The quest for the perfect loaf of sourdough bread continues:

Novel developments for selection of sourdough starter cultures

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Abstract

Sourdough fermentation, one of the oldest unit operations in food production, is currently experiencing a revival in bread production at the household, artisanal, and the industrial level. The expanding use of sourdough fermentation in bread production and the adaptation of fermentation to large scale industrial bread production also necessitates the development of novel starter cultures. Developments in the last years also have expanded the tools that are used to assess the metabolic potential of specific strains, species or genera of the *Lactobacillaceae* and has identified multiple ecological and metabolic traits as clade-specific. This review aims to provide an overview on the clade-specific metabolic potential of members of the *Lactobacillaceae* for use in sourdough baking, and the impact of these clade-specific traits on bread quality. Emphasis is placed on carbohydrate metabolism, including the conversion of sucrose and starch to soluble polysaccharides, conversion of amino acids, and the metabolism of organic acids. The current state of knowledge to compose multi-strain starter cultures (synthetic microbial communities) that are suitable for back-slopping will also be discussed. Taken together, the communication outlines the current tools for selection of microbes for use in sourdough baking.

Keywords: *Lactobacillus*, *Fructilactobacillus sanfranciscensis*, glutathione, exopolysaccharides, acid resistance, synthetic microbial communities, kokumi.

1. Introduction

Sourdough is a cereal dough used for production of bread, flat bread or steamed bread that includes lactic acid bacteria as major fermentation organisms. Sourdough has been used for more than 14,000 years in bread production (Arranz-Otaegui et al., 2018). The use of back-slopped sourdoughs to produce bread that was very similar to bread we consume today has been documented 2000 years ago (Plinius Major, 74AD) but is likely substantially older. In the 20th century, relatively pure cultures of baker's yeast grown on molasses were introduced as leavening agent (Brandt and Gänzle, 2006). The use of sourdough as leavening agent continued in artisanal baking, and in rye baking to achieve acidification (Brandt and Gänzle, 2006). Industrial equipment for large-scale sourdough production was first installed about 100 years ago in the Soviet Union (Brandt and Gänzle, 2006) and became common in other parts of Central, Northern and Eastern Europe in the second half of the 20th century (Meuser, 1995; Vogel et al., 1999; Wiese et al., 2009).

The microbiology of sourdough has been subject to scientific investigation since the beginning of the 20th century (Holliger, 1902; Kline and Sugihara, 1971; Spicher and Stephan, 1993). The resurgence of the commercial interest in sourdough in wheat and rye baking also has resulted in an increase of publications on sourdough: searching Pubmed with the keyword "sourdough" lists only 39 publications between 1948 and 2000 but 937 publications between 2001 and 2022 (accessed April 11th, 2023). Collectively, this body of literature that accumulated over the past 120 years informs on ecological principles of community assembly in sourdough (Gänzle and Ripari, 2016; Gänzle and Zheng, 2019), describes the nutritional and technological function of sourdough in bread making (Arora et al., 2021; Gänzle, 2014; Gobbetti and Gänzle, 2023; Moroni et al., 2009) and provides reliable data on the microbial composition of more than 700 sourdoughs that are used in bread making at the household level, or in artisanal and industrial bakeries (Arora et al., 2021;

De Vuyst et al., 2023, 2021; Gänzle and Zheng, 2019). The most recent comprehensive compilation of lactobacilli is provided by De Vuyst and co-authors (De Vuyst et al., 2023). With all this information available, what necessitates an update on selection of lactic starter cultures for use in (steamed) bread production? Several developments in recent years fundamentally changed how sourdough is used, and how cultures are selected:

First, the use of sourdough fermentation in baking is changing and expanding. Until the 20th century, the main function of sourdough was its use as leavening agent. The use of sourdough as sole leavening agent dictates fermentation procedures that continuously maintain rapid growth and maximum leavening power of sourdough microbes by frequent back-slopping (Brandt et al., 2023; Gänzle and Ripari, 2016). These sourdoughs almost invariably include *Fructilactobacillus sanfranciscensis* and *Kazachstania humilis* as dominant members of the community of fermentation microbes (De Vuyst et al., 2016; Gänzle and Zheng, 2019). Industrial uses of sourdough for acidification of rye doughs in the 20th century were optimized for high contents of acidity; these fermentation processes select for acid-tolerant fermentation microbes of the genera *Lactobacillus* and *Limosilactobacillus* (Gänzle and Zheng, 2019). In recent years, the use of sourdough by amateur bakers that bake at the household level has increased substantially; this trend was in part fuelled by the COVID-19 pandemic which also limited the availability of baker's yeast for several weeks in spring 2020 ("sourdough - Explore - Google Trends). Baking at the household level is done with diverse fermentation protocols that meet the individuals' needs; correspondingly, sourdoughs maintained by amateur bakers are more diverse than sourdoughs maintained in bakeries (Landis et al., 2021).

An expanded use of sourdough is also observed in industrial bread production. This trend is fuelled by several factors, the need to provide "clean label" or "kitchen friendly" ingredient lists to

consumers (Asioli et al., 2017), and the aim to reduce cost for ingredients by using in-house fermentation know-how. The labels of current commercial white pan breads often list more than a dozen ingredients or additives; the use of sourdough as sole leavening agents allows simplification to include only three ingredients, flour, water and salt. In addition, national nutrition recommendations include breads both as "ultra-processed" foods, which are not recommended for frequent consumption, and as "minimally processed" foods, which are recommended as part of a healthy diet (Anonymous, n.d.; Armet et al., 2022). Sourdough fermentation is recognized as a processing step that replaces ingredients and additives to allow production of "minimally processed" whole grain breads (Gänzle, 2014; Ma et al., 2021).

Second, while conventional bread production continues to use wheat and to a lesser extend rye as sole or major cereal ingredients, flours from other cereals, pseudocereals or pulses are increasingly used in production of specialty bread, in production of gluten free bread, or to up-cycle by-products of food processing such as bran or brewer's spent grains (Katina et al., 2012; Moroni et al., 2009; Neylon et al., 2021; Pontonio et al., 2023). Current knowledge on the presence of lactic acid bacteria in cereal fermentations documents that fermentation parameters rather than the choice of the fermentation substrate impact the selection of starter culture (De Vuyst et al., 2023; Lin and Gänzle, 2014a; Pswarayi and Gänzle, 2022), correspondingly, the choice of the fermentation substrate impacts the selection of starter cultures only in few cases. *Fructilactobacillus sanfranciscensis* occurs in wheat and rye sourdoughs only (De Vuyst et al., 2023; Gänzle and Zheng, 2019). The antimicrobial activity of sorghum phytochemicals selected against *Fl. sanfranciscensis* (Sekwati-Monang et al., 2012); the high content of phenolic compounds in red or brown sorghum varieties attenuate also the growth of other lactobacilli (Pswarayi et al., 2022). As

outlined below, specific substrates may require cultures with specific metabolic traits for improved bread quality.

Third, the expanded use of genome sequence data for the phylogenetic and metabolic characterization of lactobacilli since 2015 has enabled the identification of metabolic and ecological traits of lactobacilli that are specific for specific phylogenetic clades (Duar et al., 2017; Sun et al., 2015; Zheng et al., 2020, 2015). To illustrate, until the 1990ties, sequencing of 16S rRNA genes of a bacterial isolates was a significant achievement. Metabolic traits of sourdough lactobacilli that impact bread quality were largely considered to be species specific (Hammes and Gänzle, 1998). In 2015, genome sequences for most type strains of lactobacilli were available (Sun et al., 2015; Zheng et al., 2015). In 2023, the cost for sequencing of a bacterial genome is about $ 100 (€ 68), and will likely be further reduced to be on par with the cost for Sanger sequencing of 16S rRNA genes. *In silico* genome analysis allows the high-throughput screening of several hundred strains for presence / absence of desirable or undesirable metabolic traits to prioritize few strains for subsequent screening with more labor-intensive baking applications, and has thus become a routine part of experimental designs to understand the function of sourdough microbes in bread production. Current knowledge also provides reasonable confidence to conclude on relevant metabolic traits on the basis of the phylogeny and taxonomic position of isolates (Qiao et al., 2022; Zheng et al., 2020, 2015).

In short, developments in the last decade have altered the selection criteria that are used to select sourdough cultures, and have expanded the tools that are used to assess the metabolic potential of specific strains, species or genera. This review aims to provide an overview on the clade-specific metabolic potential of members of the *Lactobacillaceae*, subsequently termed lactobacilli, which constitute a vast majority of sourdough microbes, for use in sourdough baking.

2. A tale of two tribes: Heterofermentative versus homofermentative lactobacilli.

Phylogeny. Homofermentative and heterofermentative lactobacilli form two distinct phylogenetic clades (Figure 1). Because a single node separates the two groups of organisms, the evolution of heterofermentation was a unique event and all heterofermentative lactobacilli are offspring of a shared common ancestor (Qiao et al., 2022). Homofermentative and heterofermentative lactobacilli co-exist in many of their establishment niches, which was related to resource partitioning (Tannock et al., 2012). This co-existence is characteristic for intestinal microbial communities in mammals (Walter, 2008) or in social bees (Kwong and Moran, 2016). In mice, homofermentative and heterofermentative lactobacilli were shown to co-exist over evolutionary relevant timelines (Lin et al., 2018). Homofermentative and heterofermentative lactobacilli also co-exist in fermented foods such as back-slopped dairy starter cultures that include lactococci and *Leuconostoc* species, spontaneous fermented vegetables, and in sourdoughs (Gänzle, 2022; Van Kerrebroeck et al., 2017).

Metabolism. Homofermentative lactobacilli preferentially use glucose; the use of other carbohydrates is repressed trough carbon catabolite repression (Barrangou et al., 2006; Gänzle, 2015; Monedero et al., 2007). In contrast, many heterofermentative lactobacilli preferentially utilize the disaccharides maltose and sucrose (Gänzle, 2015; Stolz et al., 1993; Teixeira et al., 2013). During growth on glucose as sole carbon source, the ATP yield of homofermentative lactobacilli is twice as high as the ATP yield of heterofermentative lactobacilli (Gänzle, 2015). In wheat and rye doughs, sucrose and maltose are the predominant carbohydrate sources and in initial stages of fermentation, oxygen and fructose, which are used as electron acceptors by heterofermentative lactobacilli, are also abundant (Gänzle, 2014); in these conditions, the energy yield of heterofermentative metabolism is higher (Figure 2) and, correspondingly,

heterofermentative lactobacilli are generally numerically abundant in sourdoughs when compared to homofermentative lactobacilli (Arora et al., 2021; Gänzle and Zheng, 2019; Van Kerrebroeck et al., 2017). The most distinguishing metabolic properties of homofermentative and heterofermentative lactobacilli are phosphofructokinase, the rate limiting enzyme of glycolysis, which is present in most homofermentative lactobacilli but absent in virtually all heterofermentative lactobacilli; the presence of mannitol dehydrogenase in most heterofermentative lactobacilli, and the presence of the two domain acetaldehyde / alcohol dehydrogenase, which converts acetyl-coA to ethanol, in most heterofermentative lactobacilli (Figure 3). Pyruvate formate lyase is exclusive to homofermentative lactobacilli (Figure 3). Mannitol-phosphate dehydrogenase, which enables the use of mannitol as carbon source, is virtually exclusive to homofermentative lactobacilli with *Periweissella* as the only heterofermentative genus that is capable of mannitol utilization (Fanelli et al., 2022) (Figure 3).

Contribution to bread quality. Does the presence of heterofermentative lactobacilli matter for bread quality? The formation of acetic acid from maltose or sucrose contributes to flavor (Hansen and Schieberle, 2005), impacts gluten properties (Clarke et al., 2004) and contributes to inhibition of mold growth (Quattrini et al., 2019) (Table 1). The reduction of oxidized glutathione to monomeric, reduced glutathione impacts gluten properties and loaf volume (Jänsch et al., 2007; Tang et al., 2017) and supports formation of the antifungal coriolic acid from fatty acid peroxides (Black et al., 2013). Last but not least, the reduction of aliphatic aldehydes that originate from lipid oxidation and decisively impact crumb flavor (Czerny and Schieberle, 2002; Hansen and Schieberle, 2005) is dependent on the metabolic activity of heterofermentative lactobacilli (Sugahara et al., 2022; Vermeulen et al., 2007a). The reduction of aldehydes originating from lipid oxidation to alcohols with a much lower impact on flavor is particularly relevant for whole grain

products which include the germ and thus a higher content of free fatty acids. Of note, redox-reactions catalyzed by heterofermentative lactobacilli impact bread quality only if they are metabolically active during the bulk and final proof (Table 1).

3. Traditional sourdough microbes: Host adapted lactobacilli, *Lactobacillus* spp., *Limosilactobacillus* spp. and *Fructilactobacillus sanfranciscensis*.

A majority of sourdoughs that are used in bakeries as sole leavening agent include *Fl. sanfranciscensis* as one of the dominant fermentation microbes (Arora et al., 2021; Gänzle and Zheng, 2019). In sourdoughs that are fermented to achieve high levels of acidity, *Limosilactobacillus* species and *Lactobacillus* species typically dominate (Gänzle and Zheng, 2019). These organisms are host adapted, have a small genome size, and are highly specialized to specific host-adapted niches (Duar et al., 2017; Li et al., 2023; Li and Gänzle, 2020) (Figure 3). The ecological conditions in their respective persistence niches apparently match the conditions in the respective sourdoughs.

What is the specific contribution of *Fl. sanfranciscensis* to bread quality? The organism, which has a genome size of only about 1.3 Mbp, grows fast if sucrose and maltose and other growth factors are present, produces acetic acid if fructose or oxygen are present, and reduces aliphatic aldehydes and glutathione (Vogel et al., 2011). The contribution of *Fl. sanfranciscensis* to CO_2 production in traditional sourdoughs was estimated as 50% while other studies attributed CO_2 production predominantly or exclusively to sourdough yeasts (Brandt et al., 2004; Häggman and Salovaara, 2008a). The presence of this organism in most sourdoughs used a sole leavening agent, and the quality of the breads produced with this organism documents that leavening power is the main selection criterion for selection of sourdough cultures unless baker's yeast is used. To date, *Fl. sanfranciscensis* has not been isolated from any source other than wheat and rye sourdoughs,

and is absent in cereal fermentations that use substrates other than wheat and rye as main ingredients (Moroni et al., 2009; Sekwati-Monang et al., 2012).

Lactobacillus and *Limosilactobacillus* species have slightly larger genome sizes when compared to *Fl. sanfranciscensis* – around 2 Mbp – and thus offer additional metabolic traits to improve bread quality. Species of both genera are fairly acid resistant and are likely to encode for arginine deiminiase, glutaminase, an enzyme that is exclusive to host-adapted lactobacilli, and glutamate decarboxylase (Li and Gänzle, 2020; Teixeira et al., 2014; Zheng et al., 2015) (Figure 3). Acid resistance increases ecological fitness of lactobacilli in the intestine of vertebrates (Krumbeck et al., 2016; Morelli, 2000) (Figure 4). In relation to bread quality, acid resistance mechanisms in lactobacilli provide ornithine, the precursor for the character impact compound of the wheat bread crust (Hansen and Schieberle, 2005; Münch and Schieberle, 1998; Thiele et al., 2002), glutamate, a key umami tastant (Su et al., 2011; Zhao et al., 2015), and γ-aminobutyrate, a bioactive compound (Coda et al., 2010; Stromeck et al., 2011). *Limosilactobacillus* is among the few genera of lactobacilli that harbor one or several γ-glutamyl-cysteine ligases Cgl (Xie and Gänzle, 2021) (Figure 3). In contrast to glutathione synthesis by homofermentative lactobacilli, Cgls of *Lm. reuteri* recognize amino acids other than cysteine as glutamyl acceptor (Pophaly et al., 2012; Xie and Gänzle, 2021). Several of the dipeptides synthesized by Cgls of *Lm. reuteri* are kokumi active, i.e. they interact with Ca-sensing receptors that mediate signal transduction from taste receptors to nerve cells (Brennan et al., 2014; Ohsu et al., 2010). This interaction enhances the sensory perception of umami, salty and sweet tastants (Toelstede et al., 2009; Zhao et al., 2016). Kokumi active γ-glutamyl dipeptides accumulate in sourdoughs fermented with *Lm. reuteri* to kokumi-active concentrations but their role in sensory properties of bread remains unclear (Tang et al., 2017; Yan et al., 2018). *Limosilactobacillus* species also produce exopolysaccharides with

glycosyl hydrolase family 70 enzymes. EPS formation by *Lm. reuteri* contributes to formation of biofilms in the forestomach of rodents (Lin et al., 2018; Sims et al., 2011); their role in bread quality is discussed below.

4. Jack-of-all trades: Nomadic lactobacilli, *Lactiplantibacillus plantarum* and the *Lacticaseibacillus casei* group.

Lactiplantibacillus plantarum has been described as a nomadic organism with the ability to persist in multiple persistence niches including plant-associated habitats and the intestine of insects or vertebrates (Martino et al., 2016). *Lp. plantarum* has been isolated from diverse fermented foods (Gänzle, 2022). Accordingly, the selection of starter cultures for just about any food fermentation can default to *Lp. plantarum* with satisfactory but not exceptional results. *Lp. plantarum* has a relatively large genome size of more than 3 Mbp and a broad metabolic potential for carbohydrate utilization and the metabolism of secondary plant metabolites, particularly phenolic compounds (Gaur and Gänzle, 2023; López de Felipe et al., 2021; Martino et al., 2016; Siezen and van Hylckama Vlieg, 2011) (Figure 5). The core genome of *Lp. plantarum* encodes for a large number of genes coding for antimicrobial resistance, making the species highly resistant to antimicrobial plant metabolites (López de Felipe et al., 2021; Pham et al., 2023; Pswarayi et al., 2022). *Lp. plantarum* has also been reported to synthesize a broad spectrum of antifungal compounds including phenyllactate from phenylalanine (Lavermicocca et al., 2000; Ryan et al., 2009b), 10-hydroxyoleic acid from linoleic acid (Y. Y. Chen et al., 2016) (Figure 3), and cyclic dipeptides (Ryan et al., 2009a). It remains unclear whether the metabolic and ecological traits of *Lp. plantarum* are shared with other lactiplantibacilli as only the closely related *Lp. pentosus* and *Lp. paraplantarum* as well as *Lp. xiangfangensis* were isolated from sourdough (De Vuyst et al., 2023).

Species of the *Lacticaseibacillus casei* group also have relatively large genome size of 2.5 – 3 Mbp and were isolated from diverse fermented foods as well as host-associated niches including the gastrointestinal tract and the human vagina (Wuyts et al., 2017). The *Lc. casei* group is characterized by oxidative stress resistance and metabolism of multiple mono- and disaccharides (Cui and Qu, 2021; Monedero et al., 2007; Wuyts et al., 2017); its potential for metabolism of phenolic compounds is somewhat smaller when compared to *Lp. plantarum* (Fig. 4). *Lc. casei* and *Lc. paracasei* but few other lacticaseibacilli occasionally occur in sourdough (De Vuyst et al., 2023).

Lp. plantarum is often the dominant homofermentative strain in spontaneous (i.e. not controlled by back-slopping or starter cultures) fermentations of cereals including wheat, rye, sorghum, corn, millet, and pseudocereals (Gänzle and Zheng, 2019; Minervini et al., 2015; Moroni et al., 2009; Pswarayi and Gänzle, 2022; Scheirlinck et al., 2008). This dominance is attributable to the stable association of *Lp. plantarum* with plants (Minervini et al., 2015; Pswarayi and Gänzle, 2019; Yu et al., 2020). *Lp. plantarum* is also often found dominant in sourdoughs that are maintained at the household level for extensive baking (Comasio et al., 2020; Landis et al., 2021) but is much less prevalent and less dominant in back-slopped sourdoughs that are maintained in bakeries (Gänzle and Zheng, 2019).

The metabolism of hydroxybenzoic and hydroxycinnamic acids by *Lp. plantarum* has been documented in wheat and rye sourdoughs as well as in multiple other cereal substrates (Gaur and Gänzle, 2023; Ripari et al., 2019) but its consequences for bread quality remain unclear. The activity of glycosyl hydrolases and particularly of β-glucosidases of *Lp. plantarum* converts glycosides of secondary metabolites to the corresponding aglycones (Landete et al., 2014). Some (phospho-) glycosyl hydrolases of lactobacilli and their cognate phosphotransferase systems were

shown to be specific for plant glycosides rather than disaccharides (Theilmann et al., 2017). The deglycosylation detoxifies cyanogenic glycosides in cassava and yams, and vicine and convicine in pulses (Lei et al., 1999; Rizzello et al., 2016). Glycosyl hydrolases of *Lp. plantarum* also contribute to the conversion of soy isoflavones to their corresponding aglycones with higher biological activity (Kim et al., 2010; Lim et al., 2020). Many of the most potent bitter tastants in plants are glycosides (Hald et al., 2019; Yan and Tong, 2023). Sourdough fermentation thus reduces the bitterness of many plant foods. Fermentative debittering is particularly relevant for red sorghum varieties and buckwheat (Campo et al., 2016; Kobue-Lekalake et al., 2007; Wu et al., 2019).

Strains of *Lp. plantarum* are frequently described as antifungal starter cultures (Axel et al., 2017; Garnier et al., 2019; Lavermicocca et al., 2000; Ryan et al., 2009b, 2008). However, the concentration of most antifungal metabolites of *Lp. plantarum* and other antifungal lactobacilli in bread remains far below the minimum inhibitory concentration (Black et al., 2013; Quattrini et al., 2019; Ryan et al., 2009b, 2009a). Correspondingly, the *in situ* inhibition of resistant spoilage organisms, particularly *Penicillium roqueforti*, is rather limited and effective mold inhibition on bread requires the additional use of preservatives or plant-derived antifungals (Gänzle and Gobbetti, 2023; Nionelli et al., 2018; Quattrini et al., 2019; Ryan et al., 2008).

5. The also-rans: *Companilactobacillus, Latilactobacillus, Pediococcus, Furfurilactobacillus, Levilactobacillus, Weissella* and *Leuconostoc*.

Research on the impact of sourdough on bread quality often defaults to the key organisms described above, however, multiple other lactobacilli including species of the homofermentative genera *Companilactobacillus*, *Latilactobacillus* and *Pediococcus*, and species of the heterofermentative genera *Furfurilactobacillus*, *Levilactobacillus*, *Weissella* and *Leuconostoc* are

also frequently present as major or minor components of microbial communities in sourdough (Arora et al., 2021; De Vuyst et al., 2023; Gänzle and Zheng, 2019). An overview of the metabolic potential of these organisms is given in Figures 3 and 5. Of these genera, *Companilactobacillus* and *Furfurilactobacillus* occur only in few other food fermentations and their metabolic potential is largely unexplored when compared to other lactobacilli (Gänzle, 2022, 2015). *Cp. crustorum* has been described as a producer of diacetyl in sourdough when citrate is present (Comasio et al., 2021). It's potential to release phenolic compounds from phenolic acid esters or glycosides rivals or even exceeds that of *Lp. plantarum* (Figure 5) but this potential remains unexploited in baking applications. Of the four *Furfurilactobacillus* species, *Ff. rossiae* and *Ff. milii* are most frequent in sourdoughs (De Vuyst et al., 2023; Simpson et al., 2022). The metabolism of phenolic acids by furfurilactobacilli is well characterized (Gaur et al., 2022, 2020) (Figure 5). In addition, strains of *Ff. milii* and *Ff. rossiae* differ from most other heterofermentative lactobacilli by the absence of mannitol dehydrogenase and the presence of ornithine decarboxylase (De Angelis et al., 2014) (Figure 3). *Ff. rossiae* encodes for the genes for conversion of lactate to 1,2 propanediol, and for conversion of 1,2 propanediol or glycerol to propionic acid and hydroxypropionic acid (Figure 3 and Figure 6); the latter pathway was shown to be operational in *Ff. rossiae* (De Angelis et al., 2014; Liang et al., 2021).

Leuconostoc species and the two *Weissella* species that most frequently occur in sourdough, *W. cibaria* and *W. confusa,* generally encode for one or multiple GH70 family enzymes that synthesize soluble polysaccharides from starch or sucrose (Figure 7). GH70 family enzymes include three distinct enzymatic activities. (i) Glucanotransferases convert starch to a soluble polymer with linear and branched α –(1→4) and α-(1→6) linkages or α –(1→4) and α-(1→3) linkages (Bai et al., 2016; Gangoiti et al., 2018b, 2017). (ii) Glucansucrases that convert sucrose to dextran,

reuteran, alternan, or mutan, polymers with predominantly α-(1→6), α –(1→4) and α-(1→6), alternating α-(1→3) and α-(1→6), or α –(1→3) and α-(1→6) linkages, respectively (Kralj et al., 2005; Meng et al., 2016; Robyt et al., 1974; van Hijum et al., 2006). (iii) Branching sucrases use sucrose and dextran as substrates to introduce α –(1→2) or α –(1→3) branching points on linear α –(1→6) chains (Moulis et al., 2016; Passerini et al., 2015) (Figure 8). Some GH70 family enzymes include two catalytic domains, a dextransucrase and a branching sucrase, which share a single glucan binding domain (Meng et al., 2018; Passerini et al., 2015) (Figure 8). Among these enzymes, glucanotransferases form a distinct phylogenetic clade that forms the evolutionary link to GH13 amylases (Meng et al., 2016)(Figure 8). Branching sucrases form a distinct phylogenetic clade among dextransucrases (Figure 8). The linkage type of the polymers formed by glucansucrases cannot be predicted from the phylogenetic position (Figure 8), it rather depends on the amino acid sequence of the four catalytic domains of GH70 family enzymes (Meng et al., 2016).

Of the GH70 family enzymes, the use of branching sucrases in baking remains unexplored. Glucans including a large proportion of α–(1→3) linkages, however, are not soluble in water (Komatsu et al., 2011) and thus unlikely to be useful in baking applications.

The use of the glucanotransferase GtfB from *Streptococcus thermophilus* in baking increased bread volume and reduced retrogradation of starch during storage of bread (Bıyıklı et al., 2023; Li et al., 2019), providing proof of concept that conversion of starch to a soluble polymer with α-(1→4) and α–(1→6) linkages improves bread quality. The conversion of starch by GtfB expressing lactobacilli during growth in sourdough remains to be explored.

The use of dextran and reuteran producing cultures, specifically *Weissella* spp., *Leuconostoc* spp. and *Lm. reuteri*, to increase the volume of bread or steamed bread and to delay staling of starch

during ageing is extensively documented (Bounaix et al., 2010; X. Y. Chen et al., 2016; Galle et al., 2012a; Galle and Arendt, 2014; Katina et al., 2009; Lin et al., 2019; Muyanja et al., 2003). Glucansucrase activity of lactobacilli, however, not only generates water-binding polysaccharides but also releases fructose, which is converted to mannitol with concomitant formation of acetic acid by most heterofermentative lactobacilli (Figure 2). In wheat baking but not in gluten free breads, the excessive accumulation of acetate in presence of sucrose largely counter-acts any beneficial effect that glucans may have on bread volume and texture (Galle et al., 2012a, 2012b; Kaditzky et al., 2008). The comparison of sourdoughs fermented with *Leuconostoc mesenteroides* and *Weissella cibaria* also revealed that sucrose metabolism by mannitol-dehydrogenase positive heterofermenters in sourdough results an excessive sour taste of bread (Müller et al., 2021). Of those lactobacilli that have been used in sourdough fermentations, dextransucrase activity without mannitol dehydrogenase activity is observed in few strains of *Lactobacillus* and *Ligilactobacillus* species, and in most strains of *W. cibaria* and *W. confusa* (Galle et al., 2010)(Figure 7). *Weissella* species produce up to 20 g dextran / kg (Hu and Gänzle, 2018) and most successful applications of EPS producing lactobacilli employed *Weissella* species (Bounaix et al., 2010; Galle et al., 2012a; Galle and Arendt, 2014; Katina et al., 2009; Lin et al., 2019; Muyanja et al., 2003).

6. The lesser gods: Secondary fermenters, *Paucilactobacillus* spp., *Secundilactobacillus* spp. and *Lentilactobacillus* spp.

The secondary fermenters thrive in hexose-depleted habitats after other lactobacilli or yeasts consumed most fermentable carbohydrates (Zheng et al., 2020). Examples include the spoilage of pickles by *Lentilactobacillus* species (Johanningsmeier and McFeeters, 2015), cheese spoilage by *Paucilactobacillus* species (Oberg et al., 2016) and beer spoilage by *Secundilactobacillus malefermentans* (Russell and Walker, 1953). Typical metabolic traits of these organisms include

the absence of disaccharide hydrolases or phosphorylases and of mannitol dehydrogenase, the presence of the pentose phosphate pathway, and the presence of pathways for lactate conversion to 1,2 propanediol and further to propanoic acid and propanol (Figure 3). Metabolism of pentoses by the pentose phosphate pathway has not been described for any heterofermentative organism; the conversion of lactate and propanediol is well documented (Krooneman et al., 2002; Oude Elferink et al., 2001). Many strains potentially also excel in harvesting energy from esters or glycosides of phenolic compounds (Figure 5) but this activity has not been verified experimentally.

Secondary fermenters of the genera *Lentilactobacillus*, *Secundilactobacillus* and *Paucilactobacillus* are not frequently isolated from sourdoughs but are known to occur occasionally (De Vuyst et al., 2023). What is their specific contribution to bread quality? First, the accumulation of acetic and propionic acids contributes to mold inhibition on bread (C. Zhang et al., 2010). Second, many species grow at 10 °C or below and thus remain metabolically active at refrigeration temperature. Last but not least, the conversion of lactate via 1,2 propanediol to propionate and acetate supports slow but sustained growth and metabolic activity throughout extended incubation times of weeks or even month (Johanningsmeier and McFeeters, 2013; Krooneman et al., 2002; C. Zhang et al., 2010). Other sourdough lactobacilli are metabolically inactive during extended incubation times and the cell counts decrease within days of entry into the stationary phase of growth (Stromeck et al., 2011; Vogel et al., 1999; J. Zhang et al., 2010). Taken together, the use of secondary fermenters may enable the formulation of sourdough starter cultures that retain viability and metabolic activity throughout refrigerated distribution and storage for use without refreshment or extended lag times.

7. The unlikely contenders.

Of the 32 genera of the *Lactobacillaceae,* only 19 are represented by genera that were identified in more than one sourdough (De Vuyst et al., 2023; Landis et al., 2021)(compare Figure 3 and Figure 5). The remaining 19 genera represent mainly highly specialized, insect adapted genera, e.g. *Bombilactobacillus, Apilactobacillus, Nicoliella* and *Convivina*, organisms with unknown ecology that are represented only by one or two species, e.g. *Nicoliellaa, Holzapfeliella, Amylolactobacillus*, *Dellaglioa* and *Acetilactobacillus*, have small genome sizes and likely are also highly specialized to specific persistence niches, and *Liquorilactobacillus* and *Periweissella*, which include predominantly motile strains (Figure 1). Their absence in the more than 1000 sourdoughs for which reliable information on the microbial community is available means that strains of these genera are unlikely contenders for use in sourdough starter cultures.

8. Safety aspects of the selection of starter cultures

The assessment of the qualified presumed safety (QPS) of food and feed cultures by the European Food Safety Authority provides a robust framework to determine the safety of novel starter cultures (Leuschner et al., 2010). Members of the *Lactobacillaceae* are generally not considered a concern for human safety; the most relevant criteria the assessment of their safety are a reliable taxonomic identification, the formation of biogenic amines, and the lack of transferrable antibiotic resistance (EFSA, 2012; Koutsoumanis et al., 2023; Leuschner et al., 2010). The taxonomic identification as well as the determination of the presence of transferrable genes encoding for antibiotic resistance are based on whole genome sequencing (Campedelli et al., 2019; Chun et al., 2018). Few species in the *Lactobacillaceae,* most frequently *Lacticaseibacillus rhamnosus*, have caused infections in critically ill individuals; these rare infections have not questioned, however, the QPS status of *Lc. rhamnosus* (Koutsoumanis et al., 2023, 2020). *Weissella* species are also

considered safe fermentation organisms despite their occasional isolation from infected patients (Kim et al., 2023; Sturino, 2018), however, *Weissella* species have not yet received regulatory approval for commercial use as food or feed cultures.

9. Social aspects of starter selection.

While most experimental sourdoughs are inoculated with only one or two strains of lactobacilli, sourdoughs that are used in bakeries or at the household level are complex communities of microbes that typically include two to six dominant microbes. Successful sourdough starter cultures also include multiple bacterial species which include both homofermentative and heterofermentative lactobacilli. Our understanding of strain properties that matter for stable co-existence with other microbes is still in its infancy. Resource partitioning between homofermentative and heterofermentative lactobacilli as discussed above is likely also of importance for the stable co-existence of these organisms in sourdoughs. A reconstituted microbial community that exhibited functional and compositional stability over multiple back-slopping steps was characterized by stable dominant strains and fluctuating satellite strains, and by transcriptome redundancy (Calabrese et al., 2022). In intestinal communities of microbes, minor constituents specialise on metabolism of substrates that are not available to dominant strains (Brochet et al., 2021); this aspect may also relate to microbial communities in sourdoughs. Until our understanding of the social context of microbial communities improves, the composition of microbial communities can be based on those combinations of species that were frequently identified in the same sourdough. *Lactobacillus* species and *Limosilactobacillus* species flourish in the same persistence niches in intestinal habitats (Duar et al., 2017; Walter, 2008) and in sourdough (Gänzle and Zheng, 2019). These genera share comparable temperature optima and acid resistance (Zheng et al., 2020) but differ with respect to the preferred substrates for

carbohydrate metabolism (see above). In sourdoughs used as leavening agent, a combination of *Fl. sanfranciscensis*, (*Companilactobacillus* or *Lp. plantarum*) and (*Furfurilactobacillus* or *Levilactobacillus*) is frequently found (De Vuyst et al., 2021; Gänzle and Zheng, 2019). *Lp. plantarum* and *Lv. brevis* or *Lm. fermentum* co-exist in many food fermentations including spontaneous sourdoughs (De Vuyst et al., 2023, 2021; Gänzle and Zheng, 2019). The stable co-existence of microbes also depends on the fermentation conditions. Frequent back-slopping selects for fast growing strains, long fermentations times select for fast growing and acid resistant strains and extended storage in the refrigerator likely selects of psychrotropic strains of lactobacilli (Landis et al., 2021; Lin and Gänzle, 2014b; Meroth et al., 2003).

10. To back-slop or not to back-slop: is this a question?

Whether or not the laborious back-slopping of sourdoughs is preferred over the use of dried sourdoughs, or of ready-to-use starter cultures that can be reactivated with only few propagation steps was not a question until baker's yeast became available: Back-slopped sourdoughs leaven bread, spontaneous sourdoughs do not, or leaven to a much lesser extent (Arranz-Otaegui et al., 2018; Brandt et al., 2004; Gänzle and Zheng, 2019; Häggman and Salovaara, 2008b). Accordingly, most sourdoughs used as leavening agent in artisanal bakeries are back-slopped. A long history of back-slopping means that many sourdoughs are older than the bakers that use them. Documented or anecdotal evidence dates multiple sourdoughs to an age from 100 years to more than 150 years of continuous back-slopping (Feinstein, 2022). The observation that sourdough yeasts are domesticated (Bigey et al., 2021) also documents that the use of long-term back-slopped sourdoughs is the default *modus operandi* in traditional sourdough baking.

Whether or not back-slopping of sourdoughs is preferred is also not a question for suppliers to the baking industry: Dried sourdoughs, or active sourdoughs that remain stable only for few

fermentation cycles generate revenue, sourdoughs that are back-slopped perpetually don't. In addition, ready-to-use sourdoughs or sourdough products provide consistent composition and performance (Brandt et al., 2023; Brandt, 2019, 2007). Achieving the same consistent performance with back-slopped sourdough requires substantial expertise in fermentation control, which is no longer present in most (industrial) baking operations.

Whether or not the use of long-term back-slopped sourdoughs remains *de rigueur* as sourdough fermentation is re-introduced into industrial baking is an open question. It is likely that each facility will find its own compromise between the convenience and consistent performance of ready-to-use sourdoughs and the cost-savings of long-term back-slopped sourdoughs (Brandt et al., 2023). Irrespective of the balance that will be struck among different baking facilities, the stability of microbial communities in sourdoughs over multiple back-slopping steps will likely become an important selection criterion for sourdough starter cultures. The difficulties in predicting this stability on the basis of current knowledge was outlined in the preceding section.

11. Conclusions

Sourdough fermentation to produce bread is one of the oldest food fermentations; at the same time, the use of sourdough in baking is an area where current commercial and societal developments open multiple new approaches for use of novel microorganisms and novel fermentation processes in bread production. The demand for gluten-free bread has also introduced a large diversity of non-conventional cereals and pseudocereals in gluten-free and conventional baking. In addition, the tools for selection of microbes for use in sourdough baking are rapidly evolving and sequence-based analyses and *in silico* prediction of the metabolic potential of specific strains or microbial communities are readily available and affordable. Taken together, novel microbes and processes in combination with unconventional substrates and improved high-throughput screening tools

provide substantial room for innovation: the quest for the perfect loaf of sourdough bread continues.

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Figure legends

Figure 1. Phylogenetic tree of type species in the *Lactobacillaceae* that were validly or effectively published in March 2023. The 33 genera in the *Lactobacillaceae* are color coded. Bootstrap support was calculated on the basis of 1000 replicates; values below 80% are indicated. The tree was calculated with methodology described in (Qiao et al., 2022; Zheng et al., 2020, 2015). The genome accession numbers are listed in Table S1, an updated list is available on www.lactobacillus.ualberta.ca.

Figure 2. Metabolism of homofermentative lactobacilli (**Panel A**) and heterofermentative lactobacilli (**Panel B**) in sourdough. ATP generating reactions are shown in red font; ATP consuming reactions in white font on red background; NADH consuming reactions are shown in blue font; NADH generating reactions are shown in white font on blue background. Enzymatic reactions are drawn to show a neutral balance of co-factors and to indicate the ATP yields of the pathways. Homofermentative lactobacilli preferentially use glucose as carbon source; most heterofermentative lactobacilli preferentially utilize sucrose and maltose through disaccharide phosphorylases. In addition, fructose, generated by yeast invertase from sucrose and fructans, and oxygen are abundant in initial stages of fermentation, enabling regeneration of reduced co-factors by mannitol dehydrogenase and NADH-peroxidase, respectively. Drawn according to (Gänzle, 2015) with modifications.

Figure 3. Heatmap showing the proportion of type strains within a genus of the *Lactobacillaceae* encoding for genes that were shown to impact bread quality (Table 1). Type strain genomes of each species were evaluated for the presence of these enzymes by BLAST analysis using available amino acid sequences of characterized enzymes as query (cut-offs: 40 % identity, 70 % coverage). Values are given as % of species per genus. The accession numbers for the query sequences are

shown in Table S2. Proteins are abbreviated as follows: Fba, Fructose-bisphosphate aldolase; Pfk, Phosphofructokinase; Pkl, Phosphoketolase; Adh2, Alcohol dehydrogenase 2; Adh1, two-domain Alcohol dehydrogenase 1 converting acetyl-CoA to ethanol; Mdh, Mannitol dehydrogenase; Mpd, Mannitol phosphate dehydrogenase; Tal, Transaldolase; Tak, Transketolase; LdhD, Lactaldehyde dehydrogenase; PduE and PduD, glycerol dehydratase medium subunits PdeE and PduD; Pfl, Pyruvate formate lyase; ADI Arginine deiminase; OrdC, Ornithine decarboxylase; Gls, Glutaminase; Gab, Glutamate decarboxylase; Cgl, Cystein glutamyl ligase; Gsr, Glutathione reductase; Ldh, linoleate hydratase. Drawn with information and methodology described by (Zheng et al., 2015).

Figure 4. Glutamine metabolism by *Lm. reuteri*. Glutamine or glutamate are imported by GadC1 or GadC2 in antiport with glutamate or GABA, respectively. Glutamate transport selects for uncharged Glu^{+0} even at pH values where Glu^{+1} is much more abundant. Deamidation of glutamine and decarboxylation of glutamate consume one proton each, thus increasing the proton gradient across the membrane. Drawn according to (Teixeira et al., 2014)

Figure 5. Heatmap showing the proportion of type strains of those genera in the *Lactobacillaceae* that are known to occur in sourdough encoding for genes related to metabolism of hydroxycinnamic acids (green), hydroxyl benzoic acids (blue) and flavonoid glycosides (purple). LP-0796, Est_1092 and HceP, hydroxycinnamic acid esterases; Par1, HcrF and HcrB, hydroxycinnamic acid reductases; Pad, hydroxycinnamic acid decarboxylase and VprA, vinyl phenol reductase; TanA and TanB, extra- and intracellular hydroxybenzoic acid esterases; LpdC, hydroxybenzoic acid decarboxylase; Ram1, Ram2, and RamAla, rhamnosidases; rBGLa. β-glucosidase; LcGUS30, β-glucuronidase. Modified from (Gaur and Gänzle, 2023).

Figure 6. Metabolic pathway of lactate and 1,2 propanediol catabolism by lactobacilli. The key enzyme of lactate conversion is lactaldehyde dehydrogenase, which converts lactate to lactaldehyde; the key enzyme of 1,2 propanediol conversion is the diol dehydratase PduCDE, which converts 1,2 propanediol and glycerol to propionaldehyde and β-hydroxypropionaldehyde, respectively. Drawn according to (Gänzle, 2015). See Figure 3 for distribution of these genes among lactobacilli.

Figure 7. Heatmap showing the proportion of type strains within a genus of the *Lactobacillaceae* encoding one or more glycosyl hydrolase family 70 enzymes as well as mannitol dehydrogenase. Representative genomes of each species were evaluated for the presence of these enzymes by BLAST analysis using available amino acid sequences of characterized enzymes as query (cut-offs: 40 % identity, 70 % coverage). Values are given as % of species per genus.

Figure 8. Phylogenetic tree calculated on the basis of aligned amino acid sequences of glycosyl hydrolase family 70 enzymes. The maximum likelihood tree was calculated by IQ-TREE multicore version 2.1.2, using 1000 bootstrap replicates and the JTT matrix-based model. If available, all enzymes are labelled with their respective NCBI accession number. Characterized enzymes used as query sequences to blast against the representative genome of each species are shown in bold letters. Background colors display type of GH70 enzyme according to (Gangoiti et al., 2018a) GtfA (red) = glucansucrases, GtfB (blue), GtfC (green), GtfD (purple) = α-glucanotransferases. Branching sucrases (yellow) were identified by conserved amino acid residues according to (Vuillemin et al., 2016). Where information is available, the outer ring displays the type of polysaccharide product synthesized by the respective enzyme: dextran (blue), reuteran (yellow), mutan (red), alternan (green).

Table 1. Contribution of specific sourdough microbes and their metabolic activities to bread quality. Entries with grey shading require metabolically active lactobacilli during proofing.

Metabolite / metabolic pathway	Impact on quality	Organisms	Enyzmes (reference)
Carbohydrate metabolism			
Formation of lactate	Dough acidification: taste and modulation of the activity of cereal enzymes	All lactobacilli	Phosphofructokinase of phosphoketolase
Formation of acetic acid	Flavor and enhanced mold-free shelf life	Heterofermentative lactobacilli	Mannitol dehydrogenase and two-domain acetaldehyde and alcohol dehydrogenase Ald2
Formation of CO_2	Leavening	Heterofermentative lactobacilli and yeasts	(Brandt et al., 2004; Häggman and Salovaara, 2008a)
Formation of exopolysaccharides	Improved bread volume, texture and reduced staling	*Lm. reuteri, Leuconostoc* spp. and *Weissella* spp.	Glucansucrases (Galle and Arendt, 2014)
Protein and amino acid metabolism			
Release of amino acids from peptides	Taste compounds and flavor precursor compounds	All lactobacilli	Peptidases (Gänzle et al., 2008)
Conversion of glutamine to glutamate	Umami taste	All lactobacilli	Glutaminases, others (Li et al., 2020; Vermeulen et al., 2007b)
Conversion of arginine to ornithine	Precursor to formation of 2-acetyl-2-pyrroline during baking	Species-specific trait of lactobacilli	Arginine deiminase pathway (Thiele et al., 2002; Zheng et al., 2015)
Conversion of glutamate to γ-butyric acid	Bioactive	Strain specific in *Lm. reuteri, Lp. plantarum, Lv. brevis* and others	Glutamate decarboxylase (Su et al., 2011)
Reduction of oxidized glutathione	Reduced gluten quality and bread volume, accelerated degradation of allergens, formation of antifungal coriolic acid	*Fl. sanfranciscensis*	Glutathione reductase (Jänsch et al., 2007)
Formation of γ-glutamyl peptides	Kokumi activity	*Limosilactobacillus* and *Lentilactobacillus* species	γ-Glutamyl-cysteine ligase (Xie and Gänzle, 2021; Yan et al., 2018)
Conversion of amino acids to flavor	Improved flavor	Yeasts	Ehrlich pathway (Dzialo et al., 2017)

volatiles			
Lipid metabolism			
Reduction of aldehydes originating from lipid oxidation to alcohols	Altered flavor	Heterofermentative lactobacilli	Alcohol dehydrogenases (Sugahara et al., 2022; Vermeulen et al., 2007a)
Hydration of unsaturated fatty acids to hydroxyl-fatty acids	Enhanced mold-free shelf life	*Lv. hammesii*, *Lp. plantarum*, others	Linoleate hydratase (Black et al., 2013)
Others			
Conversion of phenolic acids	Flavor, bioavailability of bioactives	*Lp. plantarum*, *Lv. brevis*, yeasts	Hydroxycinnamic acid reductases and decarboxylases (Gaur and Gänzle, 2023)
Ester synthesis	Flavor	Yeasts	(Dzialo et al., 2017)

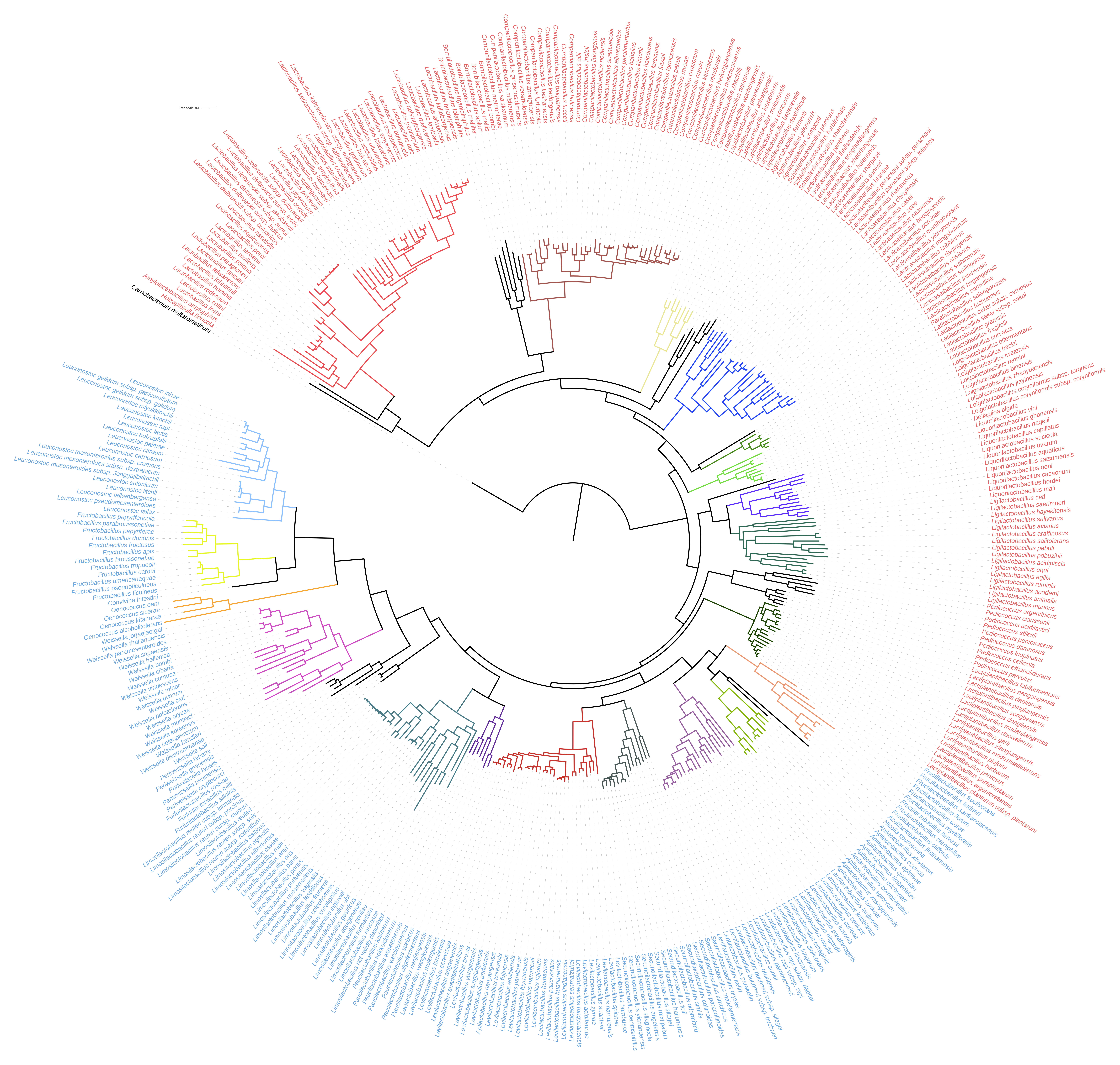
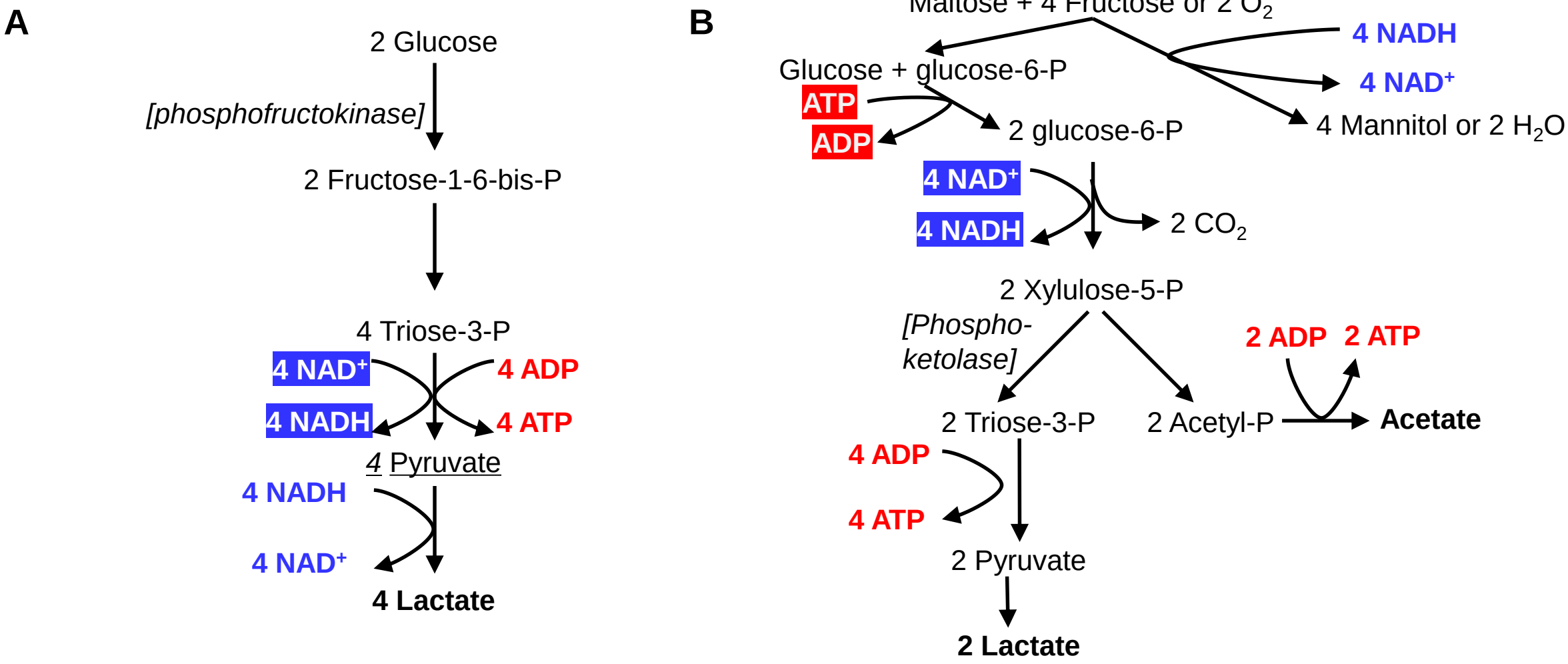

Tree scale: 0.1
Carnobacterium maltaromaticum
Holzapfeliella floricola
Amylolactobacillus amylophilus
Lactobacillus iners
Lactobacillus colini
Lactobacillus rodentium
Lactobacillus hominis
Lactobacillus johnsonii
Lactobacillus taiwanensis
Lactobacillus gasseri
Lactobacillus paragasseri
Lactobacillus psittaci
Lactobacillus mulieris
Lactobacillus jensenii
Lactobacillus porci
Lactobacillus equicursoris
Lactobacillus nasalidis
Lactobacillus delbrueckii subsp. bulgaricus
Lactobacillus delbrueckii subsp. indicus
Lactobacillus delbrueckii subsp. sunkii
Lactobacillus delbrueckii subsp. jakobsenii
Lactobacillus delbrueckii subsp. lactis
Lactobacillus delbrueckii subsp. delbrueckii
Lactobacillus corticis
Lactobacillus gigeriorum
Lactobacillus pasteurii
Lactobacillus xujianguonis
Lactobacillus kalixensis
Lactobacillus hamsteri
Lactobacillus amylolyticus
Lactobacillus intestinalis
Lactobacillus crispatus
Lactobacillus kefiranofaciens subsp. kefirgranum
Lactobacillus kefiranofaciens subsp. kefiranofaciens
Lactobacillus gallinarum
Lactobacillus helveticus
Lactobacillus acidophilus
Lactobacillus ultunensis
Lactobacillus kitasatonis
Lactobacillus amylovorus
Lactobacillus acetotolerans
Lactobacillus bombicola
Lactobacillus apis
Lactobacillus panisapium
Lactobacillus helsingborgensis
Lactobacillus kimbladii
Lactobacillus melliventris
Lactobacillus laiwuensis
Lactobacillus kullabergensis
Bombilactobacillus huangpiensis
Bombilactobacillus folatiphilus
Bombilactobacillus thymidiniphilus
Bombilactobacillus mellifer
Bombilactobacillus apium
Bombilactobacillus mellis
Bombilactobacillus bombi
Companilactobacillus metriopterae
Companilactobacillus salsicarnum
Companilactobacillus mishanensis
Companilactobacillus ginsenosidimutans
Companilactobacillus versmoldensis
Companilactobacillus zhongbaensis
Companilactobacillus furfuricola
Companilactobacillus keshanensis
Companilactobacillus kedongensis
Companilactobacillus baiquanensis
Companilactobacillus tucceti
Companilactobacillus hulinensis
Companilactobacillus allii
Companilactobacillus insicii
Companilactobacillus jidongensis
Companilactobacillus nodensis
Companilactobacillus suantsaicola
Companilactobacillus alimentarius
Companilactobacillus paralimentarius
Companilactobacillus bobalius
Companilactobacillus kimchii
Companilactobacillus halodurans
Companilactobacillus farciminis
Companilactobacillus futsaii
Companilactobacillus formosensis
Companilactobacillus pabuli
Companilactobacillus musae
Companilactobacillus crustorum
Companilactobacillus nuruki
Companilactobacillus kimchiensis
Companilactobacillus mindensis
Companilactobacillus heilongjiangensis
Companilactobacillus huachuanensis
Lapidilactobacillus zhachilii
Lapidilactobacillus nantensis
Lapidilactobacillus wuchangensis
Lapidilactobacillus gannanensis
Lapidilactobacillus achengensis
Lapidilactobacillus luobeiensis
Lapidilactobacillus mulanensis
Lapidilactobacillus concavus
Lapidilactobacillus bayanensis
Lapidilactobacillus dextrinicus
Agrilactobacillus fermenti
Agrilactobacillus yilanensis
Agrilactobacillus composti
Schleiferilactobacillus perolens
Schleiferilactobacillus harbinensis
Schleiferilactobacillus shenzhenensis
Lacticaseibacillus pantheris
Lacticaseibacillus thailandensis
Lacticaseibacillus songhuajiangensis
Lacticaseibacillus zhaodongensis
Lacticaseibacillus hulanensis
Lacticaseibacillus sharpeae
Lacticaseibacillus saniviri
Lacticaseibacillus brantae
Lacticaseibacillus paracasei subsp. paracasei
Lacticaseibacillus paracasei subsp. tolerans
Lacticaseibacillus rhamnosus
Lacticaseibacillus chiayiensis
Lacticaseibacillus casei
Lacticaseibacillus zeae
Lacticaseibacillus nasuensis
Lacticaseibacillus baoqingensis
Lacticaseibacillus porcinae
Lacticaseibacillus manihotivorans
Lacticaseibacillus yichunensis
Lacticaseibacillus mingshuiensis
Lacticaseibacillus kribbianus
Lacticaseibacillus daqingensis
Lacticaseibacillus absianus
Lacticaseibacillus suibinensis
Lacticaseibacillus suilingensis
Lacticaseibacillus jixianensis
Lacticaseibacillus hegangensis
Lacticaseibacillus camelliae
Paralactobacillus selangorensis
Latilactobacillus fuchuensis
Latilactobacillus sakei subsp. carnosus
Latilactobacillus sakei subsp. sakei
Latilactobacillus graminis
Latilactobacillus fragifolii
Latilactobacillus curvatus
Loigolactobacillus bifermentans
Loigolactobacillus backii
Loigolactobacillus iwatensis
Loigolactobacillus rennini
Loigolactobacillus binensis
Loigolactobacillus zhaoyuanensis
Loigolactobacillus jiayinensis
Loigolactobacillus coryniformis subsp. torquens
Loigolactobacillus coryniformis subsp. coryniformis
Dellaglioa algida
Liquorilactobacillus vini
Liquorilactobacillus ghanensis
Liquorilactobacillus nagelii
Liquorilactobacillus capillatus
Liquorilactobacillus sucicola
Liquorilactobacillus uvarum
Liquorilactobacillus aquaticus
Liquorilactobacillus satsumensis
Liquorilactobacillus oeni
Liquorilactobacillus cacaonum
Liquorilactobacillus hordei
Liquorilactobacillus mali
Ligilactobacillus ceti
Ligilactobacillus saerimneri
Ligilactobacillus hayakitensis
Ligilactobacillus salivarius
Ligilactobacillus aviarius
Ligilactobacillus araffinosus
Ligilactobacillus salitolerans
Ligilactobacillus pabuli
Ligilactobacillus pobuzihii
Ligilactobacillus acidipiscis
Ligilactobacillus equi
Ligilactobacillus agilis
Ligilactobacillus ruminis
Ligilactobacillus apodemi
Ligilactobacillus animalis
Ligilactobacillus murinus
Pediococcus argentinicus
Pediococcus claussenii
Pediococcus acidilactici
Pediococcus stilesii
Pediococcus pentosaceus
Pediococcus damnosus
Pediococcus inopinatus
Pediococcus cellicola
Pediococcus ethanolidurans
Pediococcus parvulus
Lactiplantibacillus fabifermentans
Lactiplantibacillus nangangensis
Lactiplantibacillus daoliensis
Lactiplantibacillus pingfangensis
Lactiplantibacillus songbeiensis
Lactiplantibacillus dongliensis
Lactiplantibacillus mudanjiangensis
Lactiplantibacillus daowaiensis
Lactiplantibacillus garii
Lactiplantibacillus xiangfangensis
Lactiplantibacillus modestisalitolerans
Lactiplantibacillus plajomi
Lactiplantibacillus herbarum
Lactiplantibacillus pentosus
Lactiplantibacillus paraplantarum
Lactiplantibacillus argentoratensis
Lactiplantibacillus plantarum subsp. plantarum
Fructilactobacillus fructivorans
Fructilactobacillus lindneri
Fructilactobacillus sanfranciscensis
Fructilactobacillus florum
Fructilactobacillus ixorae
Fructilactobacillus myrtifloralis
Fructilactobacillus hinvesii
Fructilactobacillus carniphilus
Fructilactobacillus cliffordii
Acetilactobacillus jinshanensis
Nicolia spurrieriana
Apilactobacillus xinyiensis
Apilactobacillus ozensis
Apilactobacillus apisilvae
Apilactobacillus quenuiae
Apilactobacillus timberlakei
Apilactobacillus micheneri
Apilactobacillus bombintestini
Apilactobacillus apinorum
Apilactobacillus zhangqiuensis
Apilactobacillus kunkeei
Apilactobacillus laojiaonis
Lentilactobacillus kribbianus
Lentilactobacillus senioris
Lentilactobacillus curieae
Lentilactobacillus kosonis
Lentilactobacillus parafarraginis
Lentilactobacillus hilgardii
Lentilactobacillus raoultii
Lentilactobacillus farraginis
Lentilactobacillus diolivorans
Lentilactobacillus fungorum
Lentilactobacillus kisonensis
Lentilactobacillus rapi subsp. dabitei
Lentilactobacillus rapi subsp. rapi
Lentilactobacillus parabuchneri
Lentilactobacillus sunkii
Lentilactobacillus otakiensis
Lentilactobacillus buchneri subsp. silagei
Lentilactobacillus buchneri subsp. buchneri
Lentilactobacillus parakefiri
Lentilactobacillus kefiri
Lentilactobacillus oryzae
Secundilactobacillus malefermentans
Secundilactobacillus kimchicus
Secundilactobacillus paracollinoides
Secundilactobacillus collinoides
Secundilactobacillus similis
Secundilactobacillus odoratitofui
Secundilactobacillus folii
Secundilactobacillus hailunensis
Secundilactobacillus silagei
Secundilactobacillus mixtipabuli
Secundilactobacillus angelensis
Secundilactobacillus silagincola
Secundilactobacillus yichangensis
Secundilactobacillus pentosiphilus
Secundilactobacillus bambusae
Levilactobacillus spicheri
Levilactobacillus namurensis
Levilactobacillus suantsaii
Levilactobacillus zymae
Levilactobacillus acidifarinae
Levilactobacillus tangyuanensis
Levilactobacillus senmaizukei
Levilactobacillus lindianensis
Levilactobacillus huananensis
Levilactobacillus paucivorans
Levilactobacillus humaensis
Levilactobacillus tujiorum
Levilactobacillus hammesii
Levilactobacillus fuyuanensis
Levilactobacillus parabrevis
Levilactobacillus enshiensis
Levilactobacillus fujinensis
Levilactobacillus koreensis
Apilactobacillus nanyangensis
Levilactobacillus andaensis
Levilactobacillus tongjiangensis
Levilactobacillus yonginensis
Levilactobacillus brevis
Levilactobacillus suantsaiihabitans
Levilactobacillus angrenensis
Levilactobacillus cerevisiae
Levilactobacillus lanxiensis
Levilactobacillus mulengensis
Levilactobacillus wangkuiensis
Levilactobacillus nenjiangensis
Paucilactobacillus oligofermentans
Paucilactobacillus suebicus
Paucilactobacillus vaccinostercus
Paucilactobacillus wasatchensis
Paucilactobacillus hokkaidonensis
Paucilactobacillus kaifaensis
Limosilactobacillus not validly described
Limosilactobacillus mucosae
Limosilactobacillus fermentum
Limosilactobacillus gorillae
Limosilactobacillus equigenerosi
Limosilactobacillus gastricus
Limosilactobacillus alvi
Limosilactobacillus ingluviei
Limosilactobacillus secaliphilus
Limosilactobacillus coleohominis
Limosilactobacillus frumenti
Limosilactobacillus fastidiosus
Limosilactobacillus vaginalis
Limosilactobacillus urinaemulieris
Limosilactobacillus portuensis
Limosilactobacillus pontis
Limosilactobacillus panis
Limosilactobacillus oris
Limosilactobacillus antri
Limosilactobacillus rudii
Limosilactobacillus caviae
Limosilactobacillus albertensis
Limosilactobacillus agrestis
Limosilactobacillus balticus
Limosilactobacillus reuteri subsp. rodentium
Limosilactobacillus reuteri subsp. suis
Limosilactobacillus reuteri
Limosilactobacillus reuteri subsp. murium
Limosilactobacillus reuteri subsp. porcinus
Limosilactobacillus reuteri subsp. kinnaridis
Furfurilactobacillus siliginis
Furfurilactobacillus milii
Furfurilactobacillus rossiae
Periweissella cryptocerci
Periweissella beninensis
Periweissella fabalis
Periweissella ghanensis
Periweissella fabaria
Weissella soli
Weissella diestrammenae
Weissella kandleri
Weissella coleopterorum
Weissella koreensis
Weissella muntiaci
Weissella oryzae
Weissella halotolerans
Weissella ceti
Weissella uvarum
Weissella minor
Weissella viridescens
Weissella confusa
Weissella cibaria
Weissella bombi
Weissella hellenica
Weissella sagaensis
Weissella paramesenteroides
Weissella thailandensis
Weissella jogaejeotgali
Oenococcus alcoholitolerans
Oenococcus kitaharae
Oenococcus sicerae
Oenococcus oeni
Convivina intestini
Fructobacillus ficulneus
Fructobacillus pseudoficulneus
Fructobacillus americanaquae
Fructobacillus cardui
Fructobacillus tropaeoli
Fructobacillus broussonetiae
Fructobacillus apis
Fructobacillus fructosus
Fructobacillus durionis
Fructobacillus papyriferae
Fructobacillus parabroussonetiae
Fructobacillus papyrifericola
Leuconostoc fallax
Leuconostoc pseudomesenteroides
Leuconostoc falkenbergense
Leuconostoc litchii
Leuconostoc suionicum
Leuconostoc mesenteroides subsp. Jonggajibkimchii
Leuconostoc mesenteroides subsp. dextranicum
Leuconostoc mesenteroides subsp. cremoris
Leuconostoc carnosum
Leuconostoc citreum
Leuconostoc palmae
Leuconostoc holzapfelii
Leuconostoc lactis
Leuconostoc rapi
Leuconostoc kimchii
Leuconostoc miyukkimchii
Leuconostoc gelidum subsp. gelidum
Leuconostoc gelidum subsp. gasicomitatum
Leuconostoc inhae

Figure 2.


A
2 Glucose
[phosphofructokinase]
2 Fructose-1-6-bis-P
4 Triose-3-P
4 NAD+
4 ADP
4 NADH
4 ATP
4 Pyruvate
4 NADH
4 NAD+
4 Lactate
B
Maltose + 4 Fructose or 2 O2
4 NADH
4 NAD+
Glucose + glucose-6-P
ATP
ADP
2 glucose-6-P
4 Mannitol or 2 H2O
4 NAD+
4 NADH
2 CO2
2 Xylulose-5-P
[Phospho-
ketolase]
2 ADP
2 ATP
2 Triose-3-P
2 Acetyl-P
Acetate
4 ADP
4 ATP
2 Pyruvate
2 Lactate

Genera

Lactobacillus
Amylolactobacillus
Holzapfeliella
Bombilactobacillus
Companilactobacillus
Lapidilactobacillus
Agrilactobacillus
Schleiferilactobacillus
Lacticaseibacillus
Paralactobacillus
Latilactobacillus
Loigolactobacillus
Dellaglioa
Liquorilactobacillus
Ligilactobacillus
Pediococcus
Lactiplantibacillus
Fructilactobacillus
Acetilactobacillus
Nicoliella
Apilactobacillus
Lentilactobacillus
Secundilactobacillus
Levilactobacillus
Paucilactobacillus
Limosilactobacillus
Furfurilactobacillus
Periweissella
Weissella
Oenococcus
Convivina
Fructobacillus
Leuconostoc

Fba
Pfk
Pkl
Adh2
Adh1
Mdh
Mpd
Tal
Tak
LdhD
PduE
PduD
Pfl
ADI
OrdC
Gls
Gab
Cgl
Gsr
Ldh

Genes

Proportion of genes in genera
100
75
50
25
0

Figure 4

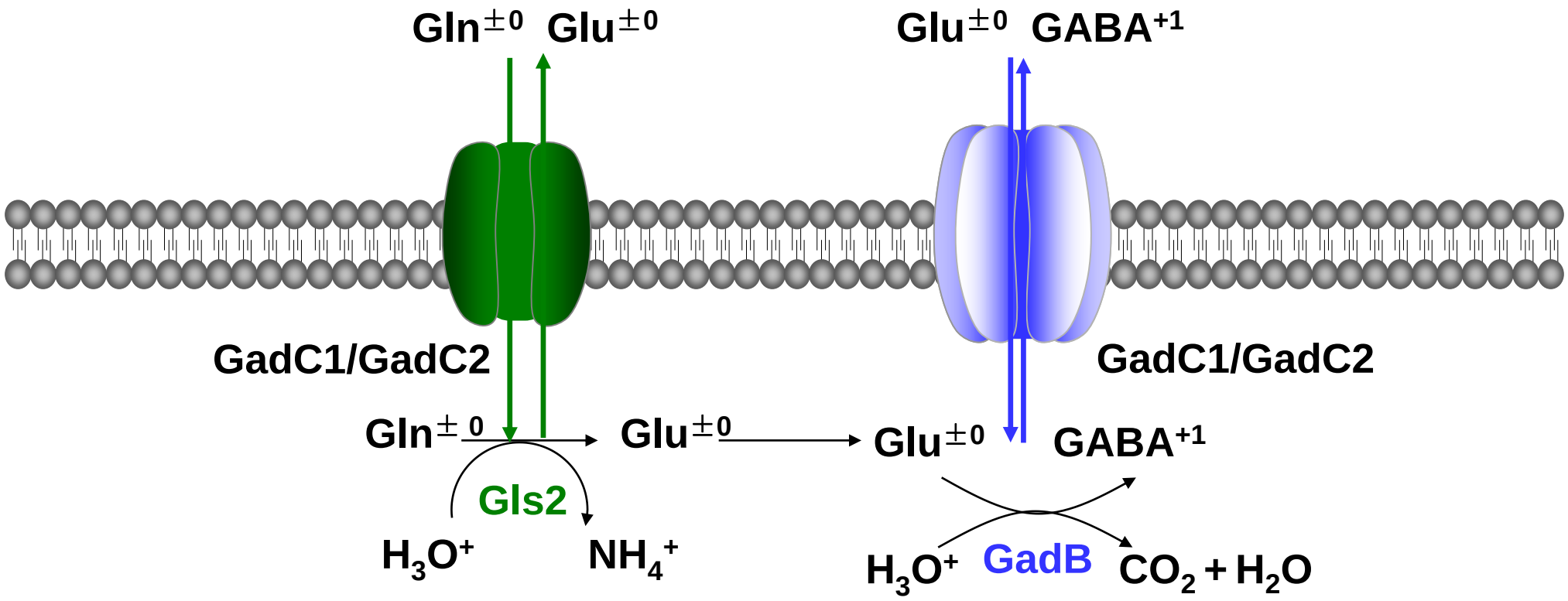
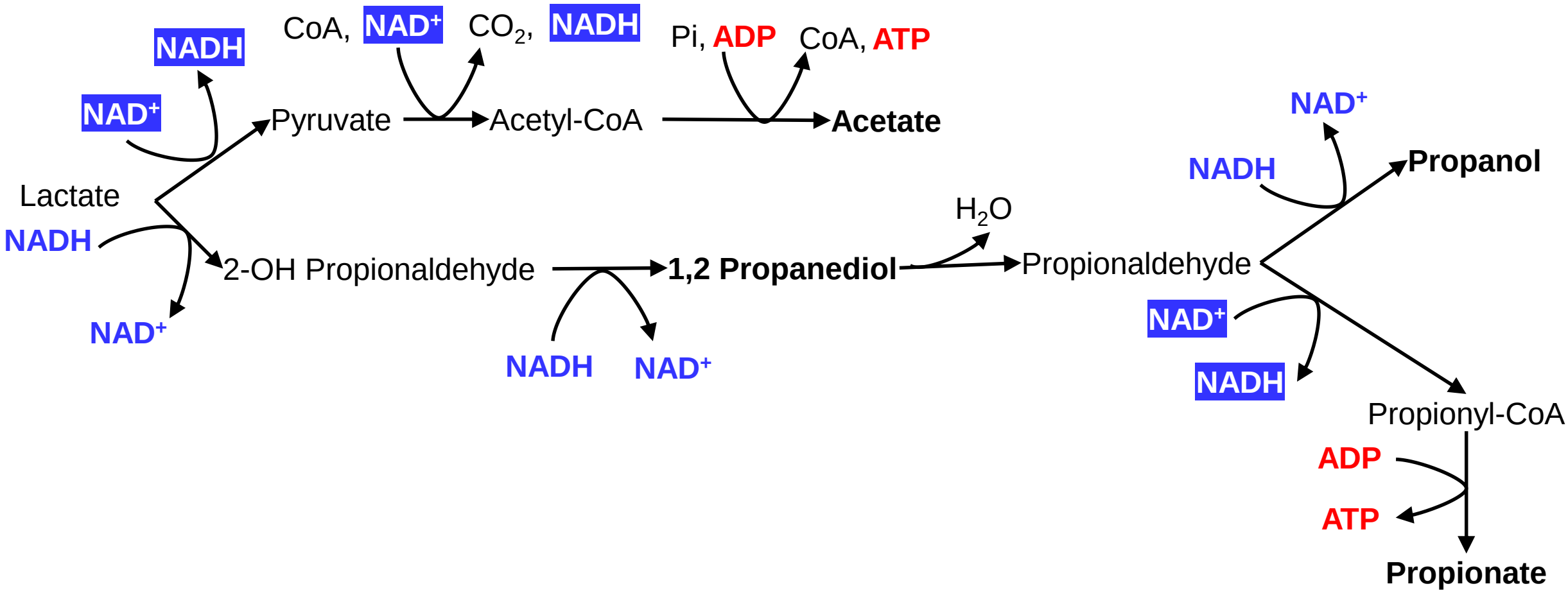

Gln±0
Glu±0
Glu±0
GABA+1
GadC1/GadC2
GadC1/GadC2
Gln±0
Glu±0
Glu±0
GABA+1
Gls2
H3O+
NH4+
H3O+
GadB
CO2 + H2O

Figure 5

Genus name	Hydroxycinnamic acid								Hydroxybenzoic acid			Glycosyl hydrolasaes				
	Lp_0796	Est_1092	HceP	Par1	HcrF	HcrB	Pad	VprA	TanA	TanB	LpdC	Ram1	Ram2	RamALa	rBGLa	LcGUS30
Lactobacillus	0	78	68	44	78	0	12	24	2	0	7	0	2	22	7	0
Companilactobacillus	100	40	60	88	0	26	74	2	0	25	71	0	11	26	100	0
Schleiferilactobacillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Lacticaseibacillus	48	4	0	20	4	0	36	12	0	0	52	0	4	0	56	12
Latilactobacillus	100	0	0	0	0	0	80	0	0	0	20	20	20	0	0	0
Loigolactobacillus	0	11	11	78	0	0	66	44	0	0	66	11	0	22	11	0
Ligilactobacillus	0	20	27	20	13	27	27	13	0	7	7	0	0	0	7	0
Liquorilactobacillus	0	42	8	25	0	33	0	0	0	0	17	0	33	17	17	0
Pediococcus	0	36	36	0	0	0	73	0	0	0	27	45	55	0	0	0
Lactiplantibacillus	100	29	52	82	6	65	100	58	23	64	94	29	47	35	6	0
Fructilactobacillus	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0
Lentilactobacillus	100	0	0	29	0	6	0	0	5	0	11	29	29	0	65	29
Secundilactobacillus	100	16	16	33	67	25	83	58	16	0	58	16	25	0	42	17
Levilactobacillus	95	0	70	0	0	4	83	12	12	62	50	0	16	4	96	25
Paucilactobacillus	100	0	14	0	0	14	71	14	0	0	85	14	14	14	43	43
Limosilactobacillus	68	7	89	0	11	0	36	21	0	4	4	4	4	0	39	18
Furfurilactobacillus	67	0	0	67	0	0	33	33	0	0	0	0	0	0	33	0
Weissella	0	26	0	0	0	0	21	0	0	0	5	0	0	0	5	0
Leuconostoc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0

Figure 6


NADH
NAD+
Lactate
NADH
NAD+
Pyruvate
CoA, NAD+ CO2, NADH
Acetyl-CoA
Pi, ADP CoA, ATP
Acetate
2-OH Propionaldehyde
NADH NAD+
1,2 Propanediol
H2O
Propionaldehyde
NAD+
NADH
Propanol
NAD+
NADH
Propionyl-CoA
ADP
ATP
Propionate

Genus	# of species in genus	Glucanotrans-ferase (starch)	Glucansucrase (sucrose)	Branching sucrase	Mannitol DH
Lactobacillus	41	10	2	2	7
Amylolactobacillus	1	0	0	0	0
Holzapfelia	1	0	0	0	0
Bombilactobacillus	2	0	0	0	0
Companilactobacillus	35	0	0	0	3
Lapidilactobacillus	7	0	0	0	0
Agrilactobacillus	2	0	0	0	50
Schleiferilactobacillus	3	0	0	0	0
Lacticaseibacillus	25	0	0	0	8
Paralactobacillus	1	0	0	0	0
Latilactobacillus	5	0	0	0	0
Loigolactobacillus	9	11	0	11	0
Dellaglioa	1	0	0	0	0
Liquorilactobacillus	12	50	50	50	0
Ligilactobacillus	15	33	33	13	0
Pediococcus	11	18	0	0	0
Lactiplantibacillus	17	12	0	0	0
Fructilactobacillus	4	25	0	0	100
Acetilactobacillus	1	0	0	0	100
Apilactobacillus	7	14	57	14	100
Lentilactobacillus	17	0	0	0	94
Secundilactobacillus	12	0	0	0	58
Levilactobacillus	24	0	0	0	100
Paucilactobacillus	7	0	0	0	0
Limosilactobacillus	28	11	14	7	100
Furfurilactobacillus	3	0	0	0	67
Periweissella	5	0	20	0	60
Weissella	19	5	11	5	53
Oenococcus	4	25	75	50	100
Convivina	1	100	100	100	100
Fructobacillus	5	0	0	0	100
Leuconostoc	16	56	94	50	81

Figure 7

Tree scale: 1

Colored ranges

- GtfA
- GtfB
- GtfC
- GtfD
- Branching sucrases

Outer ring: product

- Dextran
- Reuteran
- Mutan
- Alternan