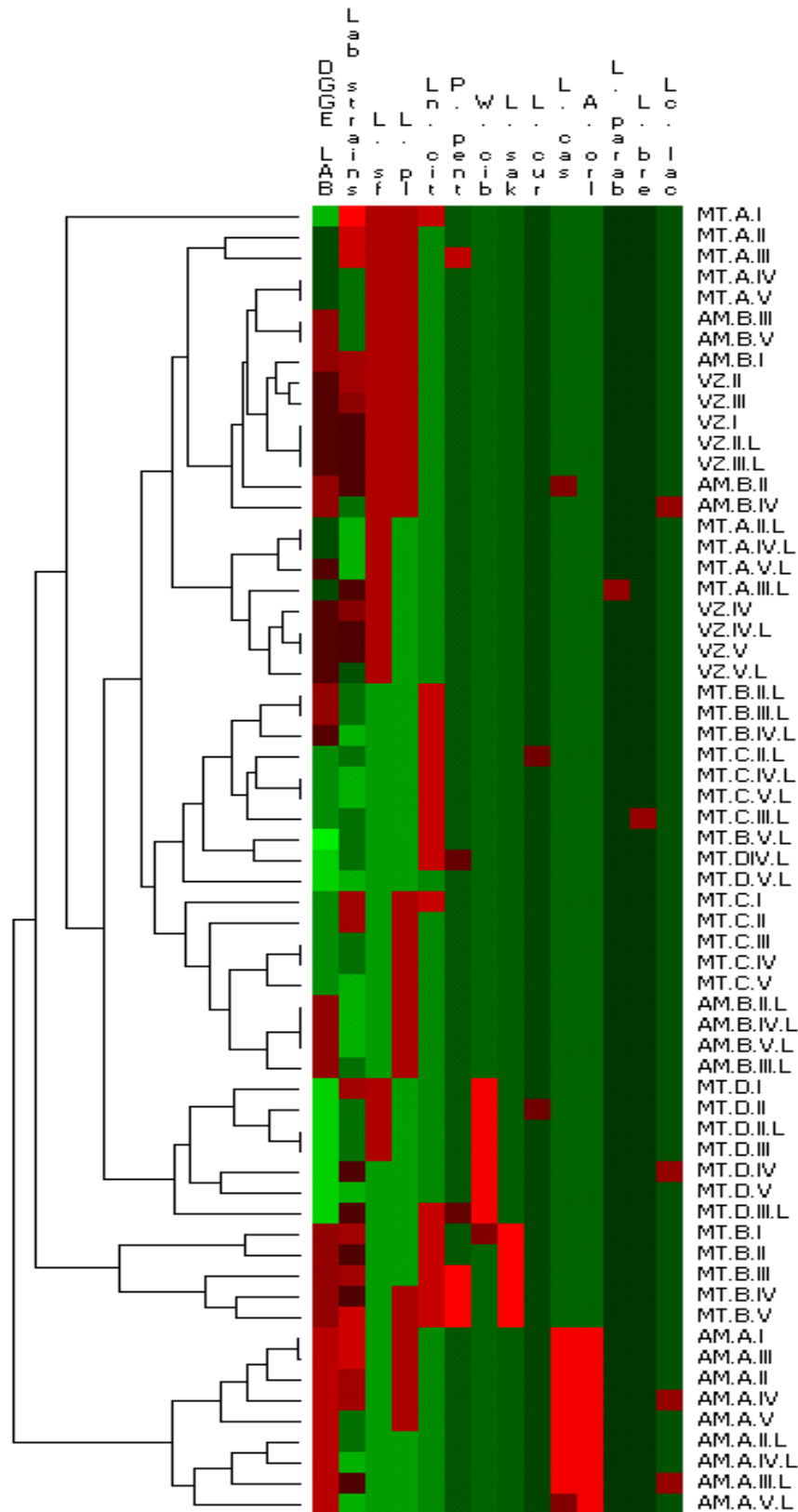


Figure 3

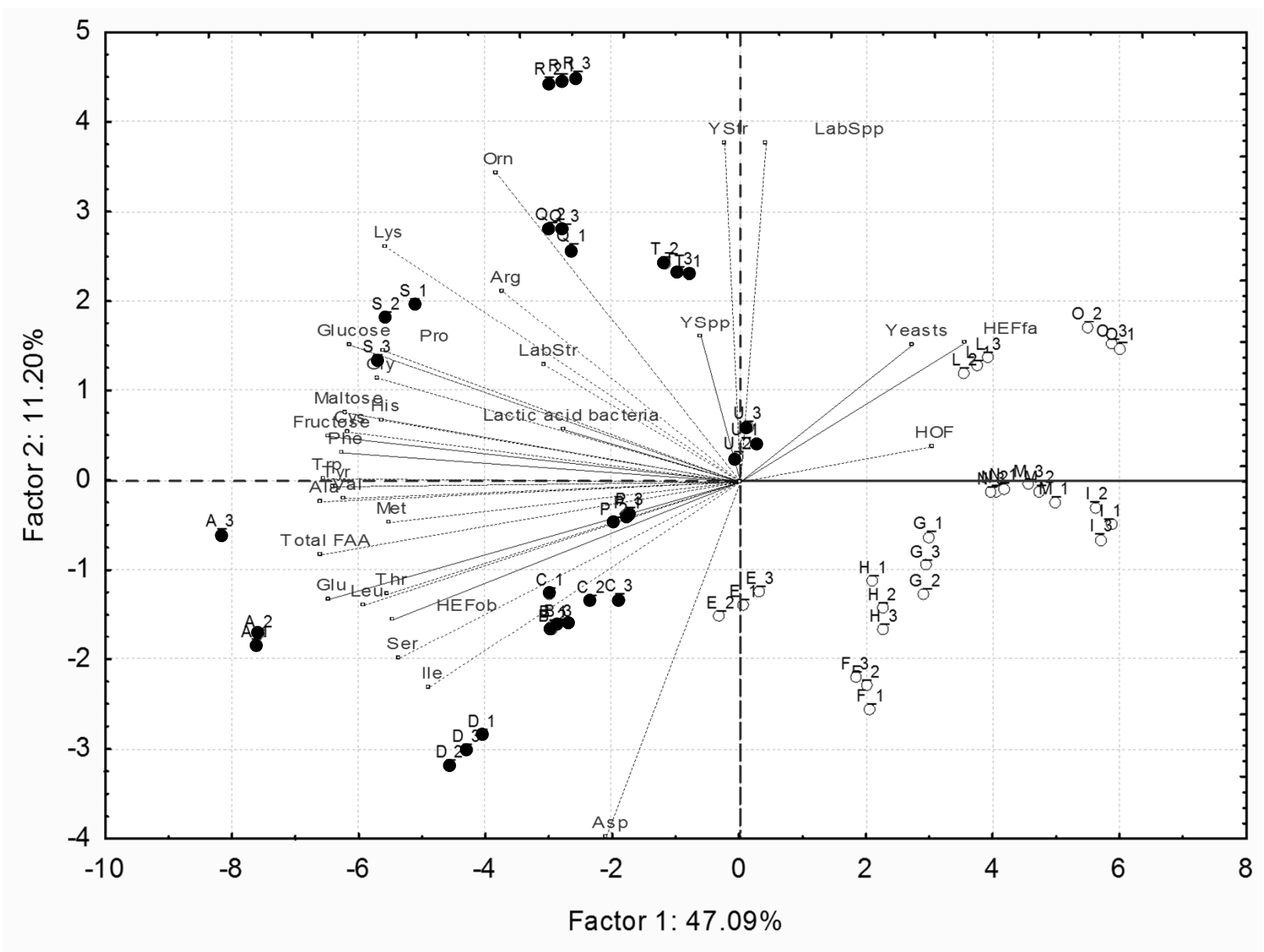


Figure 4

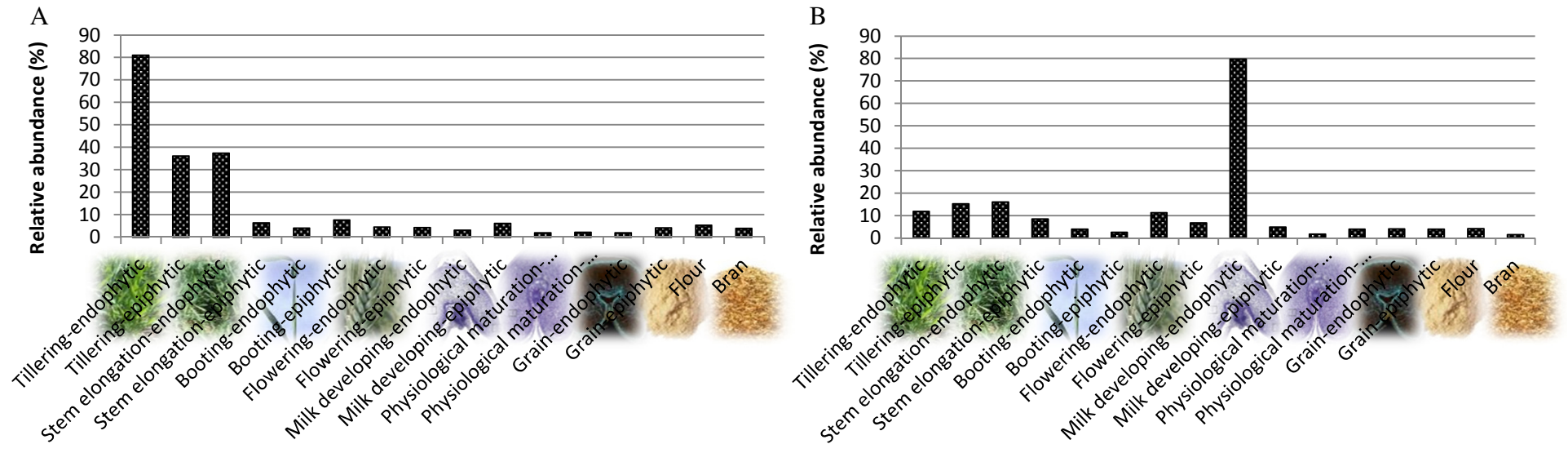


Figure 5

Figure 5.

