Composition and function of sourdough microbiota:

From ecological theory to bread quality

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Abstract

Sourdough has traditionally been used as leavening agent in artisanal baking. The production of baked and steamed cereal products increasingly employs sourdough as baking improver to achieve improved bread quality, or to obtain "clean label" products. Sourdoughs are maintained in bakeries by continuous propagation; composition and metabolic activity of sourdough microbiota and their impact on bread quality are therefore shaped by processing parameters and fermentation substrates. The diversity of fermentation processes leads to diverse compositions of sourdough microbiota. This communication explores whether concepts in community assembly support an improved understanding of the microbial ecology of sourdough. Community assembly is determined by diversification, drift, dispersal, and selection. Evidence for diversification in sourdoughs is inconclusive. Drift has been shown to shape sourdough microbiota only in specific cases. Increasing knowledge on the primary habitat of sourdough lactobacilli indicates that dispersal (limitation) is an important determinant in sourdoughs that are propagated only for short periods of time. In contrast, selection of adapted organisms mainly determines the microbiota of sourdoughs that are propagated for a long time. Bacterial metabolic traits that determine competitiveness in sourdough fermentation, i.e. effective use of maltose, exopolysaccharide formation from sucrose, the use of electron acceptors by heterofermentative lactic acid bacteria, and acid resistance mediated by arginine and glutamine conversion, also determine bread quality. The concepts in community assembly thus provide a valuable tool to understand the influence of the technology of sourdough fermentation on microbial ecology and on bread quality.

Keywords: Sourdough microbiota, microbial ecology of sourdough, dispersal, selection, competition, *L. reuteri*, *L. sanfranciscensis*.

Introduction.

The use of sourdough in baked and steamed bread production worldwide is increasing due to the improved sensory and nutritional quality of sourdough bread when compared to bread produced by straight dough processes (Gobbetti et al., 2014; Liu et al., 2016; Thiele et al., 2002; Zhao et al., 2015). Traditionally, sourdough has been used as leavening agent. The use of sourdough as baking improver in combination with baker's yeast also allows replacement of additives with "clean label" ingredients in industrial baking (Brandt, 2007). Moreover, the fermentation of sourdough in the bakery is an alternative to the use of additives (Meuser and Valentin, 2004).

Sourdough microbiota comprise yeasts and lactic acid bacteria (De Vuyst et al., 2014; Vogel et al., 1999). The metabolic activity of sourdough microbiota in combination with the enzymatic activity of the cereal substrates determines product quality (Gänzle 2014); therefore, attaining constant product quality requires control of the composition and activity of fermentation microbiota. The use of sourdough in bread production thus necessitates knowledge of the microbial ecology of sourdough and its influence on bread quality. Sourdough production by suppliers to the baking industry is performed under controlled conditions and often involves the use of defined starter cultures (Brandt, 2007). Sourdough fermentation by artisanal and industrial bakers, however, typically relies on continuous propagation or back-slopping. Fermentation control in bakeries maintains consistent technological performance of sourdough as determined by acidity, acidification or leavening capacity, or the effect on product quality, but not always achieves a constant composition of fermentation microbiota.

The differentiation between sourdough and straight-doughs is based on the contribution of lactic acid bacteria to metabolic turnover at the dough stage, which is substantial for sourdoughs but negligible or absent in straight dough processes. Moreover, comparative genomic, phylogenomic

and functional analyses that delineate origin, adaptation and competitiveness of sourdough microbiota are available only for bacteria (Su et al., 2011 and 2012; Vogel et al., 2011; Zheng et al., 2015a); therefore, this communication focuses on the bacterial ecology of sourdoughs.

Microbial ecology of sourdough: α-diversity follows fermentation technology.

The species richness in a single batch of sourdough (α-diversity) is limited; typically, less than 6 different species or strains account for more than 99% of microbial cells (reviewed by De Vuyst and Neysens, 2005; De Vuyst et al., 2014; Vogel et al., 1999). In contrast, the γ-diversity of sourdoughs, the number of species isolated from sourdoughs globally, encompasses more than 80 bacterial species, including mainly species of the *Lactobacillaceae* and *Leuconostocaceae* but also lactococci, enterococci, and streptococci (**Figure 1A,** De Vuyst and Neysens, 2005; De Vuyst and Neysens, 2014; Vogel et al., 1999). The presence of *L. plantarum* or *L. sanfranciscensis* is reported for about 50% of the sourdoughs. A β-diversity of sourdoughs cannot be defined as regional diversity (De Vuyst et al., 2014). Böcker et al. (1995) provided a suitable approach for the definition of β-diversity by differentiation of sourdough microbiota based on the technological aim of fermentation. The term “type I sourdough” has been used for sourdoughs used as leavening agent and the term “type II sourdoughs” for sourdoughs used as baking improver (Böcker et al., 1995). Microbiota of type I sourdoughs are characterized by uniformity rather than diversity. *Lactobacillus sanfranciscensis* is the key species of type I sourdoughs microbiota worldwide; this species was isolated as dominant member of the microbiota of more than 75% of the sourdoughs (**Figure 1B)**. The diversity of fermentation substrates, which increasingly include gluten-free cereals and pseudocereals, processes, and technological aims of type II sourdoughs, however, result in a high β-diversity of organisms in

type II sourdoughs. To date, however, the categorization of sourdoughs remains predominantly descriptive, and has not been based on basic concepts in community assembly.

Concepts in Community Ecology.

Concepts in community assembly aim to explain the assembly of plant or animal communities (Chave, 2004; Volkov et al., 2003). One of the most influential contributions to community ecology is the neutral theory, which implies that organisms in an ecosystem have an equivalent level of ecological fitness, and that the relative species abundance in the ecosystem is determined exclusively by random or stochastic events (drift) (Hubbell, 2001). Vellend (2010) complemented the neutral theory by a conceptual framework which explains community assembly by the four processes speciation, drift, dispersal, and selection. These terms also describe the dynamics of microbial communities (Costello et al., 2012; Nemergut et al., 2013). This communication aims to apply this conceptual framework to the microbial ecology of sourdough. The relevance of these four processes for the microbial ecology of sourdough is indicated in Table 1. Evidence for the relevance of these four processes in shaping the relative species abundance of microbial communities in sourdough is provided in the following sections.

Speciation and Diversification.

Speciation or diversification refers to the differentiation of strains and the creation of new species (Table 1). Continuous propagation of sourdoughs over long periods of time supports growth of individual strains over thousands or even millions of bacterial generations, and thus allows for evolution in response to the sourdough environment. The beginning of the use of cereal fermentations with lactic acid bacteria likely coincides with the domestication of cereals in Near Eastern Natufian cultures less than 15,000 years ago (Hayden et al., 2013). Sourdough has been used in production of leavened baked goods for more than 5000 years (Capelle et al., 2013)

and the continuous propagation of a sourdough has been documented for a period of over 100 years (Vogel et al., 2011), corresponding to an estimated 5 x 10^5 bacterial generations. Comparing the timelines for food or sourdough fermentations with the molecular clock of bacterial evolution, however, indicates that the time that passed since humans have fermented cereals does not support speciation. For example, diversification of the human pathogen *Yersinia pestis* from the clonally related *Yersinia pseudotuberculosis* occurred over a period of more than 10,000 years (Achtmann et al. 2004). The diversification of human adapted lineages of *L. reuteri* was estimated to occur in a range of 8 – 13 million years (Oh et al., 2009). The relatively minor influence of diversification on sourdough microbiota is also supported by genomic analyses of sourdough lactobacilli. The genome of a strain of *L. sanfranciscensis* remained essentially unchanged over 18 years of continuous propagation in sourdough (Ehrmann et al., 2011). *L. sanfranciscensis* was referred to as "sourdough adapted" species (Vogel et al., 2011); however, the genetic diversification that was observed in long-term back-slopped sourdoughs indicates that diversification of the species has occurred in a habitat other than sourdough (see below). Likewise, the genome content and phylogenetic position of sourdough isolates of *L. reuteri* match to host-adapted lineages of that species (Su et al., 2012, Zheng et al., 2015a). Comparative genomic analysis of sourdough isolates of *L. reuteri* and intestinal isolates of the same species revealed that sourdough specific genes or strains cannot be identified (Zheng et al., 2015a). The identification of genes that are under positive selection favouring nonsynonymous mutations, however, revealed significant differences between sourdough isolates and intestinal isolates of *L. reuteri* (Zheng et al., 2015a). Genes in the functional categories "energy production and conversion" and "carbohydrate metabolism" are under positive selection in sourdough isolates but not intestinal isolates. This may reflect adaptation of strains of *L. reuteri* to the

136 sourdough environment, or selection of a specific subset of intestinal strains during growth in
137 sourdough (Zheng et al. 2015a).

138 **Drift**

139 The term drift describes the influence of random events on community structure (Table 1). In
140 relation to the microbial ecology of sourdough, drift may be considered to encompass phage
141 contamination, or changes in the substrate properties, e.g. based on differences in the
142 composition and enzyme activities between grains harvested in different years. Contamination of
143 the sourdough from animals, humans or plants relate to dispersal (see below) but infrequent and
144 random contamination events can also be considered to contribute to drift. Several lines of
145 evidence suggest that drift shapes sourdough microbiota in specific cases.

146 Lytic bacteriophages have been identified in several sourdough isolates including
147 *L. sanfranciscensis* (Foschino et al., 2005; Ehrmann et al., 2013). The identification of plasmid-
148 encoded CRISPR elements in the genome of *L. sanfranciscensis* also supports the assumption
149 that phage contamination shapes sourdough microbiota (Vogel et al., 2011). Phage infection and
150 spread, however, is limited in solid state cereal fermentations (Foschino et al., 2005).

151 Lhomme et al. (2014) documented the evolution of sourdough microbiota in the bakery
152 environment over several months after a shift from wheat flour to gluten free flours. The
153 evolution of sourdough microbiota was characterized by stability over several weeks or month,
154 interrupted by sudden shifts of the relative species abundance that were unrelated to changes of
155 the quality of flour used for fermentation. This pattern of community assembly is consistent with
156 the assumption that relatively few "random" contamination events with competitive strains
157 altered the fermentation microbiota, followed by periods of temporal stability until the next
158 contamination event. The comparison of the evolution of sourdough microbiota under aseptic

conditions in the laboratory indicated that organic or conventional grain production influenced the development of sourdough microbiota (Rizzello et al., 2015); this finding is consistent with the assumption that the influence of the farming system on plant microbiota is a "random" factor influencing the microbial ecology of sourdough.

Further evidence for the role of drift was provided by the analysis of the ecological fitness of strains of *L. reuteri*. Strains of *L. reuteri* representing human-adapted or rodent-adapted lineages were isolated from wheat- and rye sourdoughs, respectively; these sourdoughs were processed at different fermentation conditions (Su et al., 2012). Competition experiments with these isolates demonstrated that the ecological fitness of strains from both lineages in wheat and rye sourdoughs was equivalent (Lin and Gänzle, 2014a). Their prevalence in the respective sourdoughs is thus likely attributable to random contamination events rather than lineage-specific differences in ecological fitness (Lin and Gänzle, 2014a).

Dispersal and Dispersal limitation

The term dispersal describes the movement of organisms across space (Table 1). In relation to the microbial ecology of sourdough, dispersal relates mainly to the intentional or unintentional inoculation or contamination of sourdough. As sourdough fermentation does not support speciation, the presence of microorganisms in sourdough results from the use of starter cultures, or from contamination from the primary habitat of sourdough microbiota. This assumption conforms to anecdotal reports that *de novo* fermentation of sourdoughs in bakeries is performed with the intentional use of baker's yeast, diverse plant material, or manure as inoculum. The identification of the primary habitat of sourdough organisms, however, is confounded by the fact that many sourdough lactobacilli were isolated only from sourdough (De Vuyst et al., 2014). *L sanfranciscensis* is assigned to the insect-associated *L. fructivorans* group; other sourdough

182 lactobacilli are members of the *L. reuteri* and *L. delbrueckii* groups, which comprise
183 predominantly organisms that are adapted to the intestine of warm-blooded animals, and the
184 plant-associated *L. plantarum* and *L. brevis* groups (Figure 1, De Vuyst et al., 2014; Zheng et al.,
185 2015b). Lactic acid bacteria of undefined origin, however, are also frequently found in
186 sourdough (De Vuyst and Neysens, 2005; De Vuyst et al., 2015). Lactic acid bacteria that were
187 present in sourdough were also present on the hands of bakers and in the air of the production
188 environment (Scheirlinck et al., 2009); it remains unclear, however, whether this is cause or
189 consequence of their prevalence in sourdough.
190 The molecular characterization of plant microbiota of durum wheat, followed by characterization
191 of the microbiota of laboratory-made sourdoughs that were prepared from these grains
192 demonstrated that a key sourdough species, *L. plantarum,* is also associated with plant organs
193 throughout the growth of wheat (Minervini et al., 2015). Extensive analyses of the microbiota of
194 laboratory-made sourdoughs, however, also demonstrate that flour microbiota are not the main
195 source of sourdough organisms. Propagation of *de novo* sourdoughs under laboratory conditions
196 excludes contamination from sources other than the flour. After approximately 10 propagation
197 steps, such laboratory-made *de novo* sourdoughs harbour plant-associated or ubiquitous
198 organism including pediococci and organisms of the *L. plantarum*, *L. sakei* and *L. brevis* groups
199 but typically lack *L. sanfranciscensis* and animal associated species of the *L. rossiae* and *L.*
200 *reuteri* groups (Bessmeltseva et al., 2014; Ercolini et al. 2013; Minervini et al., 2015; Rizzello et
201 al., 2015; van der Meulen 2007).
202 The *L. fructivorans* group, which comprises *L. sanfranciscensis*, is exclusively composed of
203 flower- or insect associated organisms (Zheng et al., 2015b). Fruit flies are the only known
204 source of *L. sanfranciscensis* outside of traditional sourdoughs, although this origin is

205 documented only by culture-independent analysis (Groenewald et al., 2006). Remarkably,
206 *L. sanfranciscensis* is generally absent in laboratory-made sourdoughs started with flour only
207 (Bessmeltseva et al., 2014; Ercolini et al. 2013; Minervini et al., 2015; Rizzello et al., 2015; van
208 der Meulen 2007) but strains of the species were identified in *de novo* sourdoughs that were
209 inoculated with flowers, berries, or mother of vinegar (Table 2). The absence of *L.*
210 *sanfranciscensis* in 3 of the 7 sourdoughs shown in Table 2 indicates it origin from the specific
211 contaminating material rather than the flour or the laboratory environment, which was common
212 to all 7 sourdoughs. This exceptional presence of *L. sanfranciscensis* in *de-novo* sourdoughs
213 propagated only for few fermentation cycles supports the role of insects or flowers in
214 contamination of sourdoughs with competitive bacterial strains.
215 Phylogenetic and genomic analyses of sourdough isolates of *L. reuteri* provided evidence that
216 contamination with microbiota of humans or warm-blooded animals shapes sourdough
217 microbiota (Su et al., 2012; Zheng et al., 2015a). Sourdough isolates of *L. reuteri* are assigned to
218 host-adapted lineages of this species, exhibit lineage-specific physiological traits, and retain the
219 ability to colonize the respective mammalian hosts (Su et al., 2012).
220 **Selection and ecological fitness**
221 The term selection describes the prevalence of organisms with a higher relative ecological fitness
222 over organisms with a lower relative fitness. In relation to the microbial ecology of sourdough,
223 selection results in the prevalence of competitive strains or species over a pool of contaminants
224 with lower ecological fitness (Table 1). Physiological traits that determine the ecological fitness
225 of microbial strains in sourdough were deduced from metabolic properties that differentiate
226 sourdough lactobacilli from other lactic acid bacteria (Gänzle et al., 2007; Vogel et al., 1999).
227 The fitness of specific strains or species was also analysed by competition experiments in back-

228 slopped sourdoughs that were inoculated with defined microbiota (Meroth et al., 2003). The
229 contribution of specific metabolic traits to the ecological fitness of a strain is derived from
230 competition experiments in sourdoughs inoculated with a single strain and isogenic derivatives
231 deficient in the metabolic trait of interest (Lin and Gänzle, 2014b; Su et al., 2011;).
232 The importance of process parameters on the microbial ecology of sourdough was initially
233 proposed by Böcker et al. (1995) and confirmed by Meroth et al. (2003). Short fermentation
234 cycles at ambient temperature select for *L. sanfranciscensis* (Meroth et al., 2003), reflecting the
235 rapid growth of this organism at ambient temperature and moderately acidic pH (Gänzle et al.,
236 1998). Long fermentation cycles at elevated temperatures select for organisms in the *L. reuteri*
237 group (Meroth et al., 2003), reflecting the adaptation of these organisms to warm-blooded
238 animals (Oh et al., 2009) and their high acid resistance (Teixeira et al., 2014). The level of dough
239 hydration also impacts the composition of sourdough microbiota (Di Cagno et al., 2014).
240 The comparison of the microbiota of wheat and rye sourdoughs indicates that the choice of
241 wheat or rye has little influence on the development of sourdough microbiota (De Vuyst et al.,
242 2014, Lin and Gänzle, 2014a; Van der Meulen et al., 2007; Vogel et al., 1999). The use of white
243 flour versus whole flour or bran, however, influences the buffering capacity of the dough as well
244 as the activity of cereal enzymes, and thus affects the ecological fitness of lactic acid bacteria in
245 sourdough (Meroth et al., 2003). Moreover, the use of gluten-free cereals or pseudocereals
246 strongly influences the competitiveness of lactic acid bacteria in sourdough (Lhomme et al.,
247 2014; Moroni et al., 2011). The divergence of microbiota in gluten-free sorghum sourdough was
248 attributed to the presence of antimicrobial phenolic acids, and the lack of maltogenic amylase in
249 many gluten-free cereals (Lin and Gänzle, 2014a; Sekwati Monang et al., 2012). The

concentration of antimicrobial phenolic acids in red or white sorghum is sufficient to inhibit *L. sanfranciscensis* (Sekwati Monang et al., 2012).

Sourdough lactobacilli include organisms with a very broad spectrum of carbohydrate metabolism as well as organisms which ferment only maltose and sucrose (Zheng et al., 2015b). The ability to metabolize mono- or disaccharides other than maltose or sucrose is thus not a prerequisite for ecological fitness in sourdough. The ecological fitness of heterofermentative lactic acid bacteria in sourdough is linked to the effective use of maltose by maltose-phosphorylase, sucrose metabolism, and to the use of electron acceptors to increase the energy yield of heterofermentative metabolism (reviewed by De Vuyst et al., 2009; Gänzle et al., 2007; Gänzle, 2015; Vogel et al., 1999). The role of the growth rate and co-factor regeneration for ecological fitness in type I and type II sourdoughs was demonstrated with isogenic mutants of *L. reuteri* that are defective in glycerol metabolism and exhibit a reduced growth rate in presence of glycerol (Lin and Gänzle, 2014b). Genomic analyses of *L. sanfranciscensis* and *L. reuteri* also support the role of effective maltose utilization for fitness of lactobacilli in sourdough (Vogel et al., 2011; Zheng et al.., 2015a). However, preferential maltose metabolism by maltose phosphorylase, sucrose utilization, and the use of fructose as electron acceptor are frequent metabolic traits of heterofermentative lactobacilli and not restricted to sourdough isolates (Vogel et al., 2011; Zheng et al., 2015b).

Maltose and sucrose metabolism in heterofermentative lactobacilli is not subject to carbon catabolite repression (Teixeira et al., 2013; Vogel et al., 1999) and carbohydrates are preferably transported by facilitated diffusion (Vogel et al., 1999; Vogel et al., 2011; Zheng et al., 2015b). In contrast, maltose and sucrose metabolism in homofermentative lactobacilli including *L. plantarum* is generally subject to carbon catabolite repression, and most carbohydrates are

transported by PTS systems (Gänzle, 2015; Zheng et al., 2015b). Both metabolic strategies support ecological fitness in sourdough fermentations.

Competition experiments with strains of *L. plantarum*, *L. sanfranciscensis*, or *L. reuteri* demonstrated strain-specific differences of the ecological fitness in sourdough fermentations (Lin and Gänzle, 2014a; Minervini et al., 2010; Siragusa et al., 2008). Experimental evidence delineating which metabolic properties are responsible for these strain-specific differences, however, is only beginning to emerge. The production of bacteriocins increased the ecological fitness relative of *Lactococcus lactis* in sourdough (Settani et al., 2005). Bacteriocin production, however, has been described only for few sourdough lactobacilli, suggesting that the production of antimicrobial compounds does not improve fitness relative to other highly adapted strains. A differentiated role for antimicrobial compounds in sourdough has been proposed for reutericyclin, a unique antibiotic produced by sourdough isolates of *L. reuteri* (Lin et al., 2015). Reutericyclin producing strains of *L. reuteri* persisted in an industrial sourdough over a period of 10 years (Gänzle and Vogel 2003), which argues in favour of its contribution to ecological fitness in an environment that is frequently challenged by contaminants. Reutericyclin-producing strains of *L. reuteri*, however, displayed equivalent ecological fitness in competition with isogenic reutericyclin sensitive and non-producing strains (Zheng et al., 2015a). This unexpected result may indicate that the gain in ecological fitness due to reutericyclin production is not greater than the energy investment required for reutericyclin synthesis (Zheng et al., 2015b).

Wheat and rye flours exhibit sufficient proteolytic activity to sustain growth of sourdough microbiota. Accordingly, a majority of sourdough lactic acid bacteria harbour intracellular peptidases but no extracellular proteases (Gänzle et al., 2008, Vogel et al., 2011; Zheng et al., 2015b). The conversion of arginine and glutamate, however, contribute to the strain-specific acid

296 resistance of *L. reuteri* (Teixeira et al., 2015; Su et al., 2011). Remarkably, these metabolic traits
297 mediating acid resistance are generally absent in *L. sanfranciscensis* (Zheng et al., 2015b). Acid
298 resistance mediated by glutamate decarboxylase contributes to the competitiveness of *L. reuteri*
299 in type II sourdoughs propagated with long (2 d) fermentation cycles but has no influence on the
300 competitiveness in type I sourdoughs propagated with short (12 h) fermentation cycles (Lin and
301 Gänzle, 2014b).

302 **Concepts in community assembly and bread quality: is there an association?**

303 Ecological factors influence the composition and function of sourdough microbiota. Most of the
304 metabolic traits that determine competitiveness of lactic acid bacteria also directly contribute to
305 product quality (Table 3). Acidification, leavening, and acetate formation supported by use of
306 electron acceptors are important determinants of bread quality (Gänzle et al., 2007; Hammes and
307 Gänzle, 1998) and generally determine the competitiveness of lactic acid bacteria in sourdough.
308 The formation of exopolysaccharides from sucrose is a metabolic trait that present in the
309 microbial communities of most sourdoughs (Tieking and Gänzle, 2005) and improves the texture
310 and volume of bread (Galle and Arendt, 2014). *L. reuteri* and other representatives of type II
311 sourdough microbiota employ arginine and glutamine conversion for improved acid resistance
312 and competitiveness (Thiele et al., 2002; Lin and Gänzle, 2014a); these metabolic pathways are
313 absent in *L. sanfranciscensis* and most type I sourdough microbiota (Gänzle et al., 2007).
314 Conversely, glutathione reductase is present in *L. sanfranciscensis* but not in organisms of the *L.*
315 *reuteri* group (Jänsch et al., 2007). The expression of peptidases that relate to formation of
316 bioactive peptides, and the conversion of free fatty acids to antifungal hydroxy fatty acids appear
317 to be strain specific traits that are present in type I and type II sourdough microbiota (Black et al.,
318 2013; Rizzello et al., 2008).

319 *L. reuteri* provides an example for the relevance of dispersal and drift on bread quality. All
320 human lineage strains of *L. reuteri* convert glutamine to glutamate while most rodent lineage
321 strains further decarboxylate glutamate to γ-aminobutyrate (Frese et al., 2011). This distribution
322 reflects the role of glutamate decarboxylase to ecological fitness of *L. reuteri* in rodents
323 (Krumbeck et al., 2016). Glutamate accumulation by *L. reuteri* in sourdough improves the
324 savory taste of bread (Zhao et al., 2015); however, because glutamate-decarboxylating rodent
325 lineage and human lineage strains of *L. reuteri* display comparable competitiveness in type I
326 sourdoughs (Lin and Gänzle, 2014a), the random event of contamination from humans or rodents
327 may influence bread quality.

328 **Sourdough microbiota, uniform or diverse? A synthesis.**

329 This communication explored whether concepts in community assembly facilitate the
330 understanding of factors that determine the composition and function of sourdough microbiota.
331 Three of the four processes shaping community assembly, selection, dispersal, and drift, are
332 relevant for the microbial ecology of sourdough while evidence for speciation and diversification
333 remains inconclusive (Table 1, Figure 2). The relative importance of selection and dispersal or
334 drift is dependent on the use of starter cultures, and the number of back-slopping cycles (Figure
335 2). In sourdoughs that are inoculated with starter cultures, spontaneous sourdoughs, and in
336 sourdoughs that are maintained by only a limited number of back-slopping cycles, the number of
337 bacterial generations may not suffice to selection for the most adapted strains; consequently,
338 dispersal and drift are major determinants of sourdough microbiota. These factors result in a very
339 diverse composition of sourdough microbiota (Figure 1A). Conversely, propagation of
340 sourdoughs over extended periods of time that are equivalent to thousands or millions of
341 bacterial generations eliminates the relevance of dispersal limitation and drift. Even unlikely

contamination events are likely to occur if a sourdough is maintained over several decades in the septic bakery environment (Lhomme et al., 2014; Vogel et al., 2011). The composition of microbiota in these sourdoughs is predominantly shaped by the selection of competitive organisms. Selection in sourdoughs that are maintained to achieve dough leavening without baker's yeast consistently favours *L. sanfranciscensis* (Figure 1B). Dispersal and drift thus explain the diversity of sourdough microbiota, which is caused by the diversity of processes and raw materials employed in sourdough fermentation for production of baking improvers. Selection accounts for the uniformity observed in the microbiota of sourdoughs that are maintained to achieve leavening of bread without baker's yeast. Concepts in community assembly thus provide a valuable tool to understand the influence of the technology of sourdough fermentation on microbial ecology and on bread quality.

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559 **Figure legends**

560 **Figure 1.** Graphic representation on typical sourdough lactic acid bacteria.

561 **Panel A. Diversity of sourdough microbiota.** The panel indiscriminately lists species that were
562 isolated from sourdoughs. The font size increases depending on the frequency of isolation;
563 species names are too small to be read if organisms were isolated in 4 of fewer of the 217
564 sourdoughs that were included in the graph. Isolation of one species from one sourdough was
565 counts as one report on its occurrence.

566 **Panel B. Uniformity of type I sourdough microbiota.** The panel is based on the data set as
567 Panel A, however, the following entries were removed: (i) model sourdoughs propagated in the
568 laboratory, (ii) sourdoughs that were not prepared from wheat (*Triticale* spp;) or rye, (iii)
569 sourdoughs without documented use for dough leavening. 119 of 217 sourdoughs represented in
570 Panel A match these criteria. The font size corresponds to the frequency of isolation; species
571 names are too small to be read if organisms were isolated in 3 or fewer of the 119 sourdoughs.
572 Genus names are abbreviated as follows: *A., Acetobacter; Ec., Enterococcusl L., Lactobacillus,*
573 *Lc., Lactococcus; Ln., Leuconostoc, P., Pediococcus, Sc. Streptococcus, T., Tetragenococcus;*
574 *W., Weissella.* The figure does not differentiate between *L. casei* and *L. paracasei*, *L. brevis* and
575 *L. parabrevis*, *L. alimentarius* and *L. paralimentarius,* or *L. plantarum*, *L. pentosus*, and *L.*
576 *paraplantarum*.

577 The frequency of isolations was compiled from data reviewed by, or provided by Böcker et al.,
578 1995; De Vuyst and Neysens, 2005; Sekwati-Monang et al., 2012; Lattanzi et al., 2013; De
579 Vuyst et al., 2014; Lhomme et al., 2015; Liu et al., 2016; and unpublished observations of the
580 authors for 22 sourdoughs. The word clouds were generated and evaluated with the online tools
581 available at http://www.wordle.net and http://worditout.com.

582 **Figure 2.** Effect of the four processes of community assembly, selection, dispersal, drift, and
583 speciation, on the composition of sourdough microbiota, and their effect on the quality of bread
584 Modified from Hammes and Gänzle, 1998.
585

586 **Table 1.** Concepts in community assembly and their relevance for sourdough microbiota

Concepts[1)]	Definition[1)]	Relevance for microbial ecology of sourdough
Diversification (speciation)	Generation of genetic variation	Positive or purifying selection during long-term persistence in back-slopped sourdoughs
Drift	Random changes in community structure	Effect of random events on sourdough microbiota
Dispersal	Spatial movement of organisms across space	Contamination from plant microbiota, animal or human intestinal strains, or environmental organisms
Selection	Changes in community structure caused by differences in fitness	Persistence of individual strains or species based on superior fitness

587 [1)] According to Velland, 2010, and Nemergut et al., 2013

588

589 **Table 2**. Microbiota of *de novo* sourdoughs prepared from white wheat flour and various plant
590 material. Sourdoughs were contaminated as indicated and propagated over 10 fermentation
591 cycles. Fermentation microbiota were characterized by culture-dependent analysis and high
592 resolution melting curve quantitative PCR as described (Lin and Gänzle, 2014)

***De novo* sourdough contaminated with…**[1)]	**First refreshment**[2)]	**10th refreshment**[3)]
Malus domestica flowers	*Lc mesenteroides, L. pentosus, L. graminis*	*L. plantarum, L. rossiae*
Senapis alba flowers	*Gluconobacter cerinus, L. sakei, L. graminis, Lc. mesenteroides*	*L. graminis*
Veronica persica flowers	*L. sanfranciscensis, L. plantarum, G. cerinus L. rossiae*	*L. sanfranciscensis, G. cerinus*
Crataegus monogyna berries	*L. sakei, L. curvatus, L. graminis, Lc. mesenteroides, L. sanfranciscensis*	*L. sanfranciscensis*
Myrtus communis berries	*Lc. holzapfelii, L. mesenteroides, Enterococcus hirae, A. tropicalis*	*L. sanfranciscensis*
Punica granatum fruits	*Lc. holzapfelii, L. plantarum*	*L. plantarum*
mother of natural vinegar	*A. cibinongensis, G. cerinus*	*L. sanfranciscensis*

593 [1)] Sourdoughs were contaminated with 20% of the material indicated at the first fermentation
594 cycle only.

595 [2)] *A., Acetobacter, G., Gluconobacter, L. , Lactobacillus, Lc., Leuconostoc,*

596 [3)] Sourdoughs were propagated using flour, water and a 20% inoculum form the previous cycle
597 with a dough yield of 200, a fermentation time of 2 days, and an incubation temperature of 20-
598 25°C.

599

600 **Table 3.** Effect of metabolic traits of sourdough lactic acid bacteria on their competitiveness in
601 sourdough, and on bread quality.

Metabolic trait	Ecological relevance	Contribution to bread quality	References
Conversion of carbohydrates to organic acids (and CO_2)	Carbohydrate metabolism	Flavour, texture, mould-free shelf life	Gänzle et al., 2007
Exopolysaccharide production from sucrose`	Carbohydrate metabolism; stress resistance	flavour, texture, volume	Gänzle et al., 2007; Galle and Arendt, 2014
Use of electron acceptors (e.g. mannitol, oxygen)	Growth rate of heterofermentative lactics	taste, volume	Gänzle et al., 2007
Arginine conversion to ornithine	Growth and acid resistance	crust odour	Thiele et al., 2002
Glutamine conversion to glutamate	Acid resistance	Umami taste	Zhao et al., 2015
Glutamate conversion to GABA	Acid resistance	functional ingredient	Coda et al., 2010
Metabolism of glutathione and cystine	Oxidative stress	texture, volume, taste	Jänsch et al., 2007
Peptide hydrolysis	Growth, acid resistance	bioactive peptides	Rizello et al., 2008
Conversion of lipids to hydroxy fatty acids	not known	mould free shelf life	Black et al., 2013

602

603