Lifestyles of sourdough lactobacilli – do they matter for microbial ecology and bread quality?

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Abstract.

Sourdough is used in production of (steamed) bread as leavening agent (type I sourdoughs) or as baking improver to enhance flavor, texture, and shelf life of bread (type II sourdoughs). The long-term propagation of sourdoughs eliminates dispersal limitation and consistently leads to sourdough microbiota that are composed of host adapted lactobacilli. In contrast, community assembly in spontaneous cereal fermentations is limited by dispersal and nomadic or environmental lactic acid bacteria are the first colonizers of these sourdoughs.

Propagation of sourdoughs for use as sole leavening agent (type I sourdoughs) dictates fermentation conditions that select for rapid growth. Type I wheat- and rye sourdoughs are consistently populated by insect-adapted lactobacilli, particularly *Lactobacillus sanfranciscensis,* which is characterized by a small genome size and a restricted metabolic potential. The diverse fermentation conditions employed in industrial or artisanal Type II sourdough fermentation processes also result in a more diverse microbiota. Nevertheless, type II sourdoughs are typically populated by vertebrate host adapted lactobacilli of the *L. delbrueckii* and *L. reuteri* groups. Metabolic traits of host-adapted lactobacilli that enhance competitiveness in intestinal ecosystems also provide technological functionality in bread making. Examples include formation of exopolysaccharides, arginine-, glutamine- and glutamate based mechanisms of acid resistance, and glycosyl hydrolases that reduce FODMAP levels in sourdough and sourdough bread. In conclusion, consideration of the lifestyle of sourdough lactobacilli facilitates the selection of competitive and functional sourdough starter cultures.

Keywords: Lactobacillus, sourdough, microbiota, evolution, bread leavening.

1. Introduction.

Sourdough has been traditionally used as leavening agent for bread and steamed bread. CO_2 production in sourdough is mediated by yeasts and heterofermentative lactobacilli, and is coupled to acidification (Brandt et al., 2004). Microbial metabolism in conjunction with the activity of flour enzymes also improves flavour, texture, storage life, and nutritional properties of bread (Gänzle, 2014; Gobbetti et al., 2018). The industrial production of baker's yeast allowed its use as leavening agent after the late 19th century; by the middle of the 20th century, baker's yeast had replaced sourdough as leavening agent in most applications except in some small scale artisanal bakeries, for specialty products, and in rye baking, where acidification is necessary to improve baking properties (Capelle et al., 2013). Recognition of the quality of sourdough bread and the efforts of the baking industry to offer "clean label' products led to a resurgence of sourdough use since the late 20th century. Currently, sourdough or sourdough products are used in most European baked goods with the primary aim to improve bread quality (Brandt, 2007; Pontonio et al., 2017).

Different technological aims of sourdough fermentation – leavening, acidification, or dough improver – necessitate different fermentation conditions. The traditional use of sourdough as sole leavening agent dictates frequent refreshments of the sourdough, or, in microbiological terms, fermentation conditions that maintain sourdough microbiota in the exponential phase of growth. Maintaining sourdough microbiota in a metabolically active state ensures sufficient CO_2 production and leavening power (Brandt et al., 2004). Two examples of fermentation processes to achieve leavening are shown in Figure 1. Details of the fermentation process, i.e. the number of stages per fermentation cycle, and the conditions with regards to temperature, time, water addition, and level of inoculum, are poorly documented in the scientific literature and vary widely between different bakeries; however, type I fermentation processes generally follow the principle that

63 fermentation microbiota are continuously maintained in a metabolically active state by frequent
64 refreshments. This also prevent exposure of sourdough microbiota to low pH (Böcker et al., 1995;
65 Brandt et al., 2004). The use of sourdough as sole leavening agent is generally restricted to artisanal
66 or medium scale bakeries. Industrial sourdough fermentations employ comparable fermentation
67 conditions to support leavening in combination with addition of baker's yeast. Some of the
68 sourdoughs for use as leavening agent have been maintained over long periods of time;
69 documented propagation of sourdoughs exceeds 100 years.

70 Fermentation of sourdoughs with high levels of acidity requires different fermentation conditions;
71 these sourdoughs are fermented at a high temperature for extended periods of time to achieve high
72 levels of total titrable acidity (Böcker et al., 1995, Müller et al., 2001, Rosenquist and Hansen,
73 2000, Vera et al., 2012, Viiard et al., 2013 and 2016). Dough acidification is particularly relevant
74 in rye baking to inhibit rye amylases (Brandt, 2007) and it is used predominantly in countries
75 where rye bread is popular. These sourdoughs do not allow leavening without addition of baker's
76 yeast. Comparable to the use of sourdough as leavening agent, sourdoughs that are fermented for
77 dough acidification and improved bread quality are maintained by continuous propagation to
78 achieve a consistent composition and activity of sourdough microbiota (Brandt, 2007; Zhen et al.,
79 2015b).

80 In addition to those sourdoughs that are maintained by back-slopping, spontaneous sourdough
81 fermentations are also used in bakeries. The most common practice is the use of sponge dough;
82 dough that is fermented for more than 4 h after addition of baker's yeast. Sponge doughs are
83 commonly used in wheat baking and in production of soda crackers (Sugihara et al., 1978).
84 Spontaneous fermentations of cereals are also used in the production of cereal beverages,

85 porridges, and flat bread; a large diversity of products is traditionally prepared in Africa (Todorov
86 and Holzapfel, 2015).

87 The assembly of sourdough microbiota in spontaneous fermentations is limited by dispersal, i.e.
88 community assembly depends on contaminants from plant, animal, or environmental sources
89 (Gänzle and Ripari, 2016). In contrast, microbiota of back-slopped sourdoughs are shaped by
90 selection for the most competitive microorganisms (Gänzle and Ripari, 2016). Back-slopping of
91 sourdoughs over long time periods provides opportunity for contaminants from even unlikely
92 sources to establish themselves as stable members of sourdough microbiota. Böcker et al. (1995)
93 proposed that the technological aim of sourdough fermentation – leavening or acidification –
94 determines the fermentation conditions and the composition of sourdough microbiota. The
95 concept, initially based on characterization of only a few German sourdoughs, was further
96 developed in several comprehensive reviews (De Vuyst and Neysens, 2005; De Vuyst et al., 2014;
97 Gobbetti et al., 2016; Vogel et al., 1999) to differentiate between type I sourdoughs, sourdoughs
98 used for leavening; type II sourdoughs, sourdoughs used for acidification; and type 0 sourdoughs,
99 spontaneous sourdoughs. Past reviews focused on microbiological aspects and the impact of
100 fermentation conditions on composition of sourdough microbiota (De Vuyst and Neysens, 2005;
101 De Vuyst et al., 2014; Gobbetti et al., 2016; Vogel et al., 1999) while neglecting the link between
102 technological aim and fermentation microbiota that was initially proposed by Böcker et al. (1995).

103 The present communication will initially revisit the question which lactic acid bacteria populate
104 sourdoughs. Literature data on sourdough microbiota covers more than 300 sourdoughs with
105 documented composition and use in artisanal or industrial bakeries, allowing to probe relationships
106 between the technological aim of fermentation and the composition of sourdough microbiota. In
107 addition, we address the question where to lactic acid bacteria in sourdoughs come from; i.e. we

will explore whether the competitiveness of lactic acid bacteria in sourdoughs relates to their phylogenetic position or their natural habitat. Duar et al., (2017b) proposed that lactobacilli have evolved to specific ecological niches that relate to vertebrate hosts, insects, or plants and the environment. The adaptation of lactobacilli to a specific natural habitat is typically shared by closely related species, i.e. the "lifestyle" is shared by organisms in the same phylogenetic group in the genus *Lactobacillus* (Zheng et al., 2015a). The consideration of the natural habitat of lactic acid bacteria and bifidobacteria has been instrumental in guiding the selection of bacterial strains for use as probiotics to obtain strains that are adapted to intestinal habitats (Walter et al., 2018). The use of this concept for food fermentations will improve our understanding of the factors shaping the composition and activity of sourdough microbiota to guide the selection of competitive organism with beneficial impact on bread quality**.**

2. Lactic acid bacteria in type I and type II sourdoughs

Literature data were interrogated with respect to the composition of the bacterial microbiota in sourdoughs. Studies were included in the analysis if they met the following criteria, (i) basic information on the fermentation conditions and / or the use of the sourdough was provided, (ii) isolates were obtained on suitable cultivation media, and were characterized at the species level by currently accepted methods, and (iii) information on the composition of microbiota was provided for each sourdough at the species level. The literature search aimed to provide a comprehensive overview on Type I sourdoughs and Type II sourdoughs, and additionally included examples of spontaneous fermentations, laboratory model fermentations started with flour and sterile water, and fermentations of cereals other than wheat or rye. In total, data on the composition of 315 sourdoughs was evaluated. A variable level of detail on fermentation conditions and

technological aim of the sourdough fermentation is provided by the different sources; therefore, some of the classifications as type I, type II or spontaneous sourdoughs may be ambiguous.

Literature data for the 227 sourdoughs classified as type I sourdough included mainly samples from Italy, France, Germany, Belgium, the U.S. and Canada; since 2015, data became available for Chinese sourdoughs use for production of steamed bread (Figure 2 and Table S1 of the online supplementary material). More than 95% of sourdoughs contained heterofermentative lactic acid bacteria alone or in association with homofermentative lactobacilli. *L. sanfranciscensis* was most frequently identified, 178 of the 227 sourdoughs harboured this species. Other frequent representatives include *L. plantarum* and *L. brevis*, species in the *L. alimentarius* group (*L. paralimentarius, L. crustorum, L. mindensis,* and *L. nantensis*), *Leuconostoc* spp. and *Weissella* spp. Five of the sourdoughs were maintained at the household level; here, the propagation of the sourdoughs was interrupted by refrigerated storage for several days or weeks. All of these sourdoughs harboured a combination of *L. plantarum* and *L. brevis* (Table S1).

Literature data for the 32 sourdoughs classified as type II sourdough included mainly rye sourdoughs from Finland, Estonia, Denmark, and Germany; data for few wheat sourdoughs from the U.S., China, and France are also available (Figure 3 and Table S2). Type II sourdoughs also contained heterofermentative organisms alone or in association with homofermentative lactobacilli (Table S2). Species of the *L. reuteri* group, particularly *L. pontis, L. panis, L. frumenti* and *L. reuteri* as well as the *L. delbrueckii* group organisms *L. amylovorus, L., crispatus* and *L. acidophilus* were frequently identified in type II sourdoughs (Figure 3). *L. sanfranciscensis* was identified in 3 Chinese sourdoughs that are used for dough acidification in conjunction with baker's yeast (Figure 3).

Data on the composition of spontaneous sourdoughs including model sourdoughs prepared in laboratories under aseptic conditions, sourdoughs prepared with cereals other than wheat or rye, and some cereal beverages or porridges is shown in Figure 4. *L. plantarum* and *L. fermentum* are the most frequently reported organisms; these species are particularly relevant in model sourdoughs that are started and propagated in the laboratory with flour as the only source of microorganisms (Minervini et al., 2015). *L. plantarum* and *L. fermentum* are also the most frequently isolated organisms in spontaneous African cereal fermentations, independent on whether the raw material is tef, maize, sorghum, or millet (Figure 4, Todorov and Holzapfel, 2015). Spontaneous sourdoughs also often harbour enterococci, lactococci, and pediococci (Fig. 4); these organisms are rapidly displaced by lactobacilli when spontaneous sourdoughs are back-slopped (van der Meulen et al., 2007, Hamad et al., 1997).

3. Lifestyles of sourdough lactobacilli

Food fermentations including sourdough fermentation thus do not support speciation or even adaptation below the species level (Duar et al., 2017b, Gänzle and Ripari, 2016, Zheng et al., 2015b). Lactobacilli contaminate sourdough fermentations from their primary habitats in which they form a stable population over long time periods. Large-scale comparative genomic analyses for the genus *Lactobacillus* as well as for several model species allowed identification of the primary habitats for several *Lactobacillus* spp. (Duar et al., 2017b; Martino et al., 2016; Frese et al., 2011, Krumbeck et al., 2015). The adaptation of lactobacilli to specific habitats typically represents an ecological strategy that is generally shared by phylogenetically related species (Duar et al., 2017b, Zheng et al., 2015a).

Several groups of lactobacilli have adapted to insects. Species in the *L. mellifer* and *L. kunkeei* groups and a cluster of species related to *L. apis* were isolated almost exclusively from insects.

These species are characterized by a GC of less than 40%, a small genome size of typically less than 2 Mbp, and an extremely restricted carbohydrate fermentation pattern that often includes only maltose and sucrose, and the lack of acid resistance mechanisms (Duar et al., 2017b, Filannino et al., 2016; Zheng et al., 2015a). The identification of the ecological nice for species in the *L. fructivorans* group including *L. sanfranciscensis* is less unambiguous. Many representatives, e.g. *L. lindneri*, *L. sanfranciscensis*, *L. fructivorans* and *L. homohiochii*, were initially isolated from fermented or spoiled food (Zheng et al., 2015a). Comparable to other insect-adapted lactobacilli, *L. fructivorans* group organisms have a small genome size, 1,279,300 to 1,420,000 bp, a low GC content and a very narrow carbohydrate fermentation pattern (Vogel et al., 2011; Zheng et al., 2015a). *L. fructivorans* forms stable associations with fruit flies (Wong et al., 2011) and other species originate from insects (*L. vespulae*; Hoang et al., 2015) or flowers (*L. florum* and *L. ixorae*; Endo et al., 2010; Techo et al., 2016). All isolates of *L. sanfranciscensis* originate from sourdough, however, culture-independent analyses suggested its presence in fruit flies (Groenewald et al., 2006) and grain beetles (Boiocchi et al., 2017). As a small genome size and low GC content indicate adaptation to a narrow ecological niche, *L. sanfranciscensis* and other *L. fructivorans* group organisms likely are adapted to insect hosts.

Species in the in the *L. delbrueckii* group, the *L. reuteri* group and the *L. salivarius* group are consistently associated with vertebrate hosts; examples include *L. ruminis*, *L. reuteri*, and *L. amylovorus* (Duar et al., 2017b, Frese et al., 2011; Forde, 2011; Walter, 2008). Interrogation of the experimental literature and 16S rRNA sequence databases demonstrated that adaptation to vertebrate hosts is a property that is shared across different members of a specific phylogenetic group (Duar et al., 2017b). For example, *L. helveticus* and *L. pontis* occur in cheese and sourdough fermentations but also form stable populations in the intestine of chicken and swine, respectively

198 (Duar et al., 2017b). The *Lactobacillus* species with the smallest genome size, *L. iners*, also
199 exhibits the most restricted ecological niche, the human vagina, confirming that a small genome
200 size relates to a restricted host range (Macklaim et al., 2011). Comparable to insect-adapted
201 lactobacilli, vertebrate host adapted lactobacilli have a relatively small genome size of about 2
202 Mbp. In contrast to insect associated lactobacilli, they maintain a more extensive toolset for
203 degradation of mono- di, and trisaccharides (Zhao and Gänzle, 2018; Zheng et al., 2015a) or even
204 express extracellular glycosyl hydrolases (Loponen et al., 2016). They generally also maintain
205 multiple amino-acid based mechanisms for acid resistance (Krumbeck et al., 2016; Zheng et al.,
206 2015a). *L. delbrueckii* and *L. fermentum* are exceptions to the general rule that the lifestyle of
207 lactobacilli is shared by closely related species. *L. delbrueckii* adapted to dairy environments; this
208 process included silencing of carbohydrate metabolic genes and the relatively recent acquisition
209 of lactose fermentation (El Kafsi et al., 2014). *L. fermentum* is the only species in the *L. reuteri*
210 group that is not associated with intestinal habitats (Duar et al., 2017b; Walter, 2008).

211 *L. rhamnosus* and *L. plantarum* were isolated from a broad range of habitats. For example, *L.*
212 *plantarum* was identified as member of intestinal microbiota of insects and vertebrate animals but
213 also occurs on plants and in the environment. The origin of strains of *L. plantarum* is unrelated to
214 their phylogenetic position, which indicates that the association of strains with any specific habitat
215 is only temporary (Douillard et al., 2013; Martino et al., 2016). Accordingly, their lifestyle has
216 been termed as “nomadic”, implying frequent transition from one habitat to another (Duar et al.,
217 2017b; Martino et al., 2016). *L. plantarum* and *L. rhamnosus* have a relatively large genome size,
218 which provides a broad metabolic potential and enables the organisms to temporarily persist in
219 multiple environments (Martino et al., 2016). In keeping with its broad distribution in insect,

220 animal, and plant microbiota, *L. plantarum* frequently occurs in spontaneous food fermentations
221 including sourdough fermentations (Gänzle, 2015).

222 A third group of organisms is isolated predominantly from plant or environmental sources. These
223 lactobacilli also typically have a relatively large genome size and a lower optimum and minimum
224 temperature of growth (Duar et al., 2017b). Examples include *L. buchneri*, *L. brevis*, *L. suebicus*,
225 *L. sakei*, and part of the *L. salivarius* group (Duar et al., 2017b).

226 Current knowledge does not allow identification of the ecological niche of many *Lactobacillus*
227 spp. and related organisms; examples include organisms of the *L. alimentarius* group, pediococci,
228 *Leuconostoc* spp. and *Weissella* spp. Pediococci, *Leuconostoc* spp. and *Weissella* spp. frequently
229 contaminate spontaneous plant fermentations including sourdoughs (Figure 4), suggesting an
230 environmental or plant-associated origin of these organisms. However, new species descriptions
231 in the *L. alimentarius* group also include insect isolates, and *Weissella* spp., also occur in the
232 intestine of mammals and cold-water fish (Fusco et al., 2015).

233 Overlapping the frequency of occurrence of lactobacilli in sourdough with their lifestyles provides
234 a consistent association of lifestyle with sourdough microbiota (Figure 5). Type I sourdough
235 microbiota are dominated by the insect associated *L. sanfranciscensis* (Figure 2 and Figure 5). It
236 is noteworthy that only one species is frequently found in sourdough; closely related organisms
237 were rarely (*L. fructivorans*, *L. homohiochii*) or never isolated from sourdoughs. Type II
238 sourdough microbiota are dominated by several vertebrate-host adapted species in the *L.*
239 *delbrueckii* and *L. reuteri* groups (Figure 3 and Figure 5). In particular, the species *L. amylovorus*,
240 *L. frumenti* and *L. pontis* are representatives of swine microbiota (Hu et al., 2018; Konstantinov et
241 al., 2006; Leser et al., 2002). *L. reuteri* has specialised to several host-adapted lineages; rodent as
242 well as human lineage strains were identified as stable members of sourdough microbiota (Su et

al., 2012). Spontaneous sourdoughs, laboratory sourdoughs, and sourdoughs prepared from cereals other than wheat or rye harbour environmental or nomadic organisms, particularly *L. fermentum*, *L. plantarum* and *L. brevis* (Figure 4 and Figure 5).

The significant overlap between the natural habitat of lactobacilli and their occurrence in type I sourdoughs, type II sourdoughs, or spontaneous sourdoughs reflects the ecological parameters that shape community assembly in sourdoughs. Environmental or nomadic lactobacilli are most likely to contaminate spontaneous fermentations (Gänzle and Ripari, 2016; Minervini et al., 2015, Figs. 4 and 5). In contrast, if sourdoughs are maintained in bakeries by continuous propagation over several month or years, organisms from intestinal microbiota of insects or vertebrate hosts are likely to contaminate sourdough. In type I and type II sourdoughs, community assembly is thus determined by selection for the most competitive organisms (Gänzle and Lin, 2014; Gänzle and Ripari, 2016). The dominance of a single species, *L. sanfranciscensis*, in type I sourdoughs strongly indicates highly consistent fermentation conditions in bakeries throughout the world, and a very strong selective pressure for the most rapidly growing organisms.

5. Lifestyle-associated metabolic traits impacting bread quality.

The fermentation parameters for type I sourdough fermentations select for rapidly growing organisms (Lin and Gänzle, 2014). The genome size of *L. sanfranciscensis* is among the smallest among lactobacilli (Vogel et al., 2011; Zheng et al., 2015a). *L. sanfranciscensis* nevertheless maintains a high rRNA gene density to support rapid growth (Vogel et al., 2011). Growth requirements of *L. sanfranciscensis* with respect to pH, temperature, and NaCl concentrations match conditions in type I sourdough fermentations but *L. sanfranciscensis* does not tolerate low pH or high salt concentrations (Gänzle et al., 1998).

Owing to the small genome size, the metabolism of *L. sanfranciscensis* has little to offer except acid production, gas production and co-factor regeneration. The carbohydrate fermentation pattern of insect adapted lactobacilli is generally very narrow; *L. sanfranciscensis* follow this pattern and growth of some strains is supported only by maltose (Zheng et al., 2015a; Vogel, 2011). Strain-specific metabolism of sucrose is supported by extracellular levansucrase activity (Tieking et al., 2005). In sourdough, however, *L. sanfranciscensis* is invariably associated with yeasts; sucrose hydrolysis by yeast invertase (Perlman et al., 1981) provides glucose and fructose for use by *L. sanfranciscensis*. Heterofermentative hexose metabolism by *L. sanfranciscensis* provides its key contribution to bread quality – acidification to modulate the activity of cereal enzymes (Gänzle, 2014), and CO_2 production to support leavening (Brandt et al., 2004). Cofactor regeneration supports formation of acetate which impacts taste, flavor, and the mould-free shelf life of bread (Hansen and Schieberle, 2005**)**. Of note, beneficial effects of levan production on bread texture are compensated by the associated production of excess acetate (Kaditzky et al., 2008).

Metabolic activities of *L. sanfranciscensis* related to cofactor regeneration include reduction of flavour active aldehydes to alcohols with much lower contribution to bread flavour (Gänzle, 2014; Hansen and Schieberle, 2005), and the reduction of oxidized glutathione (Jänsch et al., 2007). Glutathione and cysteine metabolism also are key elements of the oxidative stress response of *L. sanfranciscensis* (Jänsch et al., 2007; Stetina et al., 2014). The reduction of oxidized glutathione by *L. sanfranciscensis*, however, interferes with disulfide-bond mediated polymerization of gluten proteins (Jänsch et al., 2007). The volume of wheat bread is highly dependent on the quantity and quality of a gluten macropolymer (Wieser, 2007; Reinbold et al., 2008). Comparison of isogenic mutants of *L. sanfranciscensis* differing with respect to glutathione reductase activity indeed demonstrated that reduction of glutathione decreased bread volume when compared to the isogenic

glutathione-reducase negative strain (Tang et al., 2017). The glutathione reductase activity of *L. sanfranciscensis* also allows the proteolytic degradation of highly disulfide bonded allergens, e.g. ovotransferrin, that are resistant to proteolysis when the disulfide bond mediated tertiary structure is intact (Loponen et al., 2008).

The potential of *L. sanfranciscensis* to metabolise amino acids or lipids to bioactive metabolites with impact on bread quality is limited. The metabolic flux through the transaminase pathway is low when compared to *L. plantarum* (Vermeulen et al., 2006). Glutaminase, glutamate decarboxylase and arginine deiminase pathway, which mediate acid resistance in lactobacilli (Su et al., 2011; Teixeira et al., 2014), are absent in *L. sanfranciscensis* (Vogel et al., 2011; Zheng et al., 2015a). Accordingly, *L. sanfranciscensis* is rapidly eliminated from sourdough microbiota when fermentation conditions include extended incubation at low pH (Meroth et al., 2003).

Fermentation conditions in type II sourdough impose a second selective pressure, acid stress, as a consequence of prolonged incubation conditions (Lin and Gänzle, 2014; Meroth et al., 2003). Moreover, elevated fermentation temperatures typically select for lactobacilli with an optimum temperature of growth around 37°C. Vertebrate host adapted lactobacilli, the most abundant representatives of type II sourdough microbiota, are also characterized by a relatively small genome size and a high density of rRNA operons; however, genes coding for acid resistance, adhesion to mucosal surfaces, and biofilm formation are typically required for the vertebrate host adapted lifestyle (Duar et al., 2017b; Frese et al., 2011, Krumbeck et al., 2016). Acid resistance mechanisms of host adapted lactobacilli impact bread quality (Teixeira et al., 2014). Glutaminase activity of sourdough lactobacilli accumulates glutamate, an umami active taste compound (Zhao et al., 2015); further conversion of glutamate generates γ-aminobutyrate, a bioactive compound with relaxing and anti-hypertensive properties (Inoue et al., 2003; Rizzello et al., 2008). Arginine

311 conversion by the arginine deiminase pathway generates ornithine, a precursor compound for
312 formation of the character impact compound of the wheat bread crust odor, 2-acetyl-1-pyrroline
313 (Hansen and Schieberle, 2005; Thiele et al., 2002).

314 Despite their small genome size, vertebrate host adapted lactobacilli also maintain a relative large
315 spectrum of carbohydrate active enzymes for metabolism of oligosaccharides (Zheng et al., 2015a;
316 Zhao and Gänzle, 2018). The broad carbohydrate fermentation pattern by vertebrate host adapted
317 lactobacilli is used industrially to produce low-FODMAP bread through degradation of raffinose,
318 mannitol, and fructans (Loponen and Gänzle, 2018).

319 Exopolysaccharide formation by intestinal lactobacilli is essential for formation of biofilms on
320 non-secretory epithelia (Duar et al., 2017b; Frese et al., 2011). Accordingly, the formation of EPS
321 from sucrose is particularly frequent in the host-adapted *L. reuteri* and *L. delbrueckii* groups
322 (Tieking et al., 2003; Zheng et al., 2015a). EPS formation also serves other ecological roles as
323 suggested by the high frequency of glucansucrases in the *L. buchneri* group as well as *Weissella*
324 spp. and *Leuconostoc* spp. The different ecological roles are reflected in the differences of the
325 regulation of gene expression. In *L. reuteri*, reuteransucrase expression is constitutive while
326 dextransucrase expression in *Leuconostoc* spp. is induced by sucrose as well as oxidative stress
327 (Yan et al., 2016). The use of dextran-producing *Weissella* in baking applications is beneficial
328 because *Weissella* spp. often lack mannitol dehydrogenase; dextran formation is thus not
329 associated with production of excess quantities of acetate (Galle and Arendt, 2014; Galle et al.,
330 2012; Katina et al., 2008). Dextransucrase expression in *W. cibaria* 10M is not induced by sucrose
331 but responds to cold stress (Hu and Gänzle, 2018); however, current data on the regulation of
332 dextransucrase expression in *Weissella* spp. and the ecological role of dextran production are too
333 limited to provide guidance for the optimization of sourdough fermentations.

The reliable occurrence of *L. plantarum* in spontaneous cereal fermentations is linked to the stable association of this species with plants; specific strains of *L. plantarum* were also shown to persist in traditional, back-slopped sourdoughs (Minervini et al., 2015 and 2018). The relatively large genome size and genomic diversity and the corresponding metabolic versatility, however, also enable *L. plantarum* to temporarily colonize insects and vertebrate hosts, allowing probiotic applications (Schwarzer et al., 2016; Siezen and van Hycklama Vlieg, 2011; van den Nieuwboer et al., 2016). With regards to their application in cereal fermentations, strains of *L. plantarum* have an exceptionally broad capacity to metabolize phytochemicals through phenolic acid esterases, decarboxylases, and reductases, and by a diverse array of glycosyl hydrolases (Bai and Gänzle, 2015; Rodriguez et al., 2009; Santamaria et al., 2018). The ability of *L. plantarum* to convert phytochemicals likely relates to its adaptation to plants, and contributes to biochemical conversions in sorghum fermentations as well as in fruit and vegetable fermentations (Filannino et al., 2015; Svensson et al., 2010). The contribution of *L. plantarum* to conversion of phenolic compounds in wheat and rye sourdoughs, or a contribution of these conversions to bread quality remains to be demonstrated.

6. Conclusions and perspectives.

Type I sourdoughs are populated by the insect-adapted *L. sanfranciscensis* while type II sourdoughs are populated by vertebrate-host adapted organisms of the *L. delbrueckii* and *L. reuteri* groups. The ecological fitness of lactobacilli in sourdoughs and their impact on bread quality is dependent on niche-specific metabolic traits that accommodate rapid growth and CO_2 production from maltose and sucrose in case of *L. sanfranciscensis*, and amino-acid dependent mechanisms of acid resistance in *L. reuteri* and allied organisms. Remarkably, the stable association of *L. reuteri* group organisms with *L. delbrueckii* group organisms in the intestine of rodents, swine,

and poultry is maintained in type II sourdough microbiota (Konstantinov et al., 2006; Leser et al., 2002; Walter, 2008). It is tempting to speculate that the *L. alimentarius* group organisms *L. mindensis*, *L. paralimentarius, L. nantensis* and *L. crustorum*, which are often associated with *L. sanfranciscensis* in type I sourdoughs, also share the natural habitat with *L. sanfranciscensis*.

While the composition of sourdough microbiota in traditional sourdoughs is very consistent, literature data also documents that a large diversity of lactobacilli grow in sourdoughs (De Vuyst and Neysens, 2005; De Vuyst et al., 2014; Gobbetti et al., 2016). Alternative choices of fermentation conditions will allow persistence of alternative sourdough microbiota with specific metabolic properties that are not represented by "traditional" sourdough microbiota. For example, co-fermentation of *L. diolivorans* and *L. buchneri* supported propionate formation in sourdoughs (Zhang et al., 2010), and the use of specific grain fractions in sourdough fermentations supports degradation of fructans by lactobacilli with extracellular fructan hydrolases (Loponen et al., 2016; Loponen and Gänzle, 2018). Other proposed applications of sourdough are also dependent on the choice of non-traditional fermentation cultures with specific metabolic properties (Denkova et al., 2014; Gobbetti et al., 2018; Jakob et al., 2012). The use of non-conventional lactic acid bacteria that do not occur in sourdoughs, e.g. *L. buchneri* and *L. diolivorans* (Zhang et al., 2010), propionibacteria or acetic acid bacteria in conjunction with baker's yeast allows adaptation of a traditional food fermentation to the contemporary requirements of industrial food production.

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Figure 1. Two examples of sourdough fermentation processes used to achieve leavening of bread without addition of baker's yeast. **Panel A**, three stage sourdough process (Tang et al., 2017). Tang et al. performed fermentations at 30°C; in bakery practice, the fermentation stages are performed at 22 – 28°C; **Panel B**; sourdough process for production of Colomba (Raimundi et al., 2017). Other type I sourdough fermentation processes follow a different regimen with respect to incubation time and temperature, and inoculum level, however, all sourdoughs that are fermented to achieve leavening of dough without baker's yeast continuously maintain sourdough microbiota in a metabolically active state.

Figure 2. Occurrence of lactic acid bacteria in wheat and rye sourdoughs used as sole leavening agent. Shown is the percentage of 227 sourdoughs containing the species indicated on the x-axis. Species are shown only if they were identified in 3 or more sourdoughs. Data were compiled from Böcker et al., 1995, Corsetti et al, 2001, Ehrmann et al., 2003, Ferchichi et al., 2007, Foschino et al., 1999, Garofalo et al., 2008 Lattanzi et al. 2013, Lhomme et al., 2015 and 2016, Liu et al., 2016, Kitihara et al., 2005, Kline and Sugihara, 1971, Meroth et al., 2003, Michel et al., 2016, Minervini et al. 2012, Palla et al., 2017, Raimondi et al., 2017, Randazzo et al., 2005, Ripari et al, 2016, Scheierlinck et al., 2007, Spicher, 1987, Yang et al., 2017, Zhang et al., 2015, and unpublished observations for 27 sourdoughs. The composition of all 227 sourdoughs is listed in Table S1 of the online supplementary material.

Figure 3. Occurrence of lactic acid bacteria in wheat and rye sourdoughs used for acidification of wheat and rye sourdoughs, or for production of baking improvers. Shown is the percentage of 32 sourdoughs containing the species indicated on the x-axis. Species are shown only if they were identified in 2 or more sourdoughs. Data were compiled from Böcker et al., 1995, Ferchichi et al., 2007, Meroth et al., 2003, Müller et al., 2001, Rosenquist and Hansen, 2000, Vera et al., 2012,

731 Viiard et al., 2013 and 2016, and unpublished observations for 17 sourdoughs. The composition
732 of all 32 sourdoughs is listed in Table S2 of the online supplementary material.

733 **Figure 4.** Occurrence of lactic acid bacteria in spontaneous sourdoughs, model sourdoughs
734 prepared under sterile laboratory conditions, or in cereal fermentations with substrates other than
735 wheat or rye. Shown is the percentage of 54 sourdoughs containing the species indicated on the x-
736 axis. Species are shown only if they were identified in 2 or more sourdoughs. Data were compiled
737 from the reviews of De Vuyst and Neysens, 2005 and De Vuyst et al., 2014 as well as Gassem,
738 1999, Hamad et al., 1992, Madoroba et al., 2011, Muyanja et al., 2003, Sekwati-Monang and
739 Gänzle, 2011, and unpublished observations for 5 sourdoughs. The composition of all 56
740 sourdoughs is listed in Table S3 of the online supplementary material.

741 **Figure 5.** Phylogenomic analysis of *Lactobacillus, Pediococcus, Weissella* and *Leuconostoc*
742 species based on the concatenated protein sequences of 99 single-copy core genes. *Eggerthia*
743 *catenaformis* was used as an outlier for the phylogenetic analysis. The maximum likelihood tree
744 was inferred by PhyML as described (Zheng et al., 2015a) using the 187 species of *Lactobacillus*
745 and *Pediococcus* for which genome sequence data was available on the NCBI database on May
746 31, 2018, and four *Leuconostoc* and *Weissella* species that occur frequently in sourdough.
747 Members of the same phylogenetic group (Zheng et al., 2015a) are indicated by the same color for
748 branches, and the type strain of each group is printed in bold. The species names of
749 homofermentative species are printed in red; names of heterofermentative species are printed in
750 blue. Outer rings provide information on genomic features, the lifestyle of the species, and their
751 occurrence in sourdoughs as follows: (inside to outside): The color gradient in red represents the
752 GC content of each genome sequence; higher GC contents are indicated by darker shading. The
753 solid circles in brown represent genome sizes; the area of the circle correlates with the genome

size. The second ring indicates the natural habitats of the species as vertebrate host-adapted (red), insect-adapted (orange), nomadic (green), environmental (blue) or unassigned (white). The assignment of species to lifestyle was based on Duar et al. (2017b); new species in the *L. kunkeei* and *L. fructivorans* groups were assigned based on the source of isolation; *L. equicursorum* and *L. acetotolerans* were assigned to vertebrate-host adapted lifestyles as intestinal ecosystems represent the only (non-food) origin of the species (Luo et al., 2015; O' Donnell, 2013). The orange circles represents the frequency of the occurrence of species in type I sourdoughs; the circle area was calculated with data shown in Figure 2. The red circles represent the frequency of the occurrence of species in type II sourdoughs; the circle area was calculated with data shown in Figure 3. The blue circles represent the frequency of the occurrence of species in selected spontaneous sourdoughs and related cereal fermentations; the circle area was calculated with data shown in Figure 4.

Figure 1

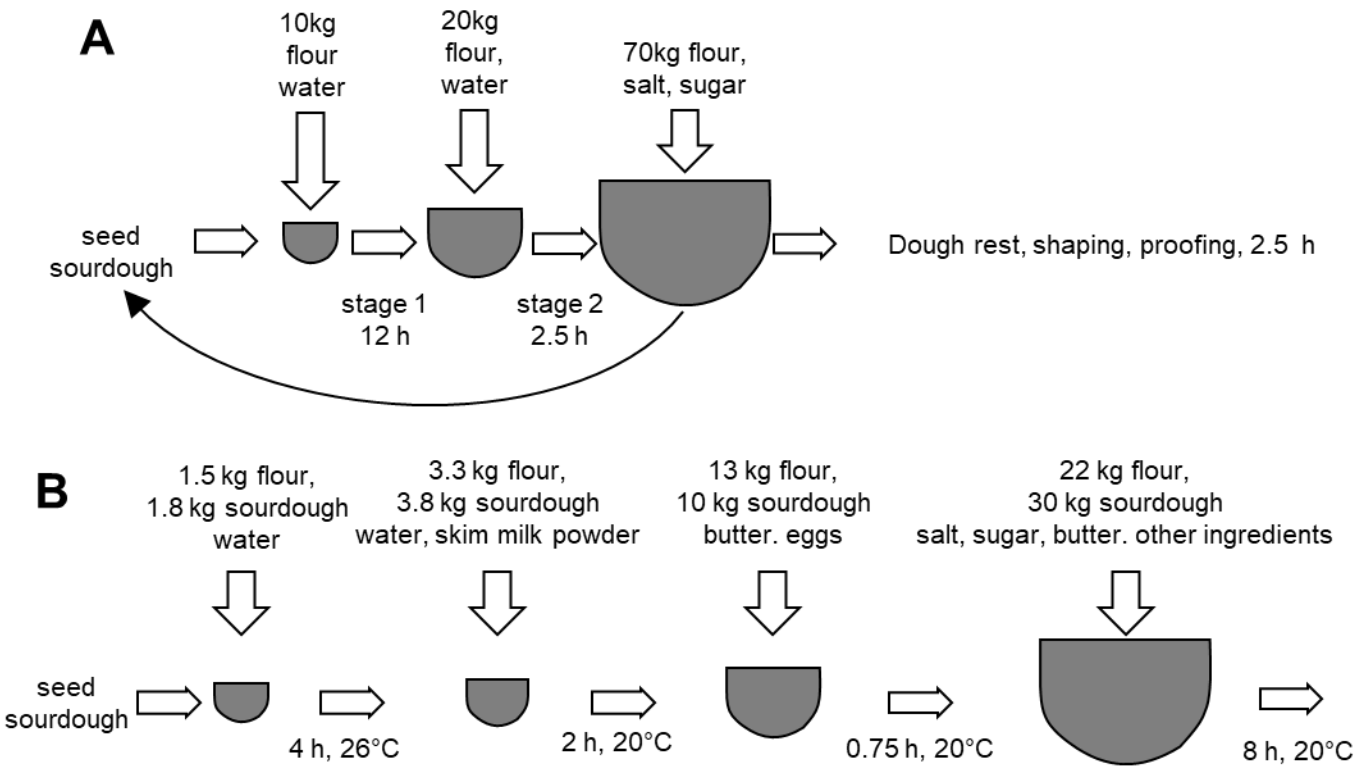
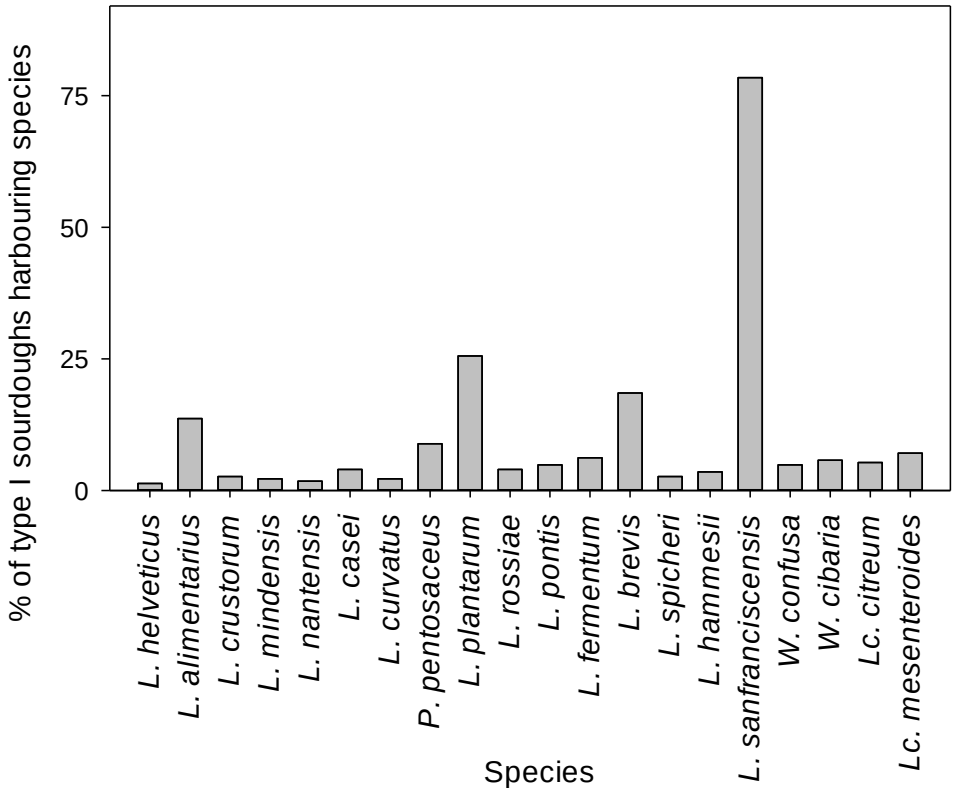
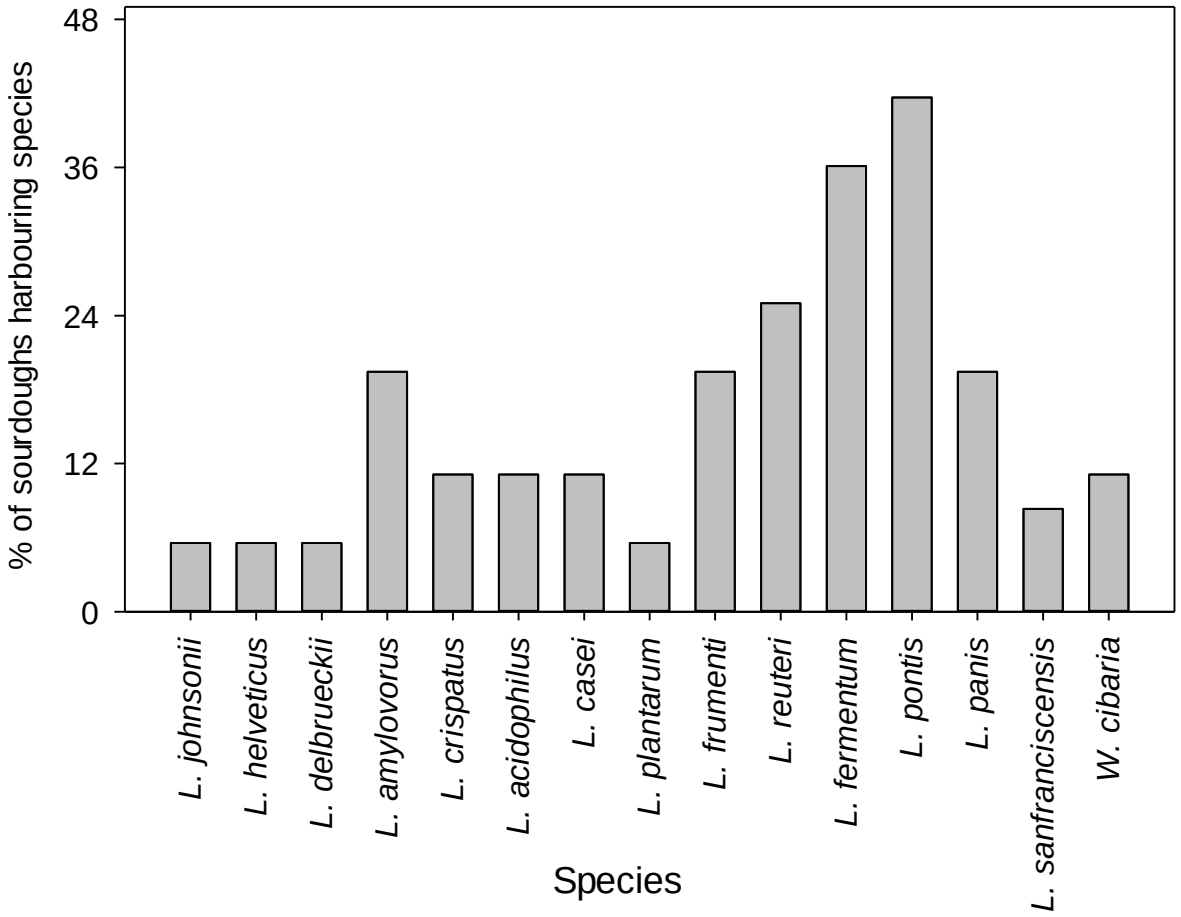

A
10kg flour water
20kg flour, water
70kg flour, salt, sugar
seed sourdough
stage 1 12 h
stage 2 2.5 h
Dough rest, shaping, proofing, 2.5 h
B
1.5 kg flour, 1.8 kg sourdough water
3.3 kg flour, 3.8 kg sourdough water, skim milk powder
13 kg flour, 10 kg sourdough butter. eggs
22 kg flour, 30 kg sourdough salt, sugar, butter. other ingredients
seed sourdough
4 h, 26°C
2 h, 20°C
0.75 h, 20°C
8 h, 20°C

Figure 2.


% of type I sourdoughs harbouring species
0
25
50
75
L. helveticus
L. alimentarius
L. crustorum
L. mindensis
L. nantensis
L. casei
L. curvatus
P. pentosaceus
L. plantarum
L. rossiae
L. pontis
L. fermentum
L. brevis
L. spicheri
L. hammesii
L. sanfranciscensis
W. confusa
W. cibaria
Lc. citreum
Lc. mesenteroides
Species

773 **Figure 3.**



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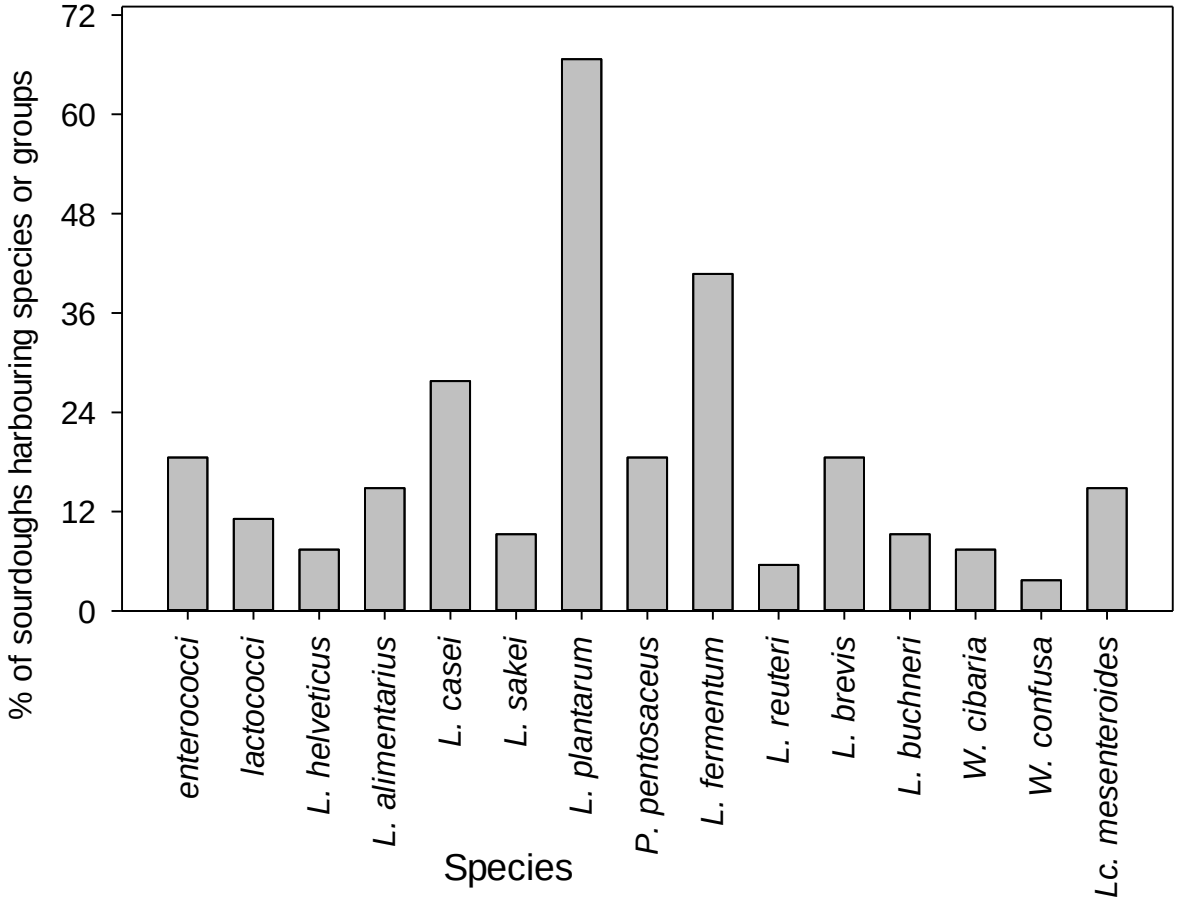
775

776 **Figure 4.**



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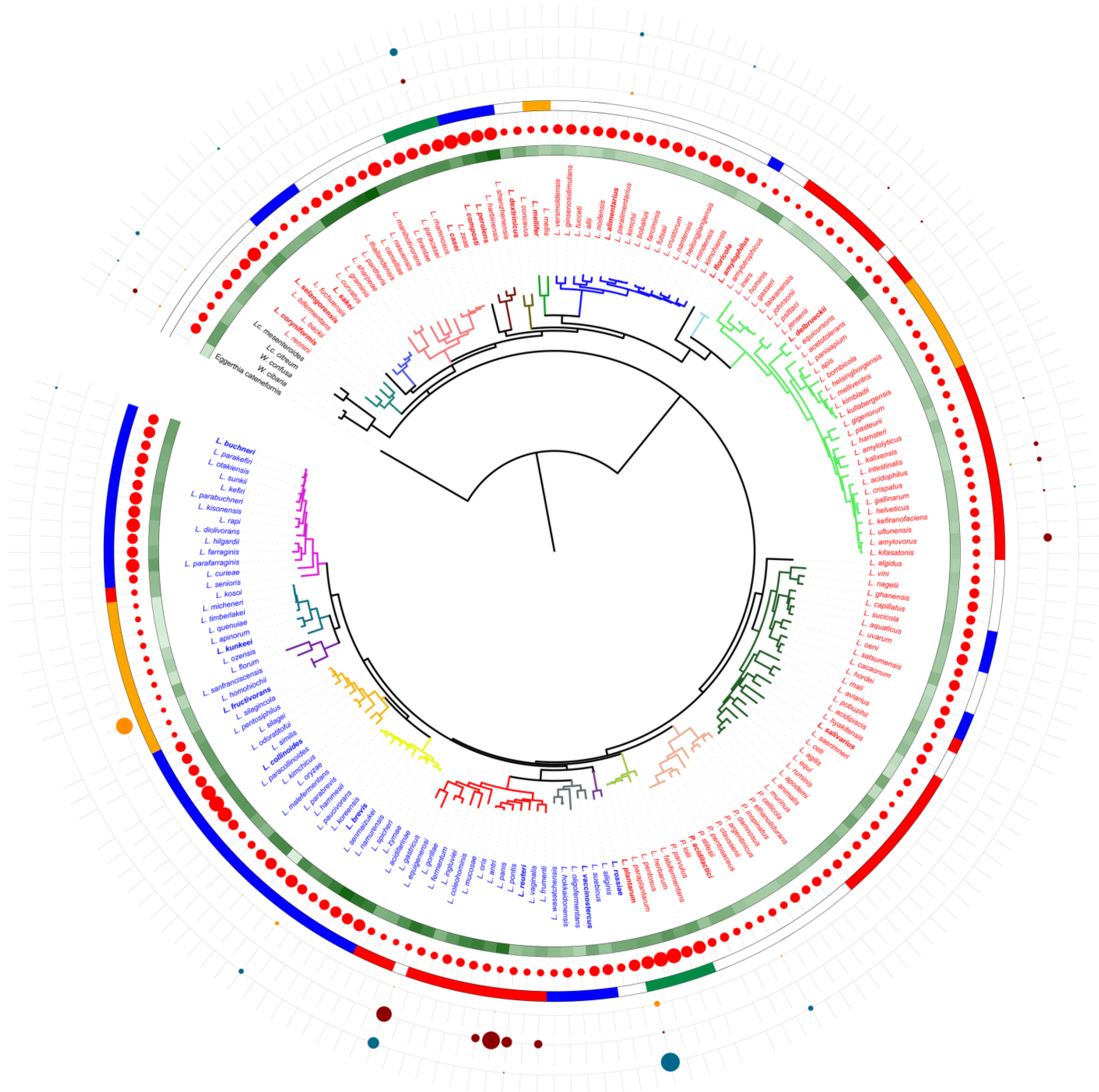

Eggerthia catenaformis
W. cibaria
W. confusa
Lc. citreum
Lc. mesenteroides
L. rennini
L. coryniformis
L. backii
L. bifermentans
L. selangorensis
L. fuchuensis
L. sakei
L. curvatus
L. graminis
L. sharpeae
L. pantheris
L. thailandensis
L. camelliae
L. nasuensis
L. manihotivorans
L. brantae
L. paracasei
L. rhamnosus
L. zeae
L. casei
L. composti
L. perolens
L. harbinensis
L. shenzhenensis
L. dextrinicus
L. concavus
L. mellifer
L. mellis
L. versmoldensis
L. ginsenosidimutans
L. furfuricola
L. allii
L. nodensis
L. alimentarius
L. paralimentarius
L. kimchii
L. bobalius
L. farciminis
L. futsaii
L. crustorum
L. nantensis
L. heilongjiangensis
L. mindensis
L. kimchiensis
L. floricola
L. amylophilus
L. amylotrophicus
L. iners
L. hominis
L. gasseri
L. taiwanensis
L. johnsonii
L. psittaci
L. jensenii
L. delbrueckii
L. equicursoris
L. acetotolerans
L. panisapium
L. apis
L. bombicola
L. helsingborgensis
L. melliventris
L. kimbladii
L. kullabergensis
L. gigeriorum
L. pasteurii
L. hamsteri
L. amylolyticus
L. kalixensis
L. intestinalis
L. acidophilus
L. crispatus
L. gallinarum
L. helveticus
L. kefiranofaciens
L. ultunensis
L. amylovorus
L. kitasatonis
L. algidus
L. vini
L. nagelii
L. ghanensis
L. capillatus
L. sucicola
L. aquaticus
L. uvarum
L. oeni
L. satsumensis
L. cacaonum
L. hordei
L. mali
L. aviarius
L. pobuzihii
L. acidipiscis
L. hayakitensis
L. salivarius
L. saerimneri
L. agilis
L. ceti
L. equi
L. ruminis
L. apodemi
L. animalis
L. murinus
P. cellicola
P. ethanolidurans
P. inopinatus
P. damnosus
P. argentinicus
P. claussenii
P. pentosaceus
P. stilesii
P. acidilactici
P. lolii
P. parvulus
L. fabifermentans
L. herbarum
L. pentosus
L. paraplantarum
L. plantarum
L. rossiae
L. siliginis
L. suebicus
L. vaccinostercus
L. oligofermentans
L. hokkaidonensis
L. wasatchensis
L. frumenti
L. vaginalis
L. reuteri
L. pontis
L. panis
L. antri
L. oris
L. mucosae
L. coleohominis
L. ingluviei
L. fermentum
L. gorillae
L. equigenerosi
L. gastricus
L. acidifarinae
L. zymae
L. spicheri
L. namurensis
L. senmaizukei
L. brevis
L. koreensis
L. paucivorans
L. hammesii
L. parabrevis
L. malefermentans
L. oryzae
L. kimchicus
L. paracollinoides
L. collinoides
L. similis
L. odoratitofui
L. silagei
L. pentosiphilus
L. silagincola
L. fructivorans
L. homohiochii
L. sanfranciscensis
L. florum
L. ozensis
L. kunkeei
L. apinorum
L. quenuiae
L. timberlakei
L. micheneri
L. kosoi
L. senioris
L. curieae
L. parafarraginis
L. farraginis
L. hilgardii
L. diolivorans
L. rapi
L. kisonensis
L. parabuchneri
L. kefiri
L. sunkii
L. otakiensis
L. parakefiri
L. buchneri