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1 **Review of lactose and galactose**
2 **metabolism in Lactic Acid Bacteria**
3 **dedicated to expert genomic annotation**
4

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20 **ABSTRACT**

21 Background

22 The term LAB (Lactic Acid Bacteria) relates to the metabolic capabilities of microorganisms
23 to ferment various carbohydrates predominantly into lactic acid. LAB are involved in a large
24 number of industrial food fermentations. Their primary contribution is rapid acid production and
25 acidification of foods. The ability of starter cultures to metabolize lactose and the resulting
26 galactose is therefore a major aspect of starter selection. Genome sequencing and the use of
27 microbial genomics are powerful tools to rapidly screen for functions of interest in the starter
28 culture industry.

29 Scope and approach

30 The annotation of the metabolic pathway encoding genes requires thorough knowledge of
31 the literature and a vast comprehension of the metabolic pathways and of the level of genetic
32 variability that LAB can exhibit. In this review an overview of lactose and galactose metabolism
33 as a basis for expert genomic annotation is presented. It includes a detailed literature overview of
34 the mechanism of internalization, subsequent metabolization of these carbohydrates and the
35 underlying genetics. In addition, a comparative genomic analysis of genes related to the Leloir
36 and Tagatose-6-P pathways is described in 237 LAB and other related Gram-positive genomes
37 with low GC content available in the MicroScope platform.

38 Key findings and Conclusions

39 This analysis delineates the extent of genetic variability that can be expected for future
40 genomic investigation of LAB regarding lactose and galatose metabolism.

41

42 **Keywords:** lactose; galactose; genome annotation; Leloir pathway; Tagatose-6Phosphate
43 pathway; Lactic Acid bacteria

44 **1. Introduction**

45 The term LAB (Lactic Acid Bacteria) relates to the metabolic capabilities of
46 microorganisms to ferment various carbohydrates predominantly into lactic acid (Klaenhammer,
47 Barrangou, Buck, Azcarate-Peril, & Altermann, 2005; Liu, 2003). LAB form a heterogeneous
48 group of microorganisms that belong to the genera *Aerococcus*, *Carnobacterium*, *Enterococcus*,
49 *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*,
50 *Tetragenococcus*, *Vagococcus* and *Weissella* (Axelsson, 2004; Pot, 2008; Stackebrandt &
51 Teuber, 1988; Stiles & Holzapfel, 1997). LAB are naturally found in a variety of natural habitats,
52 including plants, meat and milk environment. They are involved in a large number of industrial
53 food fermentations where they are used as starter and adjunct cultures. Their primary
54 contribution is rapid acid production and acidification of foods, but metabolic processes
55 accompanying the growth of LAB impact also flavor, nutrition, and texture quality of a variety
56 of fermented foods (Axelsson, 2004; Klaenhammer et al., 2005).

57 Lactose is a disaccharide consisting of the combination of two simple sugars, α/β -D-glucose
58 and β -D-galactose, linked by a β (1-4) glycosidic bond and is the main carbon source in milk
59 (Kumar, Weatherly, & Beaman, 1991). Utilization of lactose is mainly related to the
60 fermentation processes by LAB and it is well documented (Cocaign-Bousquet, Garrigues,
61 Loubiere, & Lindley, 1996; Kandler, 1983; Leroy & De Vuyst, 2004; Neves, Pool, Kok, Kuipers,
62 & Santos, 2005; Wu, Cheung, & Shah, 2015). Galactose utilization is important at the industrial
63 level where its accumulation in the product is a major problem for producers as well as for
64 consumers (Wu et al., 2015). Galactose is a carbohydrate not only found in dairy products as a
65 subunit of lactose molecules but also in many environments such as cereals, fruits, legumes, nuts,
66 meats, seeds, and vegetables. It is present as a free form or bound to other carbohydrate units in
67 various glycosidic linkages, such as α -1,6, β -1,3 and β -1,4, and as a component of lipids and
68 proteins in cells (for more informations, review (Acosta & Gross, 1995)). The ability of starter
69 cultures to metabolize lactose and the resulting galactose is therefore a major aspect of starter
70 selection.

71 Thus, whereas the metabolism of lactose is complex, the genetics is well known. The first
72 LAB that was completely sequenced, *Lactococcus lactis* subsp. *lactis* IL1403 came out in 2001
73 (Bolotin et al., 2001). According to ncbi, there are more than 102000 genomes available online

74 in 2017 (www.ncbi.nlm.nih.gov/genome). The study of LAB sequenced bacteria showed that
75 *Lactobacillales* present a diversity in genome size ranging from 1.8 Mbp for *Streptococcus* to 3.7
76 Mpb for *Carnobacterium* (Cailliez-Grimal et al., 2013; Cailliez-Grimal, Afzal, & Revol-Junelles,
77 2014). Some unindustrialized LAB have a high genetic diversity (Rahman et al., 2014, 2016)
78 which suggest high industrial potential.

79 Genome sequencing and the use of microbial genomics are powerful tools for
80 investigation of cellular processes (Koonin, Aravind, & Kondrashov, 2000; Wei et al., 2002).
81 The sequenced and annotated bacterial genomes permit the prediction of certain important
82 characteristics researched by industrials. Thanks to comparative genomics, it became easy to
83 search for metabolic pathways and this opens the possibility to consider the use of genomics to
84 rapidly screen for functions of interest in the starter culture industry. Indeed, *Lactobacillus*
85 *helveticus* CNRZ32 had been discovered to possess homologs of genes involved in the
86 proteolysis of casein found in milk (Broadbent, Hughes, Welker, Tompkins, & Steele, 2013).
87 The research project consists in predicting the formation of metabolic compounds *via* the
88 exploration of the genomic content of CDS (Coding DNA Sequences) in comparison to
89 previously annotated genomes.

90 Although the performance of automatic annotation is constantly improving, functional and
91 relational annotations of genes still require human expertise. This is exemplified with the
92 annotation of genes associated to metabolism, where the pathways are highly interconnected.
93 Therefore, the annotation of metabolic pathways encoding genes requires a thorough dissection
94 of the literature and requires a very good knowledge of the metabolic pathways and of the level
95 of genetic variability that LAB can exhibit.

96 The aim of this review is to give an overview of the available data about lactose and
97 galactose metabolism in order to facilitate expert annotation with a special focus on LAB. To do
98 so, the literature was reviewed as well as the data available in 237 accessible genomes (complete
99 or draft, see Table S1 for accession numbers) of LAB and some Gram-positive bacteria of the
100 low GC subdivision, like *Bacillus*, *Listeria* and *Staphylococcus* (Axelsson, 2004). Genome
101 analyses were mainly based on sequence alignment and synteny analyses which were performed
102 by using the MicroScope platform as previously described (Iskandar et al., 2016; Vallenet et al.,
103 2013).

104 2. Overview of lactose and galactose metabolism

105 After internalization into the cell, galactose or lactose can be metabolized thanks to two
106 pathways: Leloir and Tagatose-6-Phosphate (Tagatose-6P) pathways (Figure 1) (Kandler, 1983).
107 The end-product of the Leloir pathway is Glucose-1P, whereas the Tagatose-6P end products are
108 triose-3 phosphates (Glyceraldehyde-3Phosphate (GPDH) and Dihydroxyacetone Phosphate
109 (DHAP)). All these end products can subsequently enter the glycolysis pathway.

110 The canonical Leloir pathway is encoded by *galPMKTE*. Galactose and lactose are
111 internalized by *galP*. Lactose is then hydrolyzed *via* a β -galactosidase (*lacZ* or *lacLM*) into
112 glucose and β -galactose (Maxwell, Kurahashi, & Kalckar, 1962; Poolman, Royer, Mainzer, &
113 Schmidt, 1989; van Rooijen, van Schalkwijk, & de Vos, 1991). The resulting galactose is
114 subsequently metabolized into glucose-1P by the action of *galM* (galactose mutarotase), *galK*
115 (galactose kinase) and *galT/galE* (galactose-1-phosphate uridylyltransferase, UDP-glucose-4-
116 epimerase). Glucose-1P is then transformed into glucose-6P, an intermediate of the glycolysis,
117 by the action of the phosphoglucomutase (encoded by *pgm*) (Figure 1).

118 In the canonical Tagatose-6P pathway, lactose-6P is hydrolyzed by a phospho- β -
119 galactosidase (encoded by *lacG*) into glucose and galactose-6P. This latter is then transformed
120 into 2 trioses (GPDH and DHAP) *via* a series of 3 reactions encoded by *lacAB* (galactose-6-
121 phosphate isomerase), *lacC* (tagatose-6-phosphate kinase) and *lacD* (tagatose-1,6-diphosphate
122 aldolase) (Figure 1).

123 Not all LAB are able to metabolize galactose. Indeed, although some strains of
124 *Streptococcus thermophilus* are described to exhibit an active Leloir pathway (Anbukkarasi et
125 al., 2014), strains of this species are generally known to partially ferment lactose. Thus,
126 following lactose cleavage by the β -galactosidase encoded by *lacLM*, galactose negative *S.*
127 *thermophilus* strains metabolize the glucose moiety, and excrete galactose in the medium thanks
128 to the antiport permease *lacS*, which allows lactose/galactose exchange (Hutkins, Morris, &
129 McKay, 1985; Poolman, 1993) (Figure 1). Similarly, *Lactobacillus delbrueckii* ATCC 11482 and
130 BAA-365 strains carry putative *galP*, *lacLM* putative genes (Table 1), and metabolize the
131 glucose and release galactose in the extracellular medium (Crow & Thomas, 1984; Kafsi et al.,
132 2014; Leong-Morgenthaler, Zwahlen, & Hottinger, 1991).

133 3. Detailed view of lactose and galactose metabolism

134 3.1.Transport

135 Lactose and galactose can enter the cells *via* either permeases encoded by *galP* or
136 phosphotranferases systems encoded by *lacFE* (Figure 1). In general, permeases are non-specific
137 transport systems (Forrest, Krämer, & Ziegler, 2011; Yan, 2013) except for *lacS* (lactose
138 permease) found in *Streptococcus thermophilus* (Poolman et al., 1989).

139 In the Leloir pathway, the galactose can enter the cell *via* the permease encoded by
140 *galP*, and the lactose *via* a permease encoded by *galP* or *lacS* (Fortina, Ricci, Mora,
141 Guglielmetti, & Manachini, 2003; Leong-Morgenthaler et al., 1991; Neves et al., 2010).
142 However, a putative *galP* gene is not always present in all genomes possessing the Leloir
143 pathway (Table 1). However, the absence of the gene *galP* does not necessarily imply a
144 deficiency in lactose/galactose use. Indeed, *Lactococcus lactis* NZ9000 inactivated in the gene
145 *galP* internalizes galactose *via* an unknown PTS system. The resulting intracellular galactose-6P
146 is subsequently dephosphorylated thanks to an unknown phosphatase, and the produced
147 galactose enters the Leloir pathway (Neves et al., 2010) (Figure 1). Similarly, the genome of
148 *Streptococcus mutans* (holding Leloir and Tagatose-6P pathways) lacks *galP* responsible for
149 galactose uptake (Zeng, Das, & Burne, 2010). Instead, it would encode 14 putative PTS systems,
150 of which one is specific for lactose (Ajdic & Ferretti, 1998). Although there is no PTS system
151 specific for galactose in *S. mutans*, some data suggest that it can be transported by the lactose
152 PTS with low affinity (Abranches, Chen, & Burne, 2004; Zeng et al., 2010).

153 On the other hand, in the Tagatose-6P pathway a PTS transporter, encoded by the genes
154 *lacFE*, transports lactose and galactose. By contrast with the permease transport system,
155 internalization through the PTS system leads to the phosphorylation of lactose and galactose
156 (Abranches et al., 2004; Zeng et al., 2010; Zeng, Martino, & Burne, 2012). The PTS system is
157 mainly encoded by *lacFE*, which can be duplicated in some cases (Table 1). It can be noticed
158 that some strains would not contain *lacFE* homologues, as exemplified by *Pediococcus*
159 *acidilactici* DSM20284, *S. agalactiae* A909 and *S. agalactiae* 2603 V/R (Table 1). It can be
160 hypothesized that for these strains lactose or galactose may enter the cell through other PTS
161 systems. Indeed, in general PTS are described as specific for one given carbohydrate. However,
162 in some cases one PTS system can have the ability to transport another carbohydrate but with

163 lower affinity (Solopova et al., 2012). Thus, in *Streptococcus gordonii* DL1 galactose can enter
164 the cell *via* PTS^{Lac}, PTS^{Man} or *via* PTS^{Gal} (Zeng et al., 2012).

165 It is generally accepted that internalisation of galactose through one transport system
166 orientate the carbon flux towards one specific pathway. Thus, galactose internalization thanks to
167 a PTS system, which lead to galactose phosphorylation, is linked to the Tagatose-6P pathway,
168 whereas galactose internalization through a permease system, is linked to the Leloir pathway.

169 3.2. Beta-galactosidases

170 Lactose or phosphorylated lactose, depending on which transporter internalized lactose
171 into the cell, prior catabolism through the Leloir or the Tagatose-6P pathway, require hydrolysis
172 by the action of a β -galactosidase or phospho β -galactosidase, respectively (Figure 1). In the
173 Leloir and Tagatose-6P pathways, these enzymes are encoded by *lacZ/lacLM* and *lacG*,
174 respectively (Premi, Sandine, & Elliker, 1972). These two hydrolases belong to the large family
175 of glycosyl hydrolases. Glycosyl hydrolases are divided into 45 families with 52 E.C. entries,
176 according to an amino acid sequence comparison of 482 sequences (Henrissat, 1991; Henrissat &
177 Bairoch, 1993). One given bacterium may contain several types of glycosyl hydrolases allowing
178 to utilize different types of carbohydrates. Four of these 45 families (1, 2, 35 and 42) contain
179 enzymes with β -galactosidase activity. The P- β -galactosidase *lacG* is a family 1 member, and
180 the β -galactosidase *lacZ* is a family 2 member. It can be noticed that β -galactosidase can be
181 produced extracellularly. Indeed, in *S. pneumoniae*, BgaA (β -1,4-galactosidase) and BgaC (β -
182 1,3-galactosidase) are cell surface enzymes. They catalyze the hydrolysis of extracellular
183 galactose-containing glycans, allowing to release free galactose which can subsequently enter
184 the cell for catabolism *via* the Tagatose-6P or the Leloir pathways (Jeong et al., 2009; Paixão et
185 al., 2015; Zähler & Hakenbeck, 2000) (Figure 1, Table 1).

186 Putative *lacG* genes are found in all genomes possessing the Tagatose-6P pathway (Table 1).
187 Accordingly, the inactivation of *lacG* gene in *S. gordonii* DL1 leads to the inability of the strain
188 to use lactose, whereas it is able to grow with galactose as carbohydrate source (Zeng et al.,
189 2012). By contrast, putative *lacZ/lacLM* genes are not always found in all genome possessing the
190 Leloir pathway suggesting the use of this pathway for galactose metabolism (Table 1). Indeed,
191 inactivation of *lacZ/lacLM* in *Streptococcus salivarius* 57.I leads to the inability of the strain to
192 use lactose while galactose can be metabolized, indicating that intracellular β -galactosidase is

193 essential for lactose metabolism (Chen, Betzenhauser, Snyder, & Burne, 2002). Among the 146
194 strains carrying putative Leloir pathway genes alone or in combination with the Tagatose-6P
195 pathway genes, less than 40 strains possess homologue of *LacZ/LM* of *S. thermophilus*. Even if
196 in these strains other β -galactosidases encoding genes can also be found in the vicinity of the
197 Leloir pathway encoding genes, it does not imply that they are able to use lactose as substrate.
198 Indeed, in *C maltaromaticum* 3.18, *Carnobacterium* sp. 17.4 and AT7 and *Enterococcus*
199 *casseliflavus* ATCC12755 putative genes encoding β -galactosidases (*bgaB* and *bgaC*) are
200 clustered with the genes putatively encoding the Leloir pathways (Figure 2A). These β -
201 galactosidases would not be able to hydrolyse lactose, instead they would use other galactose-
202 containing carbohydrate polymers (Coombs & Brenchley, 1999, 2001).

203 **3.3.Leloir pathway**

204 **3.3.1. *galM***

205 Almost all genomes studied that possess the Leloir pathway genes, present alone or in
206 combination with the Tagatose- 6P pathway genes, carry one putative *galM* gene (Table 1). The
207 gene *galM* encodes a galactose mutarotase, which is not essential because its mutation does not
208 affect the growth rate. It promotes the isomerization of β -galactose into α -galactose and the
209 isomerization can take place spontaneously (Neves et al., 2010). It can be noticed that *galM* is
210 present in strains without other *gal* and *lac* genes.

211 **3.3.2. *galK***

212 The galactokinase encoded by *galK* phosphorylates α -D-galactose resulting in
213 galactose-1P. Putative *galK* gene is essential and is found in all genome holding the Leloir
214 pathway, and not present in all genome without this pathway (Table 1). Indeed, the deletion of
215 *galK* impairs severely the ability of the strain *S. mutans* UA159 to metabolize lactose and
216 galactose (Abranches et al., 2004).

217 **3.3.3. *galT***

218 The galactose-1-phosphate uridylyltransferase encoded by *galT* convert galactose-1P to
219 glucose-1P. The gene *galT* is essential and is found in all genome holding the Leloir pathway,
220 and not present in all genome without this pathway (Table 1). Indeed, a *Lactococcus lactis*

221 NCD02054 *galT* mutant accumulates galactose-1P, which leads to the inability to grow on
222 lactose and galactose (Vaughan, Pridmore, & Mollet, 1998).

223 **3.3.4. *galE***

224 UDP-galactose 4-epimerase (*galE*) plays a role in the last step of the Leloir pathway. It
225 is an essential enzyme and homologues are present in all genomes predicted to encode the Leloir
226 pathway (Table 1). However, it can be present in bacteria without the Leloir pathway as well,
227 indeed this enzyme is also involved in other metabolic functions such as exopolysaccharide
228 synthesis (Grossiord, Luesink, Vaughan, Arnaud, & de Vos, 2003) (Figure 1).

229 **3.3.5. Regulation of the Leloir pathway genes**

230 The regulation gene *galR* encodes an activator of the *galKTE* and *lacSZLM* operons, and
231 a negative regulator of its own expression. The gene *galR* is generally present in genomes
232 holding the Leloir pathway (Table 1), except for *Bacillus subtilis* 168 and *S. gordonii* CH1
233 which are unable to utilize lactose and galactose *via* the Leloir pathways (Krispin &
234 Allmansberger, 1998; Zeng et al., 2012). However, the presence an intact copy the gene *galR*
235 does not necessarily lead to a galactose fermenting phenotype. Indeed, while some strains of *S.*
236 *thermophilus* possess all the genes necessary to build an active Leloir pathway, they are unable
237 to ferment galactose. These genes are silent thanks to the presence of a nucleotide
238 substitution in the *galK* or *galR* promoter region (Anbukkarasi et al., 2014; Vaughan, van den
239 Bogaard, Catzeddu, Kuipers, & de Vos, 2001).

240 The genes encoding the Leloir pathway are subjected to catabolic repression and are
241 regulated by CcpA which binds to catabolic-responsive elements (*cre*) sites in the promoter
242 region of the Leloir genes (Carvalho, Kloosterman, Kuipers, & Neves, 2011).

243 **3.4. Tagatose-6-phosphate pathway**

244 **3.4.1. *lacAB***

245 Galactose-6P isomerase encoded by *lacAB* transforms galactose-6P into tagatose-6P.
246 This enzyme is essential since the inactivation of these genes leads to the inability to grow on
247 lactose and galactose containing medium and leads to the accumulation of galactose-6P
248 (Abranches et al., 2004; Zeng et al., 2010, 2012). Therefore, it can be speculated that genomes
249 without *lacAB* homologue does not have an active Tagatose-6P pathway (Table 1).

250 **3.4.2. *lacC***

251 The tagatose-6-phosphate kinase, encoded by *lacC*, phosphorylates the tagatose-6P in
252 order to form tagatose-1,6diP. It is generally present in the genomes possessing the other genes
253 encoding the Tagatose-6P pathway except in *Lactobacillus johnsonii* FI9785, *Lactobacillus*
254 *murinus* ASF361 and *Pediococcus acidilactici* DSM20284 (Table 1). Inactivation of *lacC* does
255 not impair growth in medium containing lactose and/or galactose as a source of carbohydrate
256 (Abranches et al., 2004; Zeng et al., 2010, 2012). *LacC* belongs to the pfkB family of
257 carbohydrate kinases, which includes ribokinase (RbsK) and 1-phosphofructokinase (FruK)
258 (Bork, Sander, & Valencia, 1993). It may present an enzymatic activity on fructose-6P when it is
259 present in the medium (Bissett & Anderson, 1980). This might explain the presence of *lacC* in
260 several copies in genomes predicted to encode the Tagatose-6P pathway as well as in five other
261 genomes where an incomplete Tagatose-6P pathway was predicted.

262 **3.4.3. *lacD***

263 The tagatose 1,6-diphosphate aldolase, encoded by *lacD*, is essential and promotes the
264 cleavage of Tagatose-1,6diP into 2 trioses. The *lacD* mutation causes the accumulation of
265 Tagatose-1,6diP in the medium, which leads to the inhibition of growth of the cells (Abranches
266 et al., 2004; Zeng et al., 2010, 2012). Importantly, the tagatose 1,6 diP aldolase may play a role
267 in the cleavage of Fructose-1,6diP (Bissett & Anderson, 1980) which could explain the fact that
268 *lacD* can be found in genomes where an incomplete Tagatose-6P pathway was predicted (Table
269 1).

270 **3.4.4. Regulation of the Tagatose 6-P**

271 The gene *lacR* is present in all genomes possessing the Tagatose-6P pathway and
272 encode a transcriptional repressor that binds the promoter region upstream *lacA*. It was
273 suggested that its repression activity is alleviated when galactose-6P and tagatose-6P binds LacR
274 (Oskouian & Stewart, 1990; van Rooijen, Dechering, Niek, Wilmink, & de Vos, 1993; Zeng et
275 al., 2010).

276 The Tagatose-6P genes are also regulated by *lacT*, which is present in some strains
277 holding the Tagatose-6P pathway, and encodes a transcriptional antiterminator protein present
278 within the *lac* operon of many LAB, that may play a role in the regulation down-stream *lacFEG*

279 expression (Richards, Choi, Bitar, Gurjar, & Stanhope, 2013; Zeng et al., 2012). By binding the
280 mRNA transcript, LacT prevent the formation of a terminator and thus allows the RNA
281 polymerase to transcribe the downstream genes (Declerck, Vincent, Hoh, Aymerich, & van
282 Tilbeurgh, 1999). The antitermination activity of LacT is modulated by PTS dependant
283 phosphorylation : when lactose or galactose PTS permease are engaged in sugar transport, LacT
284 would be dephosphorylated, and would exert its antitermination activity (van Tilbeurgh &
285 Declerck, 2001).

286 **4. Distribution of the Leloir and the Tagatose-6P pathways at the genus and species** 287 **levels**

288 The contribution of the Leloir and the Tagatose-6P pathways to lactose and galactose
289 metabolism was investigated at the genus and at the species levels (Table 1, Figure 3 A-B). In
290 this analysis, each genus was represented by at least 3 species, and each species was represented
291 by at least 8 strains. A genome is considered as a Leloir or Tagatose-6P pathway holder, if it
292 possesses at least all the essential genes of one or the other pathway.

293 The analysis of presence and number of putative genes from Leloir (*gal* genes) or
294 Tagatose-6P (*lac* genes) pathways indicated that, among the 237 strains, 4 different groups can
295 be defined (Table 1): strains holding only the Leloir pathway genes (31%), strains holding only
296 Tagatose-6P pathway genes (14%), strains holding both pathways genes (31%) and strains
297 holding no pathway (24%).

298 Putative *gal* genes are presents in 72 strains holding the Leloir pathway alone and in 74
299 strains possessing Leloir and Tagatose-6P pathways (Table 1). Some strains can exhibit a partial
300 Tagatose-6P pathway as exemplified by *C. maltaromaticum* 3.18 which lack a *lacB* homologue.
301 It can be noticed that these *lac* homologues are dispersed in the genome and are likey involved in
302 other cellular processes than lactose and galactose metabolism (Iskandar et al., 2016).

303 The proportion of bacteria predicted to have the Leloir pathway and/or the Tagatose-6P
304 pathways is very high, of at least 80%. This is contrasting with *Listeria* where 15% of the
305 genomes analyzed within this genus were predicted to encode a functional Leloir pathway and
306 none were predicted to encode a functional Tagatose-6P pathway. This is in agreement with the
307 idea that galactose and lactose metabolism is an important component of the ecology of these

308 bacteria. However, a high heterogeneity was recorded within each genome. For instance, the
309 *Lactobacillus* genus contains bacteria that would exhibit either the Leloir pathway without the
310 Tagatose-6P, the Leloir pathway in combination with the Tagatose-6P pathway, or none of these
311 two pathways.

312 The Leloir pathway is in general highly represented within a given genus. On the other
313 hand, the Tagatose-6P pathway is less well represented since it was not found in the genera
314 *Leuconostoc*, *Listeria*, and *Weissella*. The concomitant presence of Leloir and Tagatose-6P
315 pathways is present in 4 different genus, *Enterococcus*, *Lactobacillus*, *Lactococcus* and
316 *Streptococcus*. The Tagatose-6P pathway is less well represented without the genes encoding the
317 Leloir pathway as it is indeed represented in approximately 20% of *Carnobacterium*,
318 *Lactococcus*, and *Streptococcus* genomes that do not exhibit a functional Leloir pathway (Figure
319 3.A). At the species level, the genetic profiles are rather homogenous (Figure 3.B).
320 Approximately half (5/9) of the species studied present only one profile, *i.e.* all the strains of the
321 2 species *Enterococcus faecalis* and *Streptococcus suis* are predicted to encode both pathways,
322 all the strains of the 2 species *Staphylococcus aureus* and *S. pyogenes* would encode the
323 Tagatose-6P pathway and not the Leloir pathway, and all the strains of the species *Listeria*
324 *monocytogenes* would exhibit both pathways. One third (3/9) of the species show 2 different
325 profiles (*Enterococcus faecium*, *Lactobacillus rhamnosus*, *Streptococcus sanguinis*), some
326 strains of the same species possessing only Leloir pathway and the other the two pathways
327 together. *Carnobacterium maltaromaticum* is the species exhibiting the highest intraspecific
328 variability: some strains would possess the Leloir pathway, some others the Tagatose-6P
329 pathway, one strain hold the *lac* and *gal* genes and some none of these pathways (Figure 3-B).
330 This high intraspecific variability is in agreement with the high genetic diversity observed within
331 the population of this species by MLST analysis (Rahman et al., 2014).

332 **5. Organization of *lac* and *gal* genes**

333 Lactose and galactose utilization by LAB revealed various combinations of the *gal* and
334 *lac* genes (Breidt, Hengstenberg, Finkeldei, & Stewart, 1987; Chassy & Thompson, 1983;
335 Grossiord, Vaughan, Luesink, & de Vos, 1998; Iskandar et al., 2016; Loughman & Caparon,
336 2007; Rosey & Stewart, 1992). While the *gal* genes are usually located on the chromosome, *lac*

337 genes of the Tagatose-6P pathway may be plasmidic or chromosomal. Although genes present
338 high homology and conserved genomic synteny, the co-localizations are not always the same.
339 The figures 2 A-B-C show the possible arrangements between the different genera and species.

340 **6. Function of Leloir and Tagatose-6-P associated genes**

341 Diversity within genomes can be relied to the functionality of each pathway in the cell.
342 In fact, these two pathways play different roles in lactose and galactose metabolism.

343 The catabolysis of lactose and galactose *via* the Leloir pathway is described for
344 *Lactococcus garviae* ATCC 49156, *L. lactis* IL 1403, *L. lactis* SK11, *L. lactis* MG1363 (Fortina,
345 Ricci, & Borgo, 2009), *L. lactis* ATCC7962 (Vaughan et al., 1998), *Lactobacillus helveticus*
346 ATCC 15009^T (Fortina et al., 2003), *S. thermophilus* (Anbukkarasi et al., 2014) (Table 1). By
347 contrast, in strains of *Lactobacillus casei* (Bettenbrock, Siebers, Ehrenreich, & Alpert, 1999),
348 *Lactobacillus rhamnosus* (Tsai & Lin, 2006), *L. lactis* (Siezen et al., 2005), *S. mutans*
349 (Jagusztyn-Krynicka et al., 1992) (Table 1) lactose and galactose are catabolysed thanks to a
350 Tagatose-6P pathway encoded by one *lac* gene cluster. In the strain *S. gordonii* CH1, the *lac*
351 cluster is present in two copies, each cluster being specialized in the metabolism of one given
352 carbohydrate: one cluster allows lactose utilization whereas the other permits the galactose
353 metabolism (Zeng et al., 2010).

354 In some cases, *lac* and *gal* genes are present in the genome, like in *S. mutans*. It is
355 believed that the expression of the Tagatose-6P pathway in *S. mutans* is inducible by lactose
356 (Hamilton & Lebttag, 1979; Hamilton & Lo, 1978), whereas the expression of the Leloir pathway
357 can be induced by galactose in the medium or by intracellular galactose released from polymers,
358 such as melibiose (Ajdić, Sutcliffe, Russell, & Ferretti, 1996). However, this example cannot be
359 generalized to all genomes holding *lac* and *gal* genes. In *S. gordonii* CH1, *gal* genes are present
360 along with the *lac* genes, but only the *lac* genes are functional. It was hypothesized that the
361 Leloir pathway is either not sufficiently active or is crossregulated by the *lac* genes (Zeng et al.,
362 2012).

363 The functional divergence between copies of the *lac* genes can be dramatically more
364 pronounced. One example is *S. pyogenes* which exhibits two *lac* gene clusters : Lac.1 and Lac.2
365 (Ferretti et al., 2001). Lac. 2 is dedicated to carbohydrate utilization and exhibit a canonical

366 genetic organization while Lac.1 deviated from this structure during evolution. The Lac.1 cluster
367 is devoided of *lacG*, and the kinase encoding gene *lacC.1* is truncated. The gene *lacD.1* is a
368 functional gene and predicted to encode a tagatose-1,6-bisphosphate aldolase. However, even if
369 LacD.1 retains a aldolase activity *in vitro*, LacD.1 regulates virulence genes *in vivo* (Loughman
370 & Caparon, 2006, 2007).

371 The Leloir pathway can be associated to anabolism in addition to carbohydrate
372 catabolism. Indeed, the *galTE* product produces UDP-galactose when it converts galactose-1P to
373 glucose-1P. The UDP-galactose can be subsequently used for exopolysaccharide synthesis
374 (Boels, Ramos, Kleerebezem, & De Vos, 2001; Boels, van Kranenburg, Hugenholtz,
375 Kleerebezem, & de Vos, 2001; Harutoshi, 2013).

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379 **References**

- 380 Abranches, J., Chen, Y.-Y. M., & Burne, R. A. (2004). Galactose Metabolism by *Streptococcus*
381 *mutans*. *Applied and Environmental Microbiology*, 70(10), 6047–6052.
382 <https://doi.org/10.1128/AEM.70.10.6047-6052.2004>
- 383 Acosta, P., & Gross, K. (1995). Hidden Sources of Galactose in the Environment. *European*
384 *Journal of Pediatrics*, 154(7), S87–S92. <https://doi.org/10.1007/BF02143811>
- 385 Ajdic, D., & Ferretti, J. J. (1998). Transcriptional regulation of the *Streptococcus mutans gal*
386 operon by the *GalR* repressor. *Journal of Bacteriology*, 180(21), 5727–5732.
- 387 Ajdić, D., Sutcliffe, I. C., Russell, R. R., & Ferretti, J. J. (1996). Organization and nucleotide
388 sequence of the *Streptococcus mutans* galactose operon. *Gene*, 180(1–2), 137–144.

389 Anbukkarasi, K., Nanda, D. K., UmaMaheswari, T., Hemalatha, T., Singh, P., & Singh, R.
390 (2014). Assessment of expression of Leloir pathway genes in wild-type galactose-
391 fermenting *Streptococcus thermophilus* by real-time PCR. *European Food Research and*
392 *Technology*, 239(5), 895–903. <https://doi.org/10.1007/s00217-014-2286-9>

393 Axelsson, L. (2004). Lactic acid bacteria: classification and physiology. In *Lactic Acid Bacteria:*
394 *Microbiological and Functional Aspects*. (pp. 1–66). Salminen, S., von Wright, A.,
395 Ouwehand, A.

396 Bettenbrock, K., Siebers, U., Ehrenreich, P., & Alpert, C.-A. (1999). *Lactobacillus casei* 64H
397 Contains a Phosphoenolpyruvate-Dependent Phosphotransferase System for Uptake of
398 Galactose, as Confirmed by Analysis of ptsH and Differentgal Mutants. *Journal of*
399 *Bacteriology*, 181(1), 225–230.

400 Bissett, D. L., & Anderson, R. L. (1980). Lactose and D-galactose metabolism in *Staphylococcus*
401 *aureus* . III. Purification and properties of D-tagatose-6-phosphate kinase. *The Journal of*
402 *Biological Chemistry*, 255(18), 8745–8749.

403 Boels, I. C., Ramos, A., Kleerebezem, M., & De Vos, W. M. (2001). Functional analysis of the
404 *Lactococcus lactis galU* and *galE* genes and their impact on sugar nucleotide and
405 exopolysaccharide biosynthesis. *Applied and Environmental Microbiology*, 67(7), 3033–
406 3040. <https://doi.org/10.1128/AEM.67.7.3033-3040.2001>

407 Boels, I. C., van Kranenburg, R., Hugenholtz, J., Kleerebezem, M., & de Vos, W. M. (2001).
408 Sugar catabolism and its impact on the biosynthesis and engineering of
409 exopolysaccharide production in lactic acid bacteria. *International Dairy Journal*, 11(9),
410 723–732. [https://doi.org/10.1016/S0958-6946\(01\)00116-9](https://doi.org/10.1016/S0958-6946(01)00116-9)

411 Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarne, K., Weissenbach, J., ... Sorokin, A.
412 (2001). The Complete Genome Sequence of the Lactic Acid Bacterium *Lactococcus*
413 *lactis* ssp. *lactis* IL1403. *Genome Research*, 11(5), 731–753.
414 <https://doi.org/10.1101/gr.169701>

415 Bork, P., Sander, C., & Valencia, A. (1993). Convergent evolution of similar enzymatic function
416 on different protein folds: the hexokinase, ribokinase, and galactokinase families of sugar
417 kinases. *Protein Science : A Publication of the Protein Society*, 2(1), 31–40.

418 Breidt, F., Hengstenberg, W., Finkeldei, U., & Stewart, G. C. (1987). Identification of the genes
419 for the lactose-specific components of the phosphotransferase system in the *lac* operon of
420 *Staphylococcus aureus*. *The Journal of Biological Chemistry*, 262(34), 16444–16449.

421 Broadbent, J. R., Hughes, J. E., Welker, D. L., Tompkins, T. A., & Steele, J. L. (2013). Complete
422 Genome Sequence for *Lactobacillus helveticus* CNRZ 32, an Industrial Cheese Starter
423 and Cheese Flavor Adjunct. *Genome Announcements*, 1(4).
424 <https://doi.org/10.1128/genomeA.00590-13>

425 Cailliez-Grimal, C., Afzal, M. I., & Revol-Junelles, A.-M. (2014). *Carnobacterium*. In
426 *Encyclopedia of Food Microbiology*, Elsevier (Vol. 1, pp. 379–383). Batt, C. A.,
427 Tortorello, M. L.

428 Cailliez-Grimal, C., Chaillou, S., Anba-Mondoloni, J., Loux, V., Afzal, M. I., Rahman, A., ...
429 Borges, F. (2013). Complete Chromosome Sequence of *Carnobacterium maltaromaticum*
430 LMA 28. *Genome Announcements*, 1(1), e00115-12.
431 <https://doi.org/10.1128/genomeA.00115-12>

- 432 Carvalho, S. M., Kloosterman, T. G., Kuipers, O. P., & Neves, A. R. (2011). CcpA Ensures
433 Optimal Metabolic Fitness of *Streptococcus pneumoniae*. *PLOS ONE*, 6(10), e26707.
434 <https://doi.org/10.1371/journal.pone.0026707>
- 435 Chassy, B. M., & Thompson, J. (1983). Regulation and characterization of the galactose-
436 phosphoenolpyruvate-dependent phosphotransferase system in *Lactobacillus casei*.
437 *Journal of Bacteriology*, 154(3), 1204–1214.
- 438 Chen, Y.-Y. M., Betzenhauser, M. J., Snyder, J. A., & Burne, R. A. (2002). Pathways for
439 lactose/galactose catabolism by *Streptococcus salivarius*. *FEMS Microbiology Letters*,
440 209(1), 75–79.
- 441 Cocaign-Bousquet, M., Garrigues, C., Loubiere, P., & Lindley, N. D. (1996). Physiology of
442 pyruvate metabolism in *Lactococcus lactis*. *Antonie Van Leeuwenhoek*, 70(2–4), 253–
443 267.
- 444 Coombs, J. M., & Brenchley, J. E. (1999). Biochemical and phylogenetic analyses of a cold-
445 active β -galactosidase from the lactic acid bacterium *Carnobacterium piscicola* BA.
446 *Applied and Environmental Microbiology*, 65(12), 5443–5450.
- 447 Coombs, J. M., & Brenchley, J. E. (2001). Characterization of Two New Glycosyl Hydrolases
448 from the Lactic Acid Bacterium *Carnobacterium piscicola* Strain BA. *Applied and*
449 *Environmental Microbiology*, 67(11), 5094–5099.
450 <https://doi.org/10.1128/AEM.67.11.5094-5099.2001>
- 451 Crow, V. L., & Thomas, T. D. (1984). Properties of a *Streptococcus lactis* strain that ferments
452 lactose slowly. *Journal of Bacteriology*, 157(1), 28–34.

453 Declerck, N., Vincent, F., Hoh, F., Aymerich, S., & van Tilbeurgh, H. (1999). RNA recognition
454 by transcriptional antiterminators of the BglG/SacY family: functional and structural
455 comparison of the CAT domain from SacY and LicT. *Journal of Molecular Biology*,
456 294(2), 389–402. <https://doi.org/10.1006/jmbi.1999.3256>

457 Ferretti, J. J., McShan, W. M., Ajdic, D., Savic, D. J., Savic, G., Lyon, K., ... McLaughlin, R.
458 (2001). Complete genome sequence of an M1 strain of *Streptococcus pyogenes*.
459 *Proceedings of the National Academy of Sciences of the United States of America*, 98(8),
460 4658–4663. <https://doi.org/10.1073/pnas.071559398>

461 Forrest, L. R., Krämer, R., & Ziegler, C. (2011). The structural basis of secondary active
462 transport mechanisms. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1807(2),
463 167–188. <https://doi.org/10.1016/j.bbabi.2010.10.014>

464 Fortina, M. G., Ricci, G., & Borgo, F. (2009). A Study of Lactose Metabolism in *Lactococcus*
465 *garvieae* Reveals a Genetic Marker for Distinguishing between Dairy and Fish Biotypes.
466 *Journal of Food Protection*, 72(6), 1248–1254.

467 Fortina, M. G., Ricci, G., Mora, D., Guglielmetti, S., & Manachini, P. L. (2003). Unusual
468 Organization for Lactose and Galactose Gene Clusters in *Lactobacillus helveticus*.
469 *Applied and Environmental Microbiology*, 69(6), 3238–3243.
470 <https://doi.org/10.1128/AEM.69.6.3238-3243.2003>

471 Grossiord, B., Luesink, E. J., Vaughan, E. E., Arnaud, A., & de Vos, W. M. (2003).
472 Characterization, expression, and mutation of the *Lactococcus lactis galPMKTE* genes,
473 involved in galactose utilization via the Leloir pathway. *Journal of Bacteriology*, 185(3),
474 870–878. <https://doi.org/10.1128/JB.185.3.870-878.2003>

- 475 Grossiord, B., Vaughan, E. E., Luesink, E., & de Vos, W. M. (1998). Genetics of galactose
476 utilisation via the Leloir pathway in lactic acid bacteria. *Lait*, 78(1), 77–84.
- 477 Hamilton, I. R., & Lebtog, H. (1979). Lactose metabolism by *Streptococcus mutans*: evidence for
478 induction of the tagatose 6-phosphate pathway. *Journal of Bacteriology*, 140(3), 1102–
479 1104.
- 480 Hamilton, I. R., & Lo, G. C. (1978). Co-induction of beta-galactosidase and the lactose-P-
481 enolpyruvate phosphotransferase system in *Streptococcus salivarius* and *Streptococcus*
482 *mutans*. *Journal of Bacteriology*, 136(3), 900–908.
- 483 Harutoshi, T. (2013). Exopolysaccharides of Lactic Acid Bacteria for Food and Colon Health
484 Applications. In J. M. Kongo (Ed.), *Lactic Acid Bacteria - R & D for Food, Health and*
485 *Livestock Purposes*. InTech. Retrieved from [http://www.intechopen.com/books/lactic-](http://www.intechopen.com/books/lactic-acid-bacteria-r-d-for-food-health-and-livestock-purposes/exopolysaccharides-of-lactic-acid-bacteria-for-food-and-colon-health-applications)
486 [acid-bacteria-r-d-for-food-health-and-livestock-purposes/exopolysaccharides-of-lactic-](http://www.intechopen.com/books/lactic-acid-bacteria-r-d-for-food-health-and-livestock-purposes/exopolysaccharides-of-lactic-acid-bacteria-for-food-and-colon-health-applications)
487 [acid-bacteria-for-food-and-colon-health-applications](http://www.intechopen.com/books/lactic-acid-bacteria-r-d-for-food-health-and-livestock-purposes/exopolysaccharides-of-lactic-acid-bacteria-for-food-and-colon-health-applications)
- 488 Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence
489 similarities. *Biochemical Journal*, 280(Pt 2), 309–316.
- 490 Henrissat, B., & Bairoch, A. (1993). New families in the classification of glycosyl hydrolases
491 based on amino acid sequence similarities. *Biochemical Journal*, 293, 781–788.
- 492 Hutkins, R., Morris, H. A., & McKay, L. L. (1985). Galactokinase activity in *Streptococcus*
493 *thermophilus*. *Applied and Environmental Microbiology*, 50(4), 777–780.
- 494 Iskandar, C. F., Cailliez-Grimal, C., Rahman, A., Rondags, E., Remenant, B., Zagorec, M., ...
495 Revol-Junelles, A.-M. (2016). Genes associated to lactose metabolism illustrate the high

496 diversity of *Carnobacterium maltaromaticum*. *Food Microbiology*, 58, 79–86.
497 <https://doi.org/10.1016/j.fm.2016.03.008>

498 Jagusztyn-Krynicka, E. K., Hansen, J. B., Crow, V. L., Thomas, T. D., Honeyman, A. L., &
499 Curtiss, R. (1992). *Streptococcus mutans* serotype c tagatose 6-phosphate pathway gene
500 cluster. *Journal of Bacteriology*, 174(19), 6152–6158.

501 Jeong, J. K., Kwon, O., Lee, Y. M., Oh, D.-B., Lee, J. M., Kim, S., ... Kang, H. A. (2009).
502 Characterization of the *Streptococcus pneumoniae* BgaC Protein as a Novel Surface -
503 Galactosidase with Specific Hydrolysis Activity for the Gal 1-3GlcNAc Moiety of
504 Oligosaccharides. *Journal of Bacteriology*, 191(9), 3011–3023.
505 <https://doi.org/10.1128/JB.01601-08>

506 Kafsi, H. E., Binesse, J., Loux, V., Buratti, J., Boudebouze, S., Dervyn, R., ... Guchte, M. van
507 de. (2014). Genome Sequence of *Lactobacillus delbrueckii* subsp. *lactis* CNRZ327, a
508 Dairy Bacterium with Anti-Inflammatory Properties. *Genome Announcements*, 2(4),
509 e00328-14. <https://doi.org/10.1128/genomeA.00328-14>

510 Kandler, O. (1983). Carbohydrate metabolism in lactic acid bacteria. *Antonie van Leeuwenhoek*,
511 49(3), 209–224. <https://doi.org/10.1007/BF00399499>

512 Klaenhammer, T. R., Barrangou, R., Buck, B. L., Azcarate-Peril, M. A., & Altermann, E. (2005).
513 Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS*
514 *Microbiology Reviews*, 29(3), 393–409. <https://doi.org/10.1016/j.fmrre.2005.04.007>

515 Koonin, E.V., Aravind, L., & Kondrashov, A.S. (2000). The Impact of Comparative Genomics on
516 Our Understanding of Evolution. *Cell*, 101, 573–576. doi:10.1016/S0092-8674(00)80867-3

517 Krispin, O., & Allmansberger, R. (1998). The *Bacillus subtilis* *galE* gene is essential in the
518 presence of glucose and galactose. *Journal of Bacteriology*, 180(8), 2265–2270.

519 Kumar, A., Weatherly, M. R., & Beaman, D. C. (1991). Sweeteners, flavorings, and dyes in
520 antibiotic preparations. *Pediatrics*, 87(3), 352–360.

521 Leong-Morghenthaler, P., Zwahlen, M., & Hottinger, H. (1991). Lactose Metabolism in
522 *Lactobacillus bulgaricus* - Analysis of the Primary Structure and Expression of the Genes
523 Involved. *Journal of Bacteriology*, 173(6), 1951–1957.

524 Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food
525 fermentation industry. *Trends in Food Science & Technology*, 15(2), 67–78.
526 <https://doi.org/10.1016/j.tifs.2003.09.004>

527 Liu, S.-Q. (2003). Practical implications of lactate and pyruvate metabolism by lactic acid
528 bacteria in food and beverage fermentations. *International Journal of Food*
529 *Microbiology*, 83(2), 115–131. [https://doi.org/10.1016/S0168-1605\(02\)00366-5](https://doi.org/10.1016/S0168-1605(02)00366-5)

530 Loughman, J. A., & Caparon, M. G. (2006). A novel adaptation of aldolase regulates virulence in
531 *Streptococcus pyogenes*. *The EMBO Journal*, 25(22), 5414–5422.
532 <https://doi.org/10.1038/sj.emboj.7601393>

533 Loughman, J. A., & Caparon, M. G. (2007). Comparative functional analysis of the *lac* operons
534 in *Streptococcus pyogenes*. *Molecular Microbiology*, 64(2), 269–280.
535 <https://doi.org/10.1111/j.1365-2958.2007.05663.x>

536 Maxwell, E., Kurahashi, K., & Kalckar, H. (1962). Enzymes of the Leloir Pathway. *Methods in*
537 *Enzymology*, 5, 174–189. [https://doi.org/10.1016/S0076-6879\(62\)05204-0](https://doi.org/10.1016/S0076-6879(62)05204-0)

- 538 Neves, A. R., Pool, W. A., Kok, J., Kuipers, O. P., & Santos, H. (2005). Overview on sugar
539 metabolism and its control in *Lactococcus lactis* - The input from in vivo NMR. *Fems*
540 *Microbiology Reviews*, 29(3), 531–554. <https://doi.org/10.1016/j.femsre.2005.04.005>
- 541 Neves, A. R., Pool, W. A., Solopova, A., Kok, J., Santos, H., & Kuipers, O. P. (2010). Towards
542 Enhanced Galactose Utilization by *Lactococcus lactis*. *Applied and Environmental*
543 *Microbiology*, 76(21), 7048–7060. <https://doi.org/10.1128/AEM.01195-10>
- 544 Oskouian, B., & Stewart, G. C. (1990). Repression and catabolite repression of the lactose
545 operon of *Staphylococcus aureus*. *Journal of Bacteriology*, 172(7), 3804–3812.
- 546 Paixão, L., Oliveira, J., Veríssimo, A., Vinga, S., Lourenço, E. C., Ventura, M. R., ... Neves, A.
547 R. (2015). Host Glycan Sugar-Specific Pathways in *Streptococcus pneumoniae*: Galactose
548 as a Key Sugar in Colonisation and Infection. *PLoS ONE*, 10(3).
549 <https://doi.org/10.1371/journal.pone.0121042>
- 550 Poolman, B. (1993). Energy transduction in lactic acid bacteria. *FEMS Microbiology Reviews*,
551 12(1–3), 125–147. <https://doi.org/10.1111/j.1574-6976.1993.tb00015.x>
- 552 Poolman, B., Royer, T. J., Mainzer, S. E., & Schmidt, B. F. (1989). Lactose transport system of
553 *Streptococcus thermophilus*: a hybrid protein with homology to the melibiose carrier and
554 enzyme III of phosphoenolpyruvate-dependent phosphotransferase systems. *Journal of*
555 *Bacteriology*, 171(1), 244–253.
- 556 Pot, B. (2008). The taxonomy of lactic acid bacteria. In *Bactéries lactiques, de la génétique aux*
557 *ferments*. (Lavoisier, pp. 1–152). G. Corrieu, F.M. Luquet.
- 558 Premi, L., Sandine, W. E., & Elliker, P. R. (1972). Lactose-hydrolyzing enzymes of
559 *Lactobacillus* species. *Applied Microbiology*, 24(1), 51–57.

560 Price, C. E., Zeyniyev, A., Kuipers, O. P., & Kok, J. (2012). From meadows to milk to mucosa –
561 adaptation of *Streptococcus* and *Lactococcus* species to their nutritional environments.
562 *FEMS Microbiology Reviews*, 36(5), 949–971. [https://doi.org/10.1111/j.1574-](https://doi.org/10.1111/j.1574-6976.2011.00323.x)
563 6976.2011.00323.x

564 Rahman, A., Cailliez-Grimal, C., Bontemps, C., Payot, S., Chaillou, S., Revol-Junelles, A.-M., &
565 Borges, F. (2014). High Genetic Diversity among Strains of the Unindustrialized Lactic
566 Acid Bacterium *Carnobacterium maltaromaticum* in Dairy Products as Revealed by
567 Multilocus Sequence Typing. *Applied and Environmental Microbiology*, 80(13), 3920–
568 3929. <https://doi.org/10.1128/AEM.00681-14>

569 Rahman, A., El Kheir, S. M., Back, A., Mangavel, C., Revol-Junelles, A.-M., & Borges, F.
570 (2016). Repeat-based Sequence Typing of *Carnobacterium maltaromaticum*.
571 *International Journal of Food Microbiology*, 226, 1–4.
572 <https://doi.org/10.1016/j.ijfoodmicro.2016.03.003>

573 Richards, V. P., Choi, S. C., Bitar, P. D. P., Gurjar, A. A., & Stanhope, M. J. (2013).
574 Transcriptomic and genomic evidence for *Streptococcus agalactiae* adaptation to the
575 bovine environment. *BMC Genomics*, 14(1), 920. [https://doi.org/10.1186/1471-2164-14-](https://doi.org/10.1186/1471-2164-14-920)
576 920

577 Rosey, E. L., & Stewart, G. C. (1992). Nucleotide and deduced amino acid sequences of the
578 *lacR*, *lacABCD*, and *lacFE* genes encoding the repressor, tagatose 6-phosphate gene
579 cluster, and sugar-specific phosphotransferase system components of the lactose operon
580 of *Streptococcus mutans*. *Journal of Bacteriology*, 174(19), 6159–6170.

581 Siezen, R. J., Renckens, B., Swam, I. van, Peters, S., Kranenburg, R. van, Kleerebezem, M., &
582 Vos, W. M. de. (2005). Complete Sequences of Four Plasmids of *Lactococcus lactis*
583 subsp. *cremoris* SK11 Reveal Extensive Adaptation to the Dairy Environment. *Applied*
584 *and Environmental Microbiology*, 71(12), 8371–8382.
585 <https://doi.org/10.1128/AEM.71.12.8371-8382.2005>

586 Solopova, A., Bachmann, H., Teusink, B., Kok, J., Neves, A. R., & Kuipers, O. P. (2012). A
587 Specific Mutation in the Promoter Region of the Silent *cel* Cluster Accounts for the
588 Appearance of Lactose-Utilizing *Lactococcus lactis* MG1363. *Applied and*
589 *Environmental Microbiology*, 78(16), 5612–5621. [https://doi.org/10.1128/AEM.00455-](https://doi.org/10.1128/AEM.00455-12)
590 12

591 Stackebrandt, E., & Teuber, M. (1988). Molecular taxonomy and phylogenetic position of lactic
592 acid bacteria. *Biochimie*, 70(3), 317–324. [https://doi.org/10.1016/0300-9084\(88\)90204-0](https://doi.org/10.1016/0300-9084(88)90204-0)

593 Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current
594 taxonomy. *International Journal of Food Microbiology*, 36(1), 1–29.
595 [https://doi.org/10.1016/S0168-1605\(96\)01233-0](https://doi.org/10.1016/S0168-1605(96)01233-0)

596 Tsai, Y.-K., & Lin, T.-H. (2006). Sequence, organization, transcription and regulation of lactose
597 and galactose operons in *Lactobacillus rhamnosus* TCELL-1. *Journal of Applied*
598 *Microbiology*, 100(3), 446–459. <https://doi.org/10.1111/j.1365-2672.2005.02790.x>

599 Vallenet, D., Belda, E., Calteau, A., Cruveiller, S., Engelen, S., Lajus, A., Le Fevre, F., Longin,
600 C., Mornico, D., Roche, D., Rouy, Z., Salvignol, G., Scarpelli, C., Thil Smith, A.A.,
601 Weiman, M., & Medigue, C. (2013). MicroScope--an integrated microbial resource for

602 the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids*
603 *Research*, 41, D636–D647. <http://doi:10.1093/nar/gks1194>

604 van Rooijen, R. J., Dechering, K. J., Niek, C., Wilmink, J., & de Vos, W. M. (1993). Lysines 72,
605 80 and 213 and aspartic acid 210 of the *Lactococcus lactis* LacR repressor are involved in
606 the response to the inducer tagatose-6-phosphate leading to induction of *lac* operon
607 expression. *Protein Engineering*, 6(2), 201–206.

608 van Rooijen, R. J., van Schalkwijk, S., & de Vos, W. M. (1991). Molecular cloning,
609 characterization, and nucleotide sequence of the tagatose 6-phosphate pathway gene
610 cluster of the lactose operon of *Lactococcus lactis*. *The Journal of Biological Chemistry*,
611 266(11), 7176–7181.

612 van Tilbeurgh, H., & Declerck, N. (2001). Structural insights into the regulation of bacterial
613 signalling proteins containing PRDs. *Current Opinion in Structural Biology*, 11(6), 685–
614 693.

615 Vaughan, E. E., Pridmore, R. D., & Mollet, B. (1998). Transcriptional regulation and evolution
616 of lactose genes in the galactose-lactose operon of *Lactococcus lactis* NCD02054.
617 *Journal of Bacteriology*, 180(18), 4893–4902.

618 Vaughan, E. E., van den Bogaard, P. T. C., Catzeddu, P., Kuipers, O. P., & de Vos, W. M.
619 (2001). Activation of silent gal genes in the *lac-gal* regulon of *Streptococcus*
620 *thermophilus*. *Journal of Bacteriology*, 183(4), 1184–1194.
621 <https://doi.org/10.1128/JB.183.4.1184-1194.2001>

622 Wassenaar, T. M., & Lukjancenko, O. (2014). Comparative genomics of *Lactobacillus* and other
623 LAB. In W. H. Holzapfel & B. J. B. Wood (Eds.), *Lactic Acid Bacteria* (pp. 55–69). John

624 Wiley & Sons, Ltd. Retrieved from
625 <http://onlinelibrary.wiley.com/doi/10.1002/9781118655252.ch5/summary>

626 Wei, L., Liu, Y., Dubchak, I., Shon, J., & Park, J. (2002). Comparative genomics approaches to
627 study organism similarities and differences. *Journal of Biomedical Informatics*, *35*,
628 142–150. doi:10.1016/S1532-0464(02)00506-3

629

630 Wu, Q., Cheung, C. K. W., & Shah, N. P. (2015). Towards galactose accumulation in dairy foods
631 fermented by conventional starter cultures: Challenges and strategies. *Trends in Food*
632 *Science & Technology*, *41*(1), 24–36. <https://doi.org/10.1016/j.tifs.2014.08.010>

633 Yan, N. (2013). Structural advances for the major facilitator superfamily (MFS) transporters.
634 *Trends in Biochemical Sciences*, *38*(3), 151–159.
635 <https://doi.org/10.1016/j.tibs.2013.01.003>

636 Zähler, D., & Hakenbeck, R. (2000). The *Streptococcus pneumoniae* Beta-Galactosidase Is a
637 Surface Protein. *Journal of Bacteriology*, *182*(20), 5919–5921.

638 Zeng, L., Das, S., & Burne, R. A. (2010). Utilization of Lactose and Galactose by *Streptococcus*
639 *mutans*: Transport, Toxicity, and Carbon Catabolite Repression. *Journal of Bacteriology*,
640 *192*(9), 2434–2444. <https://doi.org/10.1128/JB.01624-09>

641 Zeng, L., Martino, N. C., & Burne, R. A. (2012). Two Gene Clusters Coordinate Galactose and
642 Lactose Metabolism in *Streptococcus gordonii*. *Applied and Environmental*
643 *Microbiology*, *78*(16), 5597–5605. <https://doi.org/10.1128/AEM.01393-12>

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645

646 **Figure and Table legends**

647 **Figure 1:** Schematic representation of the lactose and galactose metabolic pathways (according
648 to (Neves et al., 2010; Price, Zeyniyev, Kuipers, & Kok, 2012; Zeng et al., 2010)). Enzymes
649 encoded by the genes: *lacAB*, galactose-6-phosphate isomerase; *lacC*, tagatose-6-phosphate
650 kinase; *lacD*, tagatose-1,6-diphosphate aldolase; *lacEF*, EIIABC^{lac/Gal}; *lacG*, phospho- β -
651 galactosidase; *lacR*, transcriptional regulator; *lacS*, lactose permease; *lacZ*, β -galactosidase;
652 *lacLM*, β -galactosidase; *galP*, galactose permease; *galM*, galactose epimerase; *galK*, galactose
653 kinase; *galT*, galactose-1-phosphate uridyltransferase; *galE*, UDP-glucose-4-epimerase; *pgm*,
654 phosphoglucomutase; *glk*, glucokinase. GPDH: glyceraldehyde 3 phosphate, DHAP:
655 dihydroxyacetone phosphate. The dotted line symbolizes several reactions.

656 **Figure 2:** Organization and synteny between *gal* genes from the Leloir pathway and *lac* genes
657 genes from the Tagatose-6P pathway among representative strains. A. *gal* genes from the Leloir
658 pathway; B. *gal* and *lac* genes ; C. *lac* genes from the Tagatose-6P pathway. The arrows show
659 the gene direction. *galR* (regulation gene), *galK* (galactose kinase), *galM* (galactose mutarotase),
660 *galT* (Galactose-1P uridyltransferase), *galE* (UDP-glucose 4-epimerase), *lacZ/lacLM* (beta
661 galactosidase), *galP/lacS* (galactose/lactose permease), *agaR* (alpha-galactosidase), *bgaB* (beta-
662 galactosidase), *bgaC* (beta-galactosidase), *bglH* (beta-glucosidase). *lacE* (PTS system), *lacF*
663 (PTS system), *HP* (hypotetical protein), *araC* (AraC family transcriptional regulator), *pgcA*
664 (alpha-phosphoglucomutase), *ganB* (arabinogalactan endo-1,4-beta-galactosidase), *α -gal*
665 (alpha/beta hydrolase family), *msm* (multiple sugar metabolism), *lacX* (putative galactose
666 mutarotase, plasmid protein). *lacAB* (galactose isomerase), *lacC* (Tagatose6P kinase), *lacD*
667 (tagatose-1,6P aldolase), *lacFE* (PTS system), *lacG* (P-beta galactosidase), *lacR* (regulation
668 genes), *trp* (transposase), *lacT* (anti-terminator transcriptional gene).

669 **Figure 3:** Diversity of the lactose and galactose metabolic pathways within (A) genus and (B)
670 species of the different genus analyzed. The data used to build this graph were obtained from the
671 genomes hosted in the MicroScope platform and from the results described in a previous study
672 (Iskandar et al., 2016). The numbers between brackets on the X axis indicate the total number of
673 strains analyzed in each genus.

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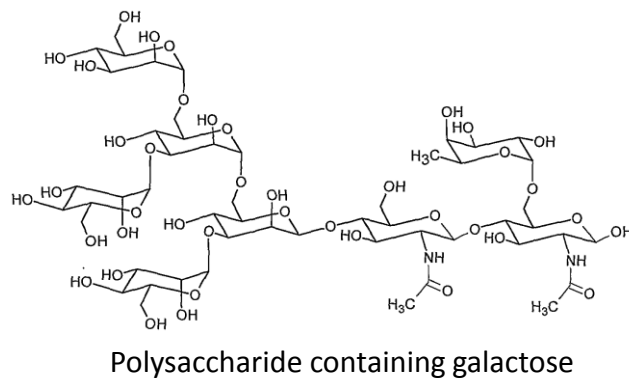

 Leloir Pathway Tagatose-6P Pathway Leloir and Tagatose -6P Pathways No Pathway

676 **Table 1 : Lactose/galactose metabolism genes present in LAB and non-LAB strains.**

677 The shadowed boxes indicate the presence of homologue: light gray indicates that only one copy
 678 of the gene is present and the dark gray indicates that more than one copy are present. The
 679 numbers indicate the number of copies of paralogues. *lacAB* (galactose isomerase), *lacC*
 680 (tagatose-6P kinase), *lacD* (tagatose-1,6P aldolase), *lacFE* (PTS system), *lacG* (P-β
 681 galactosidase), *lacR* (regulation genes), *lacS* (lactose permease), *lacLMZ* (βgalactosidase), *galP*
 682 (galactose permease), *galM* (galactose epimerase), *galK* (galactose kinase), *galT* (galactose-1P-
 683 uridyltransferase), *galE* (UDP-glucose-4-epimerase).

Figure 1: Schematic representation of the lactose and galactose metabolic pathways (according to Neves et al., 2010; Price et al., 2012; Zeng et al., 2010). Enzymes encoded by the genes: *lacAB*, galactose-6-phosphate isomerase; *lacC*, tagatose-6-phosphate kinase; *lacD*, tagatose-1,6-diphosphate aldolase; *lacEF*, EIIABC^{lac/Gal}; *lacG*, phospho-β-galactosidase; *lacR*, transcriptional regulator; *lacS*, lactose permease; *lacZ*, β-galactosidase; *lacLM*, β-galactosidase; *galP*, galactose permease; *galM*, galactose epimerase; *galK*, galactose kinase; *galT*, galactose-1-phosphate uridyltransferase; *galE*, UDP-glucose-4-epimerase; *pgm*, phosphoglucomutase; *glk*, glucokinase. GPDH: glyceraldehyde 3 phosphate, DHAP: dihydroxyacetone phosphate. The dotted line symbolizes several reactions.

Figure 1



bgaA
bgaB
bgaC

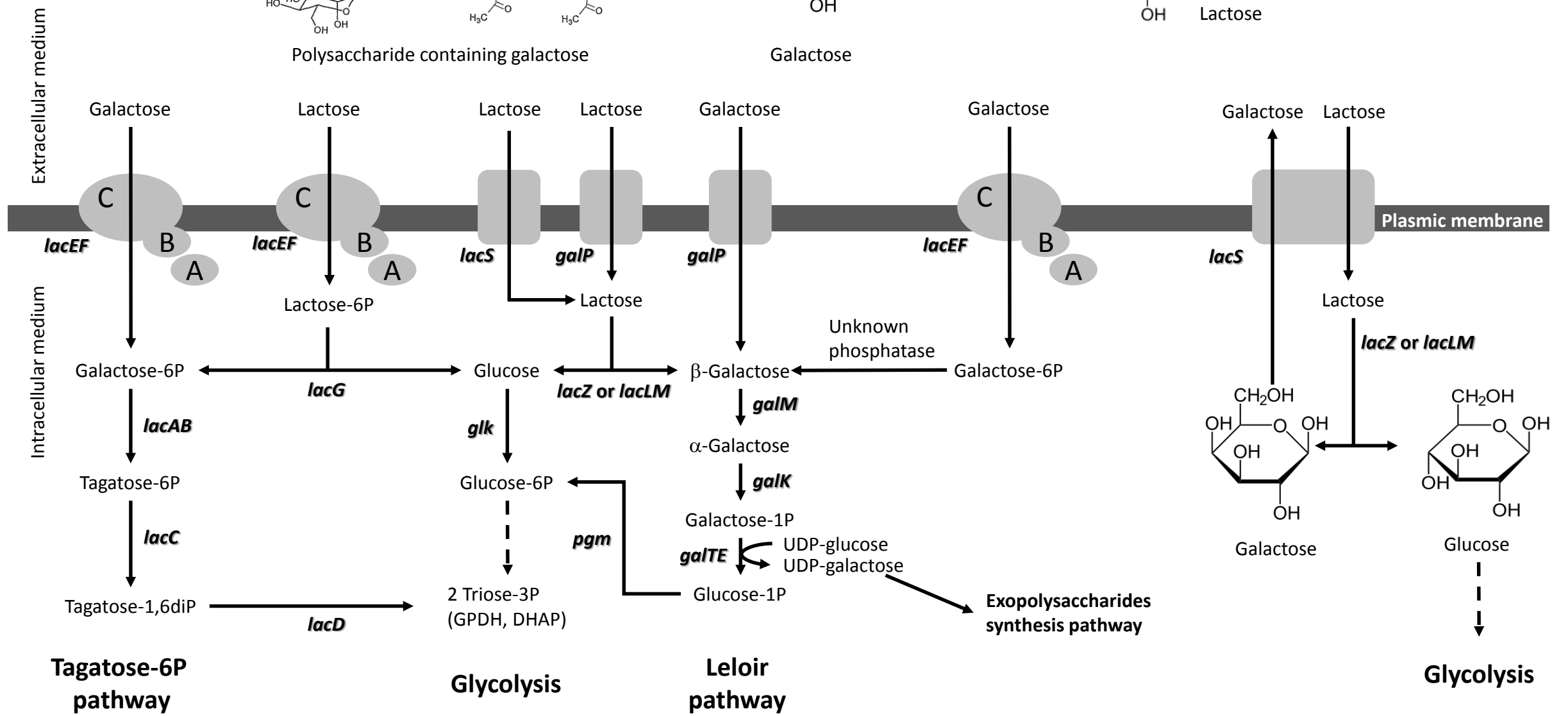
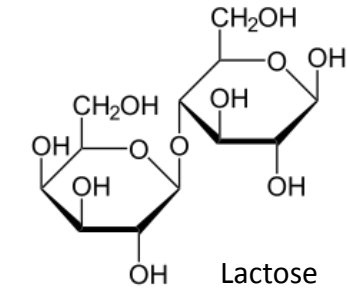
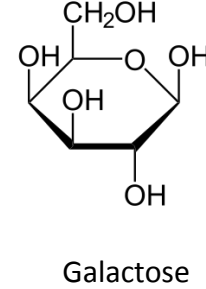


Figure 2: Organization and synteny between *gal* genes from the Leloir pathway and *lac* genes from the Tagatose-6P pathway among representative strains. A. *gal* genes from the Leloir pathway; B. *gal* and *lac* genes ; C. *lac* genes from the Tagatose-6P pathway. The arrows show the gene direction. *galR* (regulation gene), *galK* (galactose kinase), *galM* (galactose mutarotase), *galT* (Galactose-1P uridyltransferase), *galE* (UDP-glucose 4-epimerase), *lacZ/lacLM* (beta galactosidase), *galP /lacS* (galactose/lactose permease), *agaR* (alpha-galactosidase), *bgaB* (beta-galactosidase), *bgaC* (beta-galactosidase), *bglH* (beta-glucosidase). *lacE* (PTS system), *lacF* (PTS system), *HP* (hypotetical protein), *araC* (AraC family transcriptional regulator), *pgcA* (alpha-phosphoglucomutase), *ganB* (arabinogalactan endo-1,4-beta-galactosidase), *α-gal* (alpha/beta hydrolase family), *msm* (multiple sugar metabolism), *lacX* (putative galactose mutarotase, plasmid protein). *lacAB* (galactose isomerase), *lacC* (Tagatose6P kinase), *lacD* (tagatose-1,6P aldolase), *lacFE* (PTS system), *lacG* (P-beta galactosidase), *lacR* (regulation genes), *trp* (transposase), *lacT* (anti-terminator transcriptional gene).

Figure 2A

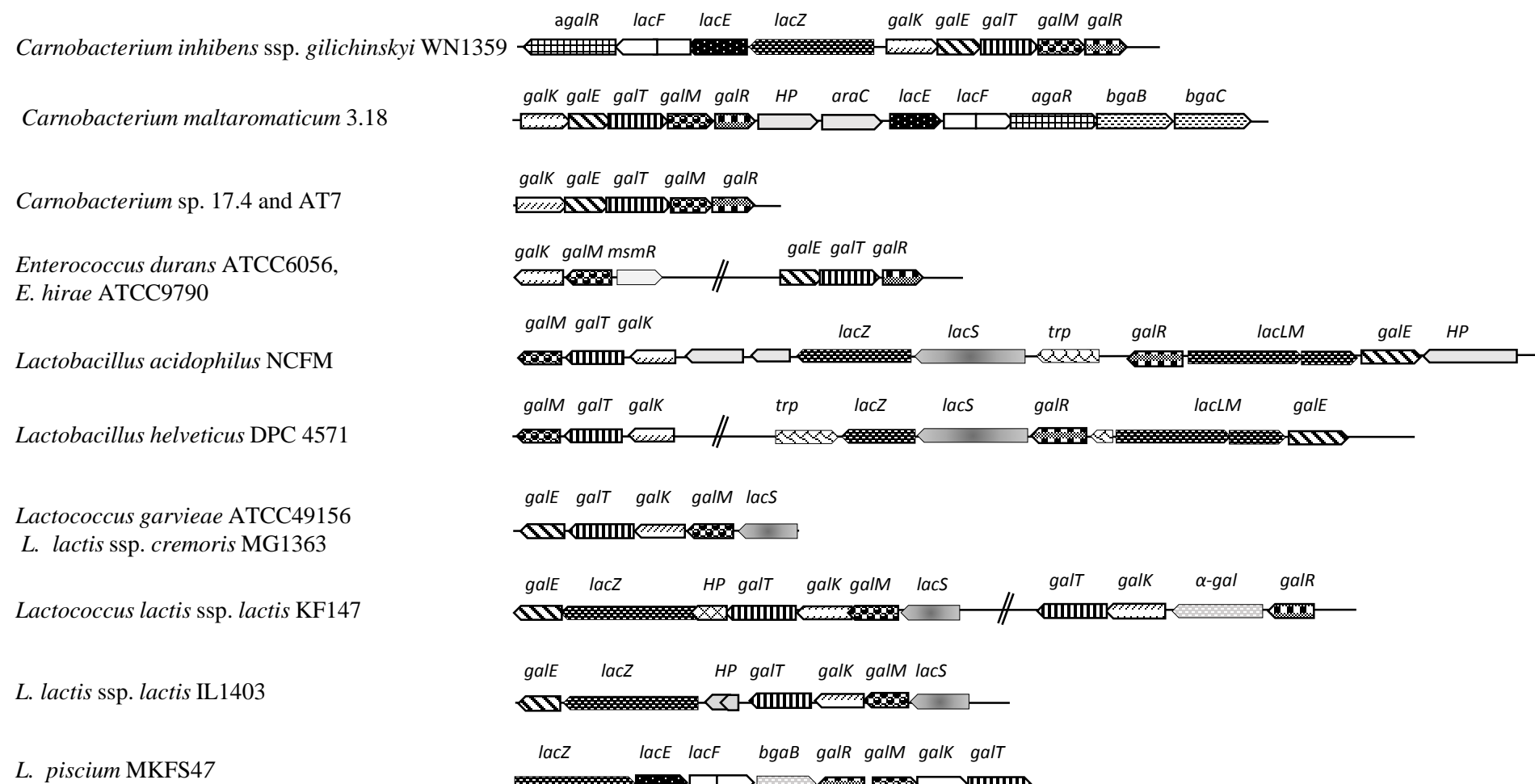
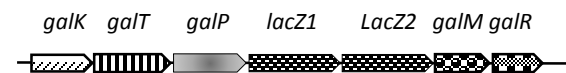


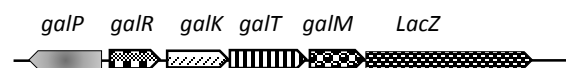
Figure 2A (continued)

Leuconostoc citreum KM20



L. gasicomitatum LMG 18811

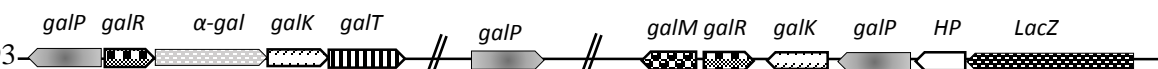
L. gelidum JB7



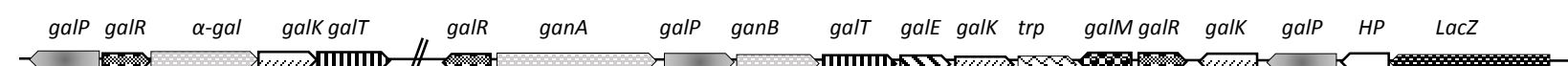
L. kimchii IMSNU11154



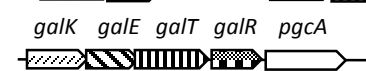
L. mesenteroides ssp. *mesenteroides* J18, ATCC8293



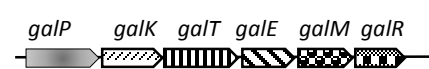
L. mesenteroides ssp. *mesenteroides* ATCC8293



Listeria grayi DSM20601



Oenococcus oeni AWRIB429, ATCC BAA-1163, PSU-1



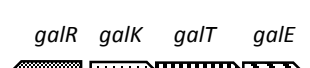
Pediococcus acidilactici DSM 20284

P. pentosaceus ATCC 25745



Streptococcus sanguinis SK353

S. salivarius SK126



Streptococcus thermophilus LMG18311

S. salivarius CCHSS3

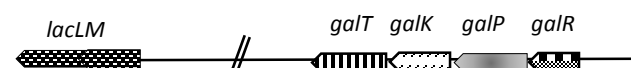


Weissella confusa LBAE C39-2



Weissella cibaria KACC 11862

W. paramesenteroides ATCC 33313

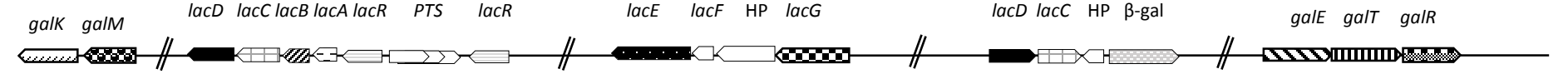


Weissella koreensis KACC 15510, KCTC 3621

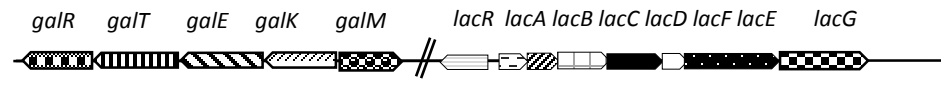


Figure 2B

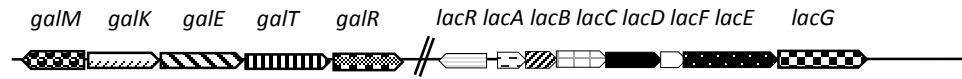
Enterococcus avium ATCC14025



Enterococcus faecalis TX1322



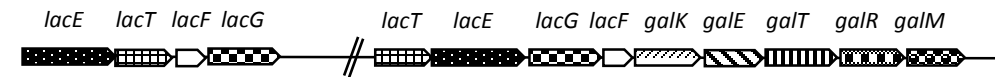
Enterococcus faecalis V583



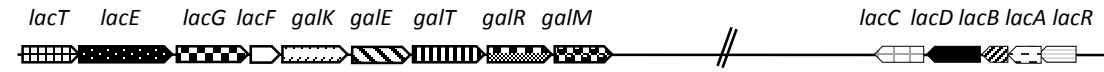
Lactobacillus rhamnosus CASL



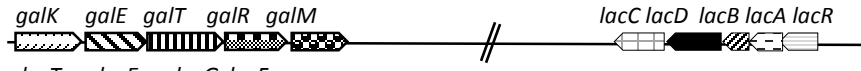
Lactobacillus rhamnosus 21052



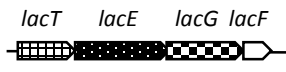
Lactobacillus casei BL23



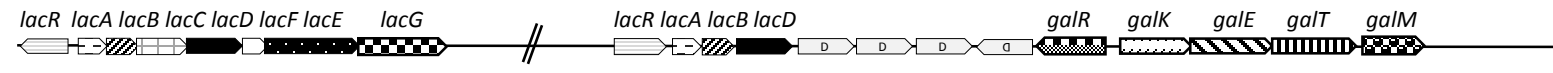
L. casei ATCC334 (chr)



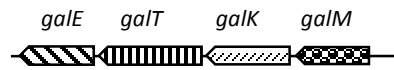
L. casei ATCC334 (pl.)



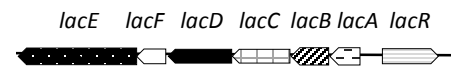
L. curvatus CRL705



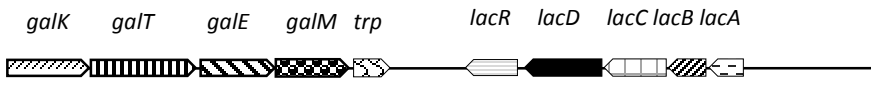
Lactococcus lactis SK11 (chr)



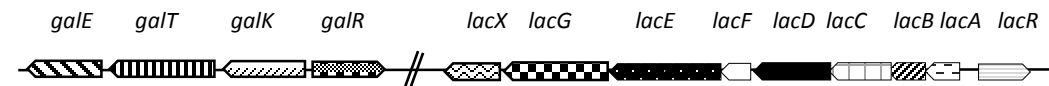
L. Lactis SK11 (pl.)



Streptococcus agalactiae A909



Streptococcus infantarius ATCC BA-102



Streptococcus gordonii CH1



Streptococcus mutans UA159

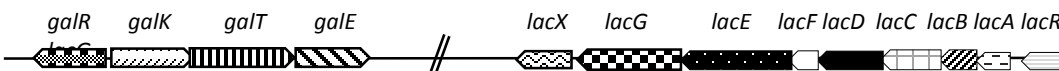


Figure 2C

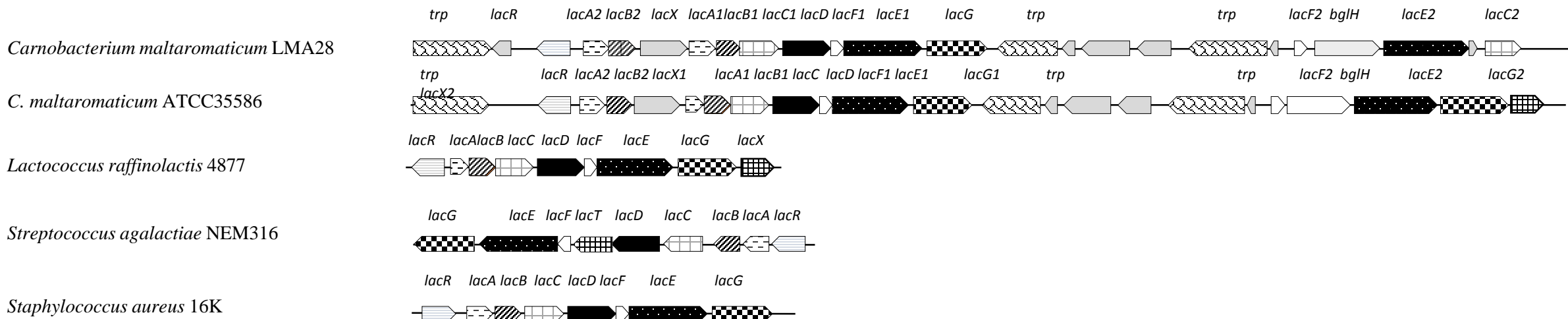


Figure 3: Diversity of the lactose and galactose metabolic pathways within (A) genus and (B) species of the different genus analyzed. The data used to build this graph were obtained from the genomes hosted in the MicroScope platform and from the results described in a previous study (Iskandar et al., 2016). **The numbers between brackets on the X axis indicate the total number of strains analyzed in each genus.**

 Leloir Pathway, Tagatose-6P pathway, Leloir and Tagatose-6P Pathways, No Pathway

Figure 3-A

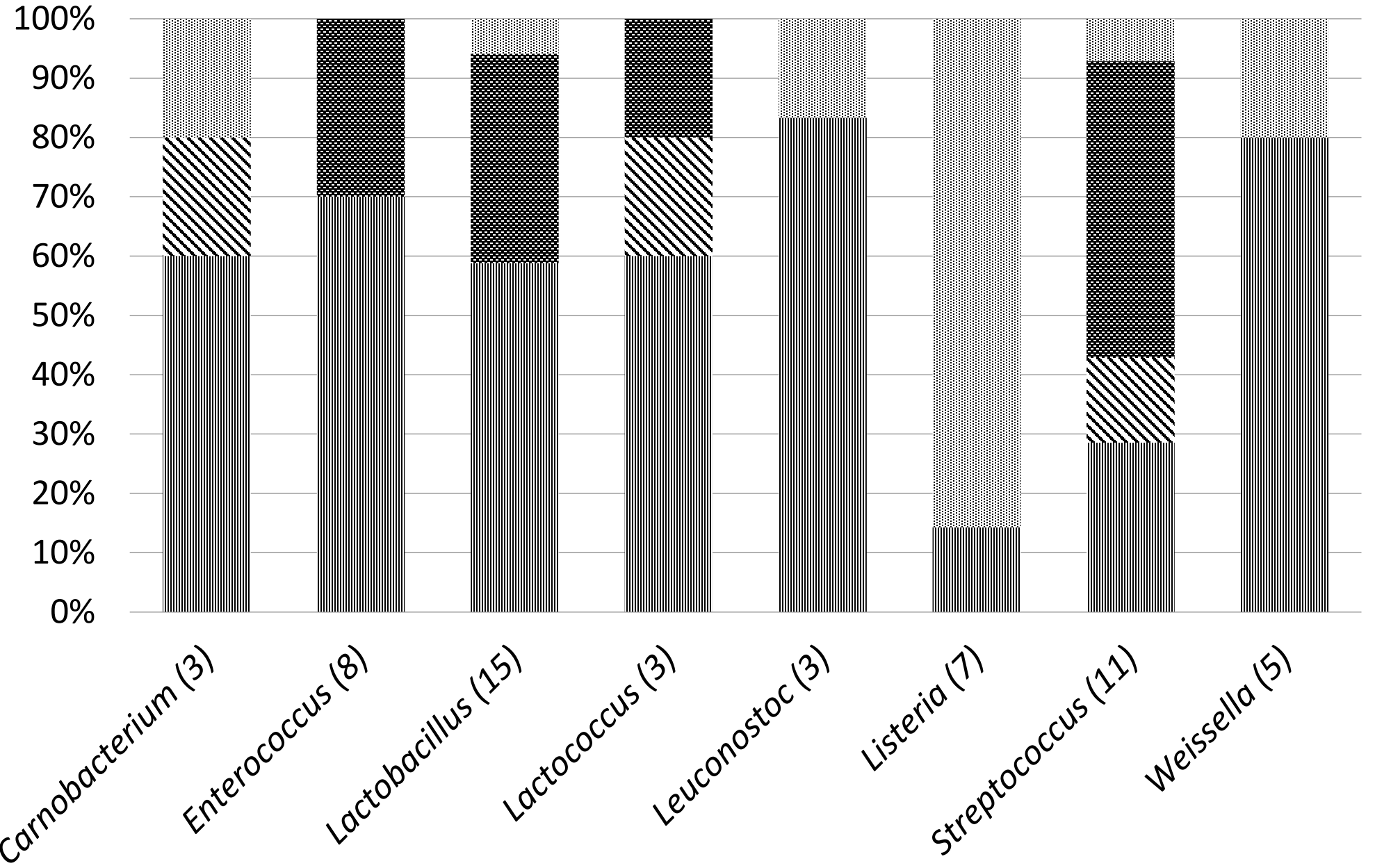


Figure 3-B

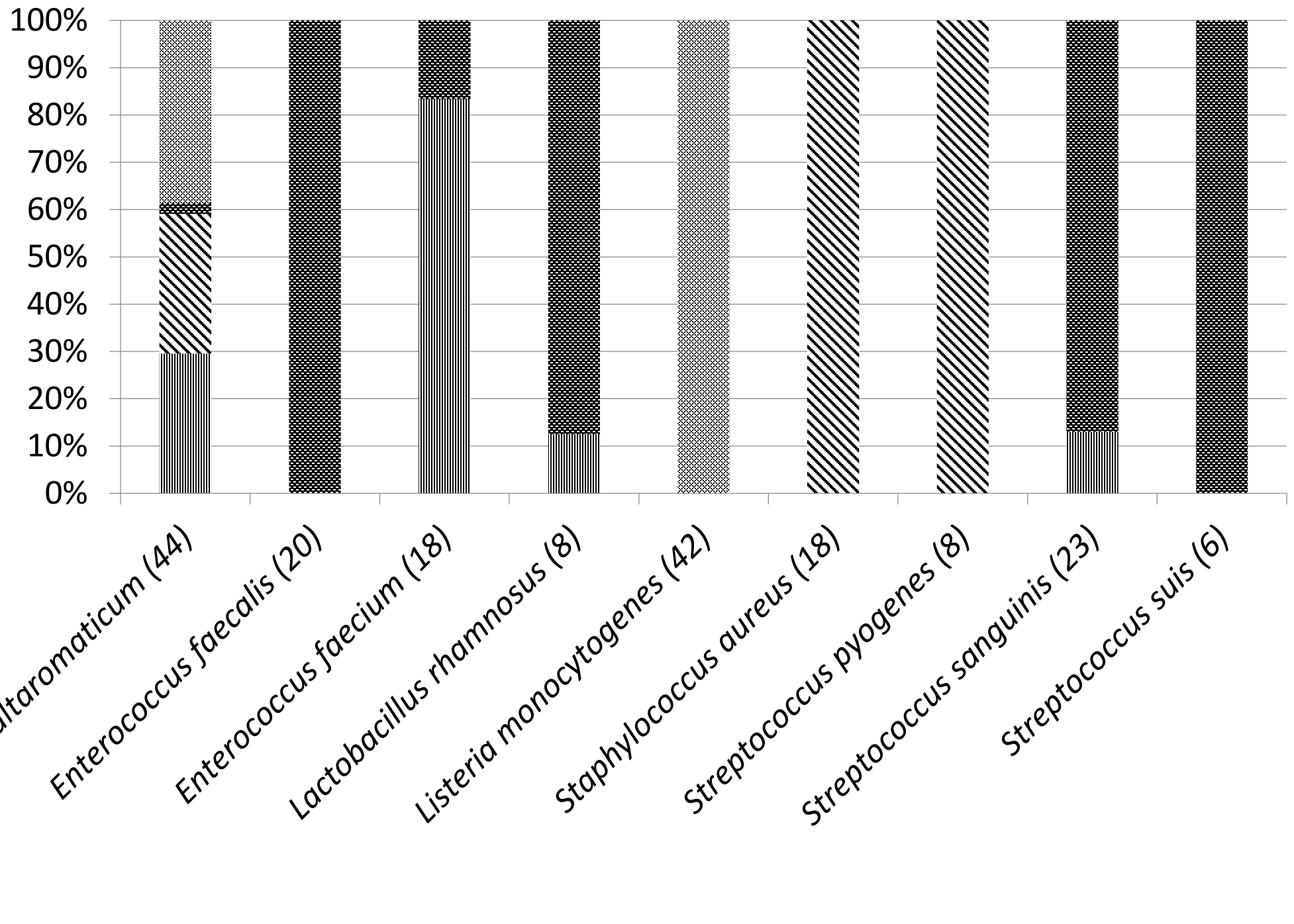


Table 1

	Tagatose-6Phosphate pathway									Leloir pathway						
	<i>lacR</i>	<i>lacA</i>	<i>lacB</i>	<i>lacC</i>	<i>lacD</i>	<i>lacE</i>	<i>lacF</i>	<i>lacG</i>	<i>lacT</i>	<i>galP</i> <i>lacS</i> <i>lacY</i>	<i>lacZ</i> <i>lacLM</i>	<i>galR</i>	<i>galM</i>	<i>galK</i>	<i>galT</i>	<i>galE</i>
<i>Bacillus subtilis</i> 168	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
<i>Carnobacterium gilichinskyi</i> WN1359	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Carnobacterium maltaromaticum</i> 3.18	1	2	0	1	2	1	1	1	0	0	0	1	1	1	1	2
<i>Carnobacterium sp.</i> 17-4	0	0	0	2	1	0	0	0	0	1	0	1	1	1	1	2
<i>Carnobacterium sp.</i> AT7	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1
<i>Enterococcus durans</i> ATCC6056	0	0	0	1	1	0	0	0	0	1	0	1	1	1	1	1
<i>Enterococcus faecium</i> D344SRF, DO NZ_AAAB, TX0133a04, TX0133B, TX0082, E1071, E1636, U0317, E1679, DO NZ_AC1Y, TX0133a01, TX0133A, TX0133C, E1162, E1039,	0	0	0	0	0	1	1	1	0	1	0	1	1	1	1	1
<i>Enterococcus hirae</i> ATCC9790	0	0	0	1	1	0	0	0	0	1	0	1	1	1	1	1
<i>Enterococcus mundtii</i> QU25	0	0	0	0	2	1	1	1	0	1	0	1	1	1	1	1
<i>Lactobacillus acidophilus</i> NCFM	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	1
<i>Lactobacillus brevis</i> ATCC27305	0	0	0	0	0	0	0	0	0	2	1	1	1	1	1	1
<i>Lactobacillus brevis</i> ATCC14869, KB290	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
<i>Lactobacillus brevis</i> ATCC367	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
<i>Lactobacillus fermentum</i> IFO3956	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Lactobacillus helveticus</i> DPC4571	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	1
<i>Lactobacillus johnsonii</i> NCC533	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	2
<i>Lactobacillus plantarum</i> ATCC14917	0	0	0	0	0	0	0	0	0	2	1	1	1	1	1	1
<i>Lactobacillus plantarum</i> JDM1, ST-III	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
<i>Lactobacillus plantarum</i> WCFS	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2
<i>Lactobacillus rhamnosus</i> ATCC5462	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
<i>Lactobacillus sakei</i> 23K	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
<i>Lactobacillus salivarius</i> ATCC11741, GJ-24, NIAS840	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2
<i>Lactobacillus salivarius</i> SMXD51, UCC118	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2
<i>Lactobacillus sp.</i> ASF360	0	0	0	0	0	0	0	0	0	2	1	1	1	1	1	3
<i>Lactococcus garvieae</i> ATCC49156	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
<i>Lactococcus lactis</i> KF147	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2	1
<i>Lactococcus lactis</i> MG1363, IL403	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Lactococcus piscium</i> MKFS47	0	0	0	0	0	1	1	0	0	0	1	1	1	1	1	1
<i>Leuconostoc citreum</i> KM20	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Leuconostoc gasicomitatum</i> LMG18811	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Leuconostoc gelidum</i> JB7	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Leuconostoc kimchii</i> IMSNU11154	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
<i>Leuconostoc mesenteroides</i> J18	0	0	0	0	0	0	0	0	0	3	1	1	1	2	1	1
<i>Leuconostoc mesenteroides</i> ATCC8293	0	0	0	0	0	0	0	0	0	3	1	1	1	3	2	2
<i>Listeria grayi</i> DSM20601	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
<i>Oenococcus oeni</i> AWRIB429, ATCC BAA- 1163, PSU-1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
<i>Pediococcus pentosaceus</i> ATCC25745	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1

<i>Streptococcus salivarius</i> CCHSS3	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
<i>Streptococcus salivarius</i> SK126	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1
<i>Streptococcus sanguinis</i> SK353	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
<i>Streptococcus thermophilus</i> CNRZ1066, LMG18311, LMD-9	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2
<i>Weissella cibaria</i> KACC11862	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
<i>Weissella confusa</i> LBAE C39-2	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1
<i>Weissella koreensis</i> KCTC3621, KACC15510	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
<i>Weissella paramesenteroides</i> ATCC33313	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
<i>Enterococcus avium</i> ATCC14025	1	1	1	2	2	2	2	2	0	1	0	1	1	1	1	1
<i>Enterococcus casseliflavus</i> ATCC12755	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
<i>Enterococcus faecalis</i> V583, TX0635, TX0411, HH22, DAPTO 512, DAPTO 516, R712, S613, TX0104, TX4248, TX1322, TX2134, TX0855, TX0860, TUSoD Ef11, TX0470, PC1.1, ATCC29200, TX0109, TX0102	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
<i>Enterococcus faecium</i> E980, PC4.1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
<i>Enterococcus faecium</i> TX1330	2	2	2	2	2	2	2	2	0	1	0	1	1	1	1	1
<i>Enterococcus italicus</i> DSM15952	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
<i>Lactobacillus curvatus</i> CRL705	1	2	2	1	2	1	1	1	0	0	0	1	1	1	1	2
<i>Lactobacillus johnsonii</i> FI 9785	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1
<i>Lactobacillus murinus</i> ASF361	1	1	1	0	2	1	1	1	1	1	1	1	1	1	1	1
<i>Lactobacillus paracasei</i> 8700:2	1	1	1	2	2	2	1	1	0	1	0	1	1	1	1	1
<i>Lactobacillus rhamnosus</i> CASL, HN001, R0011, LMS2_1, ATCC21052, LC705	1	1	1	1	1	2	2	2	2	0	0	1	1	1	1	1
<i>Lactobacillus rhamnosus</i> GG ATCC53103	1	1	1	1	2	1	1	1	1	0	0	1	1	1	1	1
<i>Lactobacillus casei</i> ATCC334	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
<i>Lactobacillus casei</i> BL23	1	1	1	1	2	1	1	1	1	0	0	1	1	1	1	1
<i>Lactococcus lactis</i> SK11	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
<i>Pediococcus acidilactici</i> DSM20284	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	1
<i>Streptococcus agalactiae</i> A909	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	2
<i>Streptococcus gordonii</i> CH1	1	2	2	2	3	2	2	1	1	0	0	0	0	1	1	1
<i>Streptococcus infantarius</i> ATCC-BA-102	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	2
<i>Streptococcus mutans</i> UA159	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	2
<i>Streptococcus pneumoniae</i> D39	1	1	1	1	1	2	2	2	1	0	0	1	1	1	2	3
<i>Streptococcus pneumoniae</i> R6, TIGR4	1	1	1	1	1	1	1	1	1	0	0	1	1	1	2	3
<i>Streptococcus sanguinis</i> ATCC29667, ATCC49296, 2908, SK36, SK335, SK678, SK1087, SK1057	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
<i>Streptococcus sanguinis</i> SK1, SK1056, SK1058, SK1059, SK115, SK160, SK340, SK405, SK408, SK49, SK72, SK150, SK330	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	2
<i>Streptococcus sanguinis</i> VMC 66	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
<i>Streptococcus suis</i> 05ZYH33, ST3, ST1, SS12, JS14, 98HAH33	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
<i>Aerococcus viridans</i> ATCC11563	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
<i>Carnobacterium maltaromaticum</i> ATCC35586	1	2	2	2	3	2	2	1	0	0	0	0	0	0	0	1
<i>Carnobacterium maltaromaticum</i> LMA28, DSM20342 MX5	1	2	2	2	1	2	2	2	0	0	0	0	0	0	0	0
<i>Lactococcus raffinolactis</i> 4877	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i> 16K, JKD6008, MRSA252, TW20, Z172, T0131, S0385, COL, N315, USA300-TSACH1516, CA-347, USA300-FPR3757, Mu50, MW2, 08BA02176, JH1, 11819-97, Newman NCTC8325	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
<i>Streptococcus agalactiae</i> 2603 V/R	1	1	1	1	1	0	0	0	0	0	0	1	0	0	0	1
<i>Streptococcus agalactiae</i> NEM 316	2	2	2	2	2	1	1	1	1	0	0	0	0	0	0	1

