1 **Impact of probiotic *Lactobacillus* spp. on autochthonous lactobacilli in weaned piglets**

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8 **Running title:** Intestinal survival of lactobacilli

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Abstract

Aims: This study aimed to determine whether host-adapted lactobacilli exhibit superior survival during intestinal transit relative to nomadic and free-living organisms, and to characterize the impact of probiotic lactobacilli on autochthonous lactobacilli.

Methods and Results. Mixed cultures of *Lactobacillus casei* K9-1 and *Lactobacillus fermentum* K9-2, or reutericyclin producing *L. reuteri* and its isogenic mutant were fed to piglets as freeze-dried culture, or as part of fermented feed. Lactobacilli in digesta and fecal samples were quantified by strain-specific qPCR, high-resolution-melting curve qPCR, and high-throughput sequencing of 16S rRNA gene sequence tags. The abundance of the host adapted *L. reuteri* in digesta and feces was higher ($P<0.05$) when compared to *L. casei* or *L. fermentum*. Feed fermentation or chemical acidification of feed reduced ($P<0.05$) cell counts of *L. salivarius* in colonic digesta. The reutericyclin producing *L. reuteri* TMW1.656 transiently reduced ($P<0.05$) the fecal abundance of lactobacilli. However, the overall impact of probiotic intervention on autochthonous lactobacilli was minor.

Conclusions. The vertebrate host-adapted *L. reuteri* survives better during intestinal transit of piglets compared to *L. casei* and *L. fermentum*.

Significance and impact. Ecology and lifestyle of *Lactobacillus* strains may be suitable criteria for selection of probiotic strains for use in swine production.

Key words: Probiotic, *L. reuteri*, feed fermentation, reutericyclin, weaned piglets, high resolution melt curve quantitative PCR

37 **Introduction**

38 Weaning piglets undergo abrupt changes in diet, social and environmental conditions at
39 weaning, and are therefore susceptible to enteric pathogens including *Escherichia coli* and
40 *Clostridium perfringens* (Gresse et al., 2017; Su et al., 2008). Feed antibiotics have been used
41 to manage pig gut microbiota, however, these also contribute to development of antibiotic
42 resistance (Wegener, 2003). Many jurisdictions restrict antibiotics to therapeutic use, therefore
43 prohibiting the prophylactic use of antibiotics and antimicrobial growth promotors. Probiotic
44 bacteria are an alternative to prophylactic antibiotics to prevent diarrheal disease in swine
45 (Valeriano et al., 2017). The efficacy of probiotic *Lactobacillus* spp. to animal health has been
46 extensively documented (for review, see Valeriano et al., 2017).

47 Selection criteria for identification of probiotic lactobacilli are currently lacking. It remains
48 unclear whether health-promoting activities are strain or species specific, or whether these
49 characteristics are generally shared among *Lactobacillus* spp. Moreover, the genus
50 *Lactobacillus* has an exceptional phylogenetic and physiological diversity. The genus
51 encompasses 24 taxonomic groups, each of these represents a diversity that is typically
52 observed in a bacterial genus (Zheng et al., 2015a). Lifestyles of *Lactobacillus* spp. were
53 distinguished as 'free-living', 'nomadic' or 'host-adapted' (Duar et al., 2017b), based on the
54 increasing availability of large-scale analysis of individual *Lactobacillis* species by large-scale
55 comparative genomics in combination with ecological studies (Duar et al., 2017a and 2017b).
56 Host adapted lactobacilli have a stable association with one or more species of vertebrate or
57 insect hosts; free living lactobacilli are adapted to environmental or plant-associated habitats;
58 nomadic lactobacilli combined a free living lifestyle with the ability to temporarily persist in

59 diverse animal or insect hosts (Duar et al., 2017b). This concept provides a rationale for
60 selection of probiotics and an ecological perspective to interpret observations in human and
61 animal trials. Host adapted *Lactobacillus* spp. are present in high cell counts throughout the GI
62 tract of pigs soon after birth and colonize the gastric epithelium (Tannock and Smith, 1970;
63 Tannock et al., 1987). Members of the *L. reuteri*-, *L. delbrueckii*- and *L. salivarius*-groups are
64 dominant; the species *L. reuteri* and *L. amylovorus* are most frequently isolated (Leser et al.,
65 2002; Su et al., 2008). *L. reuteri* is further differentiated in host-adapted lineages that colonize
66 the intestine of swine, chicken, rodents, and humans (Su et al., 2012; Wegmann et al., 2015;
67 Duar et al., 2017a).

68 This study aimed to determine whether host-adapted lactobacilli exhibit superior survival
69 during gastrointestinal transit relative to nomadic and free-living organisms, and to characterize
70 the impact of probiotic lactobacilli on autochthonous lactobacilli. The study employed *L.*
71 *reuteri*, *L. casei*, a species with a nomadic lifestyle without niche specialization (Broadbent et
72 al., 2012) and *L. fermentum*, a species with a free-living lifestyle associated with plant material
73 or environmental habitats (Duar et al., 2017b). The comparison of a reutericyclin producing
74 strains of *L. reuteri* and its isogenic reutericyclin-negative derivative (Lin et al., 2015) was used
75 to assess the impact of specific antimicrobial metabolites on autochthonous lactobacilli.

76 Vegetative cells of probiotic cultures are generally freeze-dried for use in food/feed
77 applications (Ross et al., 2005) but can be alternatively applied in feed fermentations (Yang et
78 al., 2015a; Le et al., 2016). The study therefore delivered probiotic cultures as freeze-dried
79 preparations or as fermentation organisms in fermented feed.

80 Materials and methods

81 **Microorganisms and growth conditions**

82 The reutericyclin producing *L. reuteri* TMW1.656 and the reutericyclin negative mutant *L.*
83 *reuteri* TMW1.656Δ*rtcN* (Lin et al., 2015) and two commercial probiotics, *L. casei* K9-1 and
84 *L. fermentum* K9-2 were routinely grown on modified de Man Rogosa Sharp 5 agar (Meroth et
85 al., 2003) at 37°C under anaerobic conditions. *L. reuteri* TMW1.656 is a rodent-lineage
86 representative of the vertebrate-host adapted species *L. reuteri* with documented probiotic
87 activity in swine (Yang et al., 2015a); *L. casei* has been attributed a nomadic lifestyle and *L.*
88 *fermentum* is associated with environmental habitats (Duar et al., 2017b). Food grade freeze-
89 dried cultures of *L. casei* K9-1 and *L. fermentum* K9-2 with a viable cell count of 10^9 CFU / g
90 were provided by CanBiocin Inc. (Edmonton, AB, Canada). The freeze-dried cultures were
91 stored at 4°C until use.

92 **Experimental diet preparation**

93 Feed fermentations were performed as previously described (Yang et al., 2015a). To confirm
94 the identity of the inoculum with fermentation microbiota, the pH, the viable cell counts and
95 the colony morphology of isolates from of each batch of fermented feed were monitored. Viable
96 cell counts were determined by surface plating of serially diluted samples onto MRS5 agar.

97 The phase I and II basal diets were fed sequentially in the 3-week pig trial and met
98 recommended nutrient requirements for weaning piglets (Table S1 of the online supplementary
99 material). The phase I basal diet was fed from day 1 to day 8 and the phase II basal diet from
100 day 9 to 22 (Figure S1). The basal diet was mixed with wheat, fermented feeds or probiotic
101 cultures to obtain the following dietary treatments: **Diet A**, unfermented wheat; **Diet B**,
102 unfermented wheat acidified to pH 3.8 with lactic acid and acetic acid; **Diet C**, unfermented

103 wheat with freeze-dried cultures of *L. casei* K9-1 and *L. fermentum* K9-2; **Diet D**, wheat

104 fermented with *L. casei* K9-1 and *L. fermentum* K9-2; **Diet E**, wheat fermented with *L. reuteri*

105 TMW1.656; **Diet F**, wheat fermented *L. reuteri* TMW1.656Δ*rtcN*.

106 **Animal experimentation**

107 This study was performed at the University of Alberta Swine Research and Technology Centre

108 (SRTC), University of Alberta (Edmonton, AB, Canada), approved by the University of

109 Alberta Animal Care and Use Committee for Livestock, and followed principles established

110 by the Canadian Council on Animal Care. A total of 48 crossbred castrated male piglets (Duroc

111 × Large White/ Landrace F1) were selected at weaning (21 days old). Each piglet was housed

112 in an individual metabolism pen (0.58 m width x 1.22 m length x 0.76 m height) in a

113 temperature-controlled room (28 ± 2.5^{o}C). The six dietary treatments were randomly allocated

114 to 48 piglets in a randomized block design. Each animal was housed in a single pen to provide

115 8 replicates per dietary treatment. The six experimental diets were administered for 21 days

116 and pigs were killed on day 23. Pigs had free access to feed and water during the trial. Diets

117 were provided at equal amounts twice per day.

118 For bacterial analysis, fresh feces was collected from the pen floors days 0, 7, 14 and 21. The

119 fecal samples were kept at -20°C upon. Digesta of stomach, ileum, cecum and colon were

120 collected at euthanasia and stored at -20°C. Frozen samples were thawed, mixed aseptically by

121 spatula and 2-3 g subsamples were stored at -80°C.

122 **Extraction of DNA from intestinal and fecal microbiota samples**

123 DNA was extracted from intestinal and fecal samples using QIAamp Fast DNA stool mini kit

124 (Qiagen, Inc., Valencia, CA, USA). In brief, approximately 0.2 g of sample was placed into a

125 2 mL tube filled with 0.2 g of silica beads. The sample was homogenized mechanically
126 followed by heating for 15min at 95°C. DNA extraction from pure cultures was performed
127 using Blood & Tissue Kit (Qiagen, Inc., Valencia, CA, USA).

128 **Design of strain-specific primers**

129 Strain-specific primers for *L. casei* K9-1 and *L. fermentum* K9-2 were identified by
130 comparative genomic analysis. Genome sequencing, assembly, and annotation of *L. casei* K9-1
131 and *L. fermentum* K9-2 was conducted by Fusion Genomics (Burnaby, BC, Canada) using *L.*
132 *casei* ATCC 393 and *L. fermentum* 3872 as reference genomes, respectively. Genome
133 sequences for *L. casei* K9-1 and *L. fermentum* K9-2 were compared to 33 and 19 closed
134 genomes of *L. casei* and *L. fermentum*, respectively (Table S2). Strain specific sequences (Table
135 1) were identified by alignment of all genomes of the same species using MAUVE (Darling et
136 al., 2004) (Figure S2, Table S2).

137 Strain-specific primers for *L. reuteri* strains targeted the non-ribosomal peptide synthase RtcN,
138 which is exclusive to five strains of *L. reuteri* including *L. reuteri* TMW1.656 (Table 1, Lin et
139 al., 2015). *L. reuteri* TMW1.656Δ*rtcN* was detected with primer targeting the deleted region of
140 *rtcN*. The primer binding sites are also present in the wild-type strain, however, elongation
141 times prevented amplification of the 3047 bp *rtcN* from the wild-type *L. reuteri* TMW1.656.
142 To evaluate primer specificity *in silico*, primers were analysed by BLAST against the
143 nucleotide collection available on Genbank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). PCR
144 reactions indicated that the strain-specific primers resulted in positive amplicons from genomic
145 DNA of the respective strains (Online supplementary Table 1, Figure S3).

146 ***In silico* validation of group specific primers**

147 Group-specific primers for *L. reuteri* group, *L. delbrueckii* group, and *L. salivarius* group are
148 shown in Table 1. Their specificity was verified with the probe match tool of the Ribosomal
149 Database Project (https://rdp.cme.msu.edu/). Group-specific primers were tested in PCR
150 reactions with template DNA as follows: *L. plantarum* FUA3099, *L. fermentum* K9-2, *L.*
151 *paralimentarius* FUA3121, *L. sanfranciscensis* FUA3458 and *L. casei* K9-1 were used for
152 validation of the general LAB primers; *L. ruminis* FUA3179, *L. animalis* FUA3045 were used
153 for validation of *L. salivarius* group primers; *L. reuteri* TMW1.656 and *L. vaginalis* FUA3049
154 were used for validation of *L. reuteri* group primers; *L. crispatus* DSM29598 was used for
155 validation of *L. delbrueckii* group primers.

156 **Quantitative PCR for detection of probiotic strains**

157 Quantitative PCR reactions were conducted on a 7500 Fast Real-Time PCR System (Applied
158 Biosystems, Foster City, CA, USA). Standard curves for qPCR were generated PCR amplicons
159 obtained with the same primers and genomic DNA of the respective strains as template. Six
160 10-fold serially diluted standard samples were used as template. The number of gene copies
161 for each standard was calculated based on DNA concentrations as determined using Nano-drop
162 spectrophotometer system (Thermo Fisher Scientific Inc., Wilmington, USA) and the
163 molecular weight of the PCR product. The detection limit of the assay was 10^5 gene copies per
164 g. The qPCR reaction mixture with a total volume of 25 µL contains 12.5 µL of Quanti Fast
165 SYBR Green master mix (Applied Biosystems), 0.5 µL of 10 µM forward/ reverse primers
166 (Thermo Fisher Scientific Inc.), 1 µL of template DNA and 10.5 µL of RNase-free water.
167 Technical repeats were conducted for all qPCR reactions.

168 **High-resolution melting (HRM)-qPCR for detection of *Lactobacillus* groups**

169 HRM-qPCR was conducted on Rotor-GeneQ (Qiagen, USA) using Type-it HRM PCR Kit
170 (Qiagen, USA) (Lin and Gänzle, 2014) with group specific primers (Thermo Fisher Scientific
171 Inc.; Table 1). Purified 16S rDNA amplicon derived from *L. ruminis* FUA3179, *L. reuteri*
172 TMW1.656 and *L. crispatus* DSM29598 were used as standards in quantification of *L.*
173 *salivarius* group, *L. reuteri* group, *L. delbrueckii* group, respectively (Lin and Gänzle, 2014).
174 Multiple species per PCR reaction were identified by cloning of PCR products and Sanger
175 sequencing. In brief, 16S rDNA regions were amplified with group-specific primers followed
176 by purification of the PCR products. The resultant purified 16S rDNA amplicon and the vector
177 pUC19 were ligated by T4 ligase after digestion with *SmaI* (Thermo Fisher Scientific Inc.).
178 The ligated plasmid was transformed into *E. coli* DH5α followed by plating onto LB agar
179 containing IPTG (0.2 mmol L^{-1}), X-gal (40 mg L^{-1}) and Ampicillin (50 mg L^{-1}).

180 **High throughput sequencing of 16S rDNA sequence tags**

181 Fragments of genes coding for 16S rRNA were sequenced on an Illumina MiSeq by the
182 University of Minnesota Genomics Center (Minneapolis, MN, USA). The V5-V6 domain of
183 the 16S rRNA gene was amplified using forward and reverse primers
184 GTGCCAGCMGCCGCGGTAA and CGACRRCCATGCANCACCT, respectively, and the
185 amplicons were pooled for pair-end 300-bp reads sequencing. Sequences were analysed on
186 QIIME pipeline (MacQIIME 1.9.1-20150604) (Caporaso et al., 2010). After quality control, a
187 total of 6,647,893 sequences with an average length of 266 bp were obtained, corresponding to
188 an average 34,805 sequences per sample. Operational Taxonomic Units (OTU) clustering was
189 conducted by UCLUST (Edgar, 2010) using the GreenGenes database with 97% similarity
190 threshold after quality-filtering and de-multiplexing. Low abundance OTUs with relative

191 abundance < 0.005% of the total OTUs were discarded (Bokulich et al., 2012). The OTU table
192 was filtered by filter_taxa_from otu_table.py to obtain the OTUs clustered into *Lactobacillus*.
193 The assignment of selected OTUs to phylogenetic groups in the genus *Lactobacillus* (Zheng et
194 al., 2015a) was based on BLAST analysis with the sequences in the NCBI database. The
195 relative abundance was calculated as percentage of the abundance of amplicons representing
196 specific bacterial taxa relative to the total abundance of bacterial rDNA. Mixed Procedure
197 (ProcMIXED) based on repeated measurement under randomized block design was applied to
198 normalized relative abundance of each *Lactobacillus* group.

199 **Statistical analysis**

200 Analysis of qPCR results was performed in SigmaPlot (Systat Software, San Jose, CA). Gene
201 copy numbers of administered probiotic strains, indigenous *L. reuteri* group, *L. delbrueckii*
202 group and *L. salivarius* group in intestinal contents were compared among dietary treatment
203 using two-way ANOVA. Statistical significance was assessed at an error probability of 5% (P
204 < 0.05). Results were expressed as mean ± standard deviations.

205 **Results**

206 **Strain-specific detection of probiotic strains.**

207 A strain-specific quantitative PCR assay was established to monitor the fate of probiotic strains
208 during intestinal transit. Strain-specific primers for *L. reuteri* TMW1.656 and
209 TMW1.656Δ*rtcN* target the reutericyclin biosynthesis gene cluster which is unique to 5 strains
210 of *L. reuteri* (Lin et al., 2015). The strain-specific primers for *L. casei* K9-1 and *L. fermentum*
211 K9-2 target unique sequences that were identified by comparative genomic analysis (Figure S2
212 and S3). Strains were detected in samples from pigs fed the corresponding strains, but not in

213 pigs that were fed other strains, or animals that did not receive probiotics (Table 2). The
214 unexpected presence of probiotic strains in few fecal samples of probiotic-free groups (Table
215 2) likely relates to cross-contamination during sampling or DNA handling. Strains were not
216 observed in intestinal samples of animals that did not receive the respective strain in the diet;
217 this observation excludes contamination during feed preparation.

218 **Fate of ingested probiotic strains through piglet GIT**

219 The cell counts of the strains *L. casei* K9-1, *L. fermentum* K9-2, *L. reuteri* TMW1.656 and
220 TMW1.656Δ*rtcN* in feed are shown in Table 3. The average cell counts of *L. casei* K9-1
221 supplied as freeze-dried culture (Diet C) or by feed fermentation (Diet D) were 7.46 and 8.08
222 log (CFU/g), respectively; the cell counts of *L. fermentum* K9-2 supplied in Diet C and D were
223 7.25 and 7.68 log (CFU/g), respectively. The estimated daily intake of individual probiotic
224 strains was about 10^{10} -10^{11} CFU / day. To assess survival of strains with different lifestyles,
225 probiotics were quantified with strain-specific primers in digesta obtained from the stomach,
226 the ileum, the caecum, and the colon (Figure 1) and in fecal samples (Table 3). Gene copy
227 numbers in colonic digesta were lower ($P < 0.05$) than gene copy numbers in stomach or ileal
228 digesta for all strains except *L. reuteri* TMW1.656Δ*rtcN* (Figure 1). The decline in the number
229 of *L. fermentum* K9-2 from proximal GIT (stomach) to distal GIT (colon) was largest relative
230 to other strains. Gene copy numbers of *L. fermentum* K9-2 in the cecum were lower ($P < 0.05$)
231 when delivered as freeze-dried form compared to delivery of the same strain in fermented feed
232 (Figure 1). *L. reuteri* wild-type strain had a higher ($P < 0.05$) gene copy number in the stomach
233 than its reutericyclin-negative isogenic mutant (Figure 1). Conforming to the abundance of
234 strain specific DNA in intestinal samples, higher gene copies of the *L. reuteri* strains were

235 detected in fecal samples when compared to *L. casei* K9-1 and *L. fermentum* K9-2 (Table 4).
236 Overall, the strain specific detection of lactobacilli in intestinal and fecal samples indicated that
237 the survival of vertebrate host-adapted lactobacilli is higher when compared to other
238 lactobacilli.

239 **Effect of probiotic strains on abundance and composition of autochthonous lactobacilli**

240 To characterize the impact of probiotics on autochthonous lactobacilli, the abundance and
241 composition of autochthonous lactobacilli was analysed in digesta samples collected at the end
242 of the trial. Intestinal lactobacilli all belonged to the *L. reuteri* group, the *L. delbrueckii* group,
243 or the *L. salivarius* group (Table 4). Group-specific HRM-qPCR quantified representatives of
244 these *Lactobacillus* groups. *L. reuteri* constituted the most abundant group in the stomach; the
245 *L. delbrueckii* group was most abundant in colonic digesta while the *L. salivarius* group was a
246 minor component in both intestinal compartments (Figure 2). Probiotics did not alter the
247 composition of *Lactobacillus* populations in the stomach (Figure 2A). The abundance of the *L.*
248 *salivarius* group in the colon was decreased ($P < 0.05$) in animals fed chemically acidified feed
249 or fermented feed, indicating that organic acids may contribute to this effect (Figure 2B).
250 HRM-qPCR discriminates between 16S rDNA amplicons obtained with the same primers by
251 analysis of the melting temperature (Tm), and thus discriminates between closely related
252 species which differ with respect to the Tm of amplicons. Melting peaks obtained in HRM-
253 qPCR analysis were assigned to specific *Lactobacillus* species by using reference strains,
254 Sanger sequencing of PCR amplicons, or 16S rDNA sequences from fecal samples of same
255 piglet. Two melting peaks with Tm 81.8°C and 82.9°C were consistently observed after
256 amplification of 16S rDNA from the *L. salivarius* group in colonic digesta of 10 piglets from

257 all six dietary treatments. Only one melting peak with a Tm of 81.8ºC was observed in samples
258 of remaining 38 piglets (Figure S4A). Sanger sequencing of PCR amplicons from one of the
259 piglets identified amplicons with Tm of 81.8ºC and 82.9ºC as amplicons from *L. salivarius* and
260 *L. ruminis*, respectively (Figure S4A). This assignment matched the predicted Tm of 16S rDNA
261 from *L. ruminis* and *L. salivarius*. *L. salivarius* accounted for about 99% of *L. salivarius* groups
262 organisms in fecal samples (Table 4 and data not shown), in agreement with the consistent
263 presence of the amplicon with Tm 81.8ºC in intestinal samples from all piglets (Figure S4A).
264 In colonic digesta, a single melting peak at Tm 84.1ºC was observed with *L. reuteri* group
265 specific primers (Figure S4B). This Tm matches the Tm of the reference strain of *L. reuteri,*
266 and the prevalence of OTUs assigned to *L. reuteri* in 16S rDNA sequences of fecal samples. A
267 single peak at Tm of 84.5ºC was observed in all samples with primers specific for the *L.*
268 *delbrueckii* group (Figure S4C). This Tm matches the predicted Tm of *L. amylovorus* (84.75°C),
269 *L. johnsonii* (85.0°C), as well as *L. gasseri* (84.75°C); these species can thus not be
270 differentiated by the HRM-qPCR as used in this study. OTUs assigned to *L. amylovorus* and
271 (*L. gasseri* or *L. johnsonii*) accounted for more than 97% of all sequences assigned to the *L.*
272 *delbrueckii* group in fecal samples. In short, HRM-qPCR of intestinal samples provided no
273 indication that probiotic feeding influenced the composition of intestinal lactobacilli at the
274 species level.

275 **Effect of probiotic strains on autochthonous *Lactobacillus* communities in feces**

276 The alteration of intestinal *Lactobacillus* communities in response to probiotics was further
277 analysed by high throughput sequencing of 16S rDNA sequence tags of PCR amplicons from
278 fecal samples. In keeping with prior observations with *L. reuteri* TMW1.656 in weanling

279 piglets (Yang et al., 2015b), probiotic feeding did not induce major change for total fecal
280 microbiota (Figure S5). Sequences matching to the genus *Lactobacillus* were initially analysed
281 at the level of the phylogenetic group (Zheng et al., 2015); results are shown in Table 4.
282 Identification of most lactobacilli at the species level was achieved by a combination of 16S
283 rDNA sequence data and the species-level identification with HRM-qPCR; results of the
284 species level identification are shown in Figure 3. Individual animals differed substantially
285 with respect to the *Lactobacillus* microbiota at baseline (Table 4). The abundances of
286 lactobacilli and the three major *Lactobacillus* groups fluctuated in the first weeks but stabilized
287 after week 2 (Table 4 and Figure 3). Probiotic interventions with *L. casei* and *L. fermentum* did
288 not affect the abundance of *L. reuteri* group, *L. delbrueckii* group or *L. salivarius* group (Table
289 4). Administration of the reutericyclin-positive *L. reuteri* TMW1.656 transiently decreased the
290 abundance of the *L. reuteri* group in fecal samples in comparison to control and *L. casei / L.*
291 *fermentum* fed animals (Table 4) and reduced the proportion of lactobacilli in comparison to
292 the reutericyclin-negative mutant (Figure 3). These results suggest that reutericyclin is a subtle
293 but significant modulator of the *Lactobacillus* community in pigs.

294 **Discussion**

295 The present study investigated the interaction between probiotic bacteria of non-swine origin
296 and *Lactobacillus* communities in the pig intestine. We employed probiotic *L. fermentum*, *L.*
297 *casei*, and *L. reuteri* strains in weaned piglets to i) compare the effect of freeze-dried culture
298 versus fermented cultures on probiotic efficacy in the piglet GIT; ii) develop a culture-
299 independent method for specific quantification of probiotic strains during intestinal transit; and
300 iii) to explore the *in vivo* ecological role of reutericyclin producing by *L. reuteri*. The absolute

301 and relative abundance of three indigenous *Lactobacillus* groups in various regions of the gut,
302 i.e. stomach, ileum, cecum and colon, and in feces, were detected using strain-, group-specific
303 HRM-qPCR, and 16S rDNA amplicon sequencing.

304 **Development of a strain-specific qPCR assay to enumerate probiotic strains.** Strain-
305 specific quantitative PCR differentiated probiotic strains from autochthonous lactobacilli
306 throughout the intestine of weaned piglets. The availability of genome sequences enables
307 identification of strain-specific primers by comparative genomics. Previously, typing methods,
308 such as Random Amplification of Polymorphic DNA (RAPD), Pulsed-Field Gel
309 Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP) and Suppression
310 Subtractive Hybridization (SSH) were used to distinguish between bacterial isolates (Sisto et
311 al., 2009;) (Table S3 and references therein). In addition, strain specific qPCR primers were
312 designed based on strain-specific RAPD banding patterns (Fujimoto et al., 2008), unique
313 metabolic traits (Treven et al., 2013) or ITS-sequences (Vitali et al., 2003). Studies employing
314 these assays are summarized in Table S3. Limitations of these assays include the limited
315 specificity and the requirement for bacterial culture. For example, RAPD and AFLP analyses
316 require re-isolation of strains and cover only 1 - 10% of a bacterial genome, which may be
317 insufficient for strain-level differentiation. SSH relies on the comparison to only one organism
318 (Konstantinov et al., 2005). The present study thus provides a novel approach for strain-specific
319 quantification of probiotic *L. fermentum*, *L. casei* and *L. reuteri* by qPCR.

320 **Survival of freeze-dried and fresh probiotic cultures.** The survival of *L. fermentum* K9-2 in
321 the GI tract was increased when this strain was provided as part of fermented feed compared
322 to delivery of the same strain as freeze-dried culture. In contrast, survival of *L. casei* K9-1 did

323 not depend on the form of delivery. The survival of freeze-dried probiotic strains during
324 intestinal transit is affected by multiple factors including the culture conditions and the pH at
325 harvest of probiotic bacteria, the use of cryoprotectants during freeze-drying, and the
326 composition of the food matrix used for probiotic delivery (Vinderola et al., 2012). In feed
327 applications, endospores of *Bacillus* spp. are currently preferred as probiotic additives due to
328 the resistance of *Bacillus* endospores to the high temperatures during feed production and feed
329 distribution (Krause et al., 2010). Feed fermentation with probiotic cultures is a viable
330 alternative for delivery of probiotics in animal production that eliminates the need for strain
331 preparations with high resistance to heat and dry storage (Le et al., 2016). In analogy, the use
332 of probiotic strains as starter cultures in food fermentations (Marco et al., 2017) may improve
333 their survival during intestinal transit.
334 **Lifestyle of lactobacilli relate to intestinal survival.** Strain-specific primers provided a
335 powerful tool to analyze survival of probiotic lactobacilli and their interaction with
336 autochthonous lactobacilli. The lifestyle of lactobacilli was proposed to determine their
337 suitability for probiotic applications (Duar et al., 2017b); however, this claim has not been
338 substantiated experimentally. *L. casei*, *L. fermentum* and *L. reuteri* represent nomadic, free-
339 living and host-adapted organisms, respectively (Duar et al., 2017b). The host-adapted *L.*
340 *reuteri* strain survived better in the swine intestine compared to nomadic or free-living species.
341 Our study complements and expands observations in human subjects (Frese et al., 2012). *L.*
342 *reuteri* is a symbiont of pigs; the phylogenetic clade IV of *L. reuteri* has evolved separately
343 from other host-specific clades of *L. reuteri* (Frese et al., 2011). However, genetic signatures
344 distinguishing pig-derived *L. reuteri* from strains of other host-adapted clades are lacking

345 (Wegmann et al., 2015) and clade IV pig isolates do not outcompete other strains of *L. reuteri*
346 in the pig gut (Duar et al., 2017a). The lack of swine-specific metabolic traits of *L. reuteri* may
347 account for the improved survival of *L. reuteri* TMW1.656, a sourdough isolate of the rodent-
348 specific clade III (Zheng et al., 2015b) in the pig intestine. The present study thus supports the
349 hypothesis that adaptation of lactobacilli to vertebrate hosts is a relevant criterion for selection
350 of probiotic strains (Walter et al., 2018).
351 **Impact of probiotic strains on autochthonous lactobacilli.** Despite colonization resistance
352 of intestinal microbiota, increasing evidence indicates a role of probiotic strains on modulating
353 autochthonous microbiota if strains are adapted to vertebrate hosts, or to specifically to the host
354 species. Generally, probiotics have only a limited impact on the resident gut microbiome
355 (Zhang et al., 2016; McNulty et al., 2011) and probiotic strains are detectable only for a few
356 days after intake of the probiotic ends (Derrien and van Hylckama Vlieg, 2015). However,
357 temporary or permanent persistence of probiotic strains was observed when the ecological
358 niche was not occupied by closely related species and when the probiotic strain was adapted to
359 the host species (Maldonado-Gómez et al., 2016). The present study investigated weaned
360 piglets, which undergo major shifts in intestinal microbiota including intestinal lactobacilli in
361 the first two weeks post-weaning (Pieper et al., 2006; Su et al., 2008; Yang et al., 2015b). Feed
362 fermentation and probiotic lactobacilli were subtle yet significant modulators on the population
363 of autochthonous lactobacilli. The abundance of the *L. salivarius* group was decreased by feed
364 that was acidified chemically or by fermentation (Figure 2). Strain-specific effects of probiotics
365 on composition of intestinal lactobacilli were observed only for the reutericyclin-producing *L.*
366 *reuteri* TMW1.656. Production of antimicrobial metabolites by probiotic strains is regarded as

367 an important trait for probiotic functionality; past studies particularly discussed bacteriocin
368 formation as a potential probiotic trait (Dobson et al., 2012; Riboulet-Bisson et al., 2012). *L.*
369 *reuteri* TMW1.656, a strain producing the low-molecular weight antimicrobial compound
370 reutericyclin (Gänzle, 2004), affected intestinal microbiota of piglets when compared to a
371 reutericyclin-negative wild-type strain of *L. reuteri* (Yang et al., 2015b). We investigated the
372 role of reutericyclin in shaping *Lactobacillus* populations by comparison of *L. reuteri*
373 TMW1.656 to a reutericyclin-deficient isogenic mutant. The reutericyclin producing *L. reuteri*
374 TWM1.656 persisted better in the stomach of piglets when compared to the reutericyclin-
375 negative mutant; reutericyclin-production by *L. reuteri* also altered fecal *Lactobacillus*
376 communities, indicating that reutericyclin production may displace sensitive autochthonous
377 lactobacilli.

378 In conclusion, the present study compared the persistence of lactobacilli with different
379 lifestyles in the swine intestinal tract. *L. reuteri*, a species adapted to vertebrate hosts, survives
380 better during intestinal transit of piglets compared to either the nomadic *L. casei* or to the free-
381 living *L. fermentum*. Therefore, ecology and lifestyle of *Lactobacillus* strains may be suitable
382 criteria to select probiotic strains for use in swine production. Probiotic lactobacilli had only a
383 limited impact on autochthonous lactobacilli in the swine intestine, however, reutericyclin
384 production had a subtle but significant impact on intestinal microbiota. Probiotic lactobacilli
385 that were delivered with feed fermentations persisted equal to or better in the swine intestine
386 when compared to freeze-dried cultures, indicating that feed fermentation with probiotic
387 cultures is an alternative to dried cultures or bacterial endospores.

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393 **References**

394

395 Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., Mills,
396 D.A., and Caporaso, J.G. (2012) Quality-filtering vastly improves diversity estimates from
397 Illumina amplicon sequencing. *Nat Methods* **10**, 57–59.

398 Broadbent, J. R., Neeno-Eckwall, E. C., Stahl, B., Tandee, K., Cai, H., Morovic, W.,
399 Horvath, P., Heidenreich, J., Perna, N.T., Barrangou, R., and Steele, J.L. (2012) Analysis of
400 the *Lactobacillus casei* supragenome and its influence in species evolution and lifestyle
401 adaptation. *BMC Genomics* **13**, 533.

402 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K.,
403 Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D.,
404 Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder,
405 J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld,
406 J., and Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing
407 data. *Nat Methods* **7**, 335–336.

408

409 Darling, A. C. E., Mau, B., Blattner, F. R., and Perna, N. T. (2004) Mauve: Multiple
410 alignment of conserved genomic sequence with rearrangements. *Genome Res* **14**, 1394–1403.

411 Derrien, M., and van Hylckama Vlieg, J. E. T. (2015) Fate, activity, and impact of ingested
412 bacteria within the human gut microbiota. *Trends Microbiol* **23**, 354–366.
413 Dobson, A., Cotter, P. D., Paul Ross, R., and Hill, C. (2012) Bacteriocin production: A
414 probiotic trait? *Appl Environ Microbiol* **78**, 1–6.
415 Duar, R. M., Frese, S. A., Lin, X. B., Fernando, S. C., Burkey, T. E., Tasseva, G., Peterson,
416 D.A., Blom, J., Wenzel, C.Q., Szymanski, C.M., and Walter, J. (2017a) Experimental
417 evaluation of host adaptation of *Lactobacillus reuteri* to different vertebrate species. *Appl*
418 *Environ Microbiol* **83**, e00132-17.
419 Duar, R. M., Lin, X. B., Zheng, J., Martino, M. E., Grenier, T., Perez-Munoz, M. E., Leulier,
420 F., Gänzle, M., and Walter, J. (2017b) Lifestyles in transition: evolution and natural history
421 of the genus *Lactobacillus*. *FEMS Microbiol Rev*. **41(Suppl. 1),** S27-S48.
422 Edgar, R. C. (2010) Search and clustering orders of magnitude faster than BLAST.
423 *Bioinformatics* **26**, 2460–2461.
424 Frese, S. A., Benson, A. K., Tannock, G. W., Loach, D. M., Kim, J., Zhang, M., Oh, P.L.,
425 Heng, N.C., Patil, P.B., Juge, N., Mackenzie, D.A., Pearson, B.M., Lapidus, A., Dalin, E.,
426 Tice, H., Goltsman, E., Land, M., Hauser, L., Ivanova, N., Kyrpides, N.C., and Walter, J.
427 (2011) The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus*
428 *reuteri*. *PLoS Genet* **7**. e1001314.
429 Frese, S. A., Hutkins, R. W., and Walter, J. (2012) Comparison of the colonization ability of
430 autochthonous and allochthonous strains of lactobacilli in the human gastrointestinal tract.
431 *Adv Microbiol* **2**, 399–409.
432 Fujimoto, J., Matsuki, T., Sasamoto, M., Tomii, Y., and Watanabe, K. (2008) Identification

433 and quantification of *Lactobacillus casei* strain Shirota in human feces with strain-specific
434 primers derived from randomly amplified polymorphic DNA. *Int.J Food Microbiol* **126**, 210–
435 215.
436 Gänzle, M. G. (2004) Reutericyclin: Biological activity, mode of action, and potential
437 applications. *Appl Microbiol Biotechnol* **64**, 326–332.
438 Gresse, R., Chaucheyras-Durand, F., Fleury, M. A., Van de Wiele, T., Forano, E., and
439 Blanquet-Diot, S. (2017) Gut microbiota dysbiosis in postweaning piglets: Understanding the
440 keys to health. *Trends Microbiol* **25,** 851-873.
441 Konstantinov, S. R., Smidt, H., and De Vos, W. M. (2005) Representational difference
442 analysis and real-time PCR for strain-specific quantification of *Lactobacillus sobrius* sp. nov.
443 *Appl Environ Microbiol* **71**, 7578–7581.
444 Krause, D. O., Bhandari, S. K., House, J. D., and Nyachoti, C. M. (2010) Response of
445 nursery pigs to a synbiotic preparation of starch and an anti-*Escherichia coli* K88 probiotic.
446 *Appl Environ Microbiol* **76**, 8192–8200.
447 Leser, T. D., Amenuvor, J. Z., Jensen, T. K., Lindecrona, R. H., Boye, M., and Moøller, K.
448 (2002) Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota
449 revisited. *Appl Environ Microbiol* **68**, 673–690.
450 Le, M. H. A., Galle, S., Yang, Y., Landero, J. L., Beltranena, E., Gänzle, M. G., and Zijlstra,
451 R. T. (2016) Effects of feeding fermented wheat with *Lactobacillus reuteri* on gut
452 morphology, intestinal fermentation, nutrient digestibility, and growth performance in
453 weaned pigs. *J Anim Sci* **94**, 4677–4687.
454 Lin, X. B., and Gänzle, M. G. (2014) Quantitative high-resolution melting PCR analysis for

455 monitoring of fermentation microbiota in sourdough. *Int J Food Microbiol* **186**, 42–48.

456 doi:10.1016/j.ijfoodmicro.2014.06.010.

457 Lin, X. B., Lohans, C. T., Duar, R., Zheng, J., Vederas, J. C., Walter, J., and Gänzle, M.G.

458 (2015) Genetic determinants of reutericyclin biosynthesis in *Lactobacillus reuteri*. *Appl*

459 *Environ Microbiol* **81**, 2032–2041.

460 Maldonado-Gómez, M. X., Martínez, I., Bottacini, F., O'Callaghan, A., Ventura, M., van

461 Sinderen, D., Hillmann, B., Vangay, P., Knights, D., Hutkins, R.W., and Walter, J. (2016).

462 Stable engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on

463 individualized features of the resident microbiome. *Cell Host Microbe* **20**, 515–526.

464 Marco, M.S., Heeney, D., Binda, S., Cifelli, C.J., Cotter, P.D., Foligné, B., Gänzle, M., Kort,

465 R., Pasin, G., Pihlanto, A., Smid, E.J., and Hutkins, R. (2017) Health benefits of fermented

466 foods: Microbiota and beyond. *Curr Op Biotechnol* **44**, 94-102.

467 Matsuda, K., Tsuji, H., Asahara, T., Matsumoto, K., Takada, T., and Nomoto, K. (2009)

468 Establishment of an analytical system for the human fecal microbiota, based on reverse

469 transcription-quantitative PCR targeting of multicopy rRNA molecules. *Appl Environ*

470 *Microbiol* **75**, 1961–1969.

471 Meroth, C. B., Walter, J., Hertel, C., Brandt, M. J., and Hammes, W. P. (2003) Monitoring

472 the bacterial population dynamics in sourdough fermentation processes by using PCR-

473 denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **69**, 475–482.

474 Riboulet-Bisson, E., Sturme, M. H. J., Jeffery, I. B., O'Donnell, M. M., Neville, B. A., Forde,

475 B. M., Claesson, M.J., Harris, H., Gardiner, G.E., Casey, P.G., Lawlor, P.G., O'Toole, P.W.,

476 and Ross, R.P. (2012) Effect of *Lactobacillus salivarius* bacteriocin ABP118 on the mouse

477 and pig intestinal microbiota. *PLoS One* **7,** e31113.

478 Ross, R. P., Desmond, C., Fitzgerald, G. F., and Stanton, C. (2005) Overcoming the
479 technological hurdles in the development of probiotic foods. *J Appl Microbiol*, **98**, 1410–
480 1417.

481 Sisto, A., De Bellis, P., Visconti, A., Morelli, L., and Lavermicocca, P. (2009) Development
482 of a PCR assay for the strain-specific identification of probiotic strain *Lactobacillus*
483 *paracasei* IMPC2.1. *Int J Food Microbiol* **136**, 59–65.

484 Su, M. S.-W., Oh, P. L., Walter, J., and Gänzle, M. G. (2012) Intestinal origin of sourdough
485 *Lactobacillus reuteri* isolates as revealed by phylogenetic, genetic, and physiological
486 analysis. *Appl Environ Microbiol* **78**, 6777–6780.

487 Su, Y., Yao, W., Perez-Gutierrez, O. N., Smidt, H., and Zhu, W. Y. (2008) Changes in
488 abundance of *Lactobacillus* spp. and *Streptococcus suis* in the stomach, jejunum and ileum of
489 piglets after weaning. *FEMS Microbiol Ecol* **66**, 546–555.

490 Tannock, G., Blumershine, R., and Archibald, R. (1987) Demonstration of epithelium-
491 associated microbes in the oesophagus of pigs, cattle, rats and deer. *FEMS Microbiol Lett* **45,**
492 199–203.

493 Tannock, G. W., and Smith, J. M. B. (1970) The microflora of the pig stomach and its
494 possible relationship to ulceration of the pars oesophagea. *J Comp Pathol* **80**, 359-367.

495 Treven, P., Turkova, K., Trmčić A., Obermajer, T., Rogelj, I., and Matijašić B. B. (2013)
496 Detection and quantification of probiotic strain *Lactobacillus gasseri* K7 in faecal samples by
497 targeting bacteriocin genes. *Folia Microbiol (Praha)* **58**, 623–630. doi:10.1007/s12223-013-
498 0252-8.

499 Valeriano, V. D. V., Balolong, M. P., and Kang, D. K. (2017) Probiotic roles of *Lactobacillus*
500 sp. in swine: insights from gut microbiota. *J Appl Microbiol* **122**, 554–567.
501 Vinderola, G., Zacarías, M. F., Bockelmann, W., Neve, H., Reinheimer, J., and Heller, K. J.
502 (2012) Preservation of functionality of *Bifidobacterium animalis* subsp. *lactis* INL1 after
503 incorporation of freeze-dried cells into different food matrices. *Food Microbiol* **30**, 274–280.
504 Vitali, B., Candela, M., Matteuzzi, D., and Brigidi, P. (2003) Quantitative detection of
505 probiotic *Bifidobacterium* strains in bacterial mixtures by using real-time PCR. *Syst Appl*
506 *Microbiol* **26,** 269–276.
507 Walter, J., Maldonado-Gómez, M.X., and Martínez, I. (2018). To engraft or not to engraft: an
508 ecological framework for gut microbiome modulation with live microbes. *Curr Opin*
509 *Biotechnol* **49**, 129-139.
510 Walter, J., Hertel, C., Tannock, G. W., Lis, C. M., Munro, K., and Hammes, W. P. (2001)
511 Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces
512 by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Appl*
513 *Environ Microbiol* **67**, 2578–2585.
514 Wegener, H. C. (2003) Antibiotics in animal feed and their role in resistance development.
515 *Curr Opin Microbiol* **6**, 439–445.
516 Wegmann, U., MacKenzie, D. A., Zheng, J., Goesmann, A., Roos, S., Swarbreck, D., Walter,
517 J., Crossman, L.C., and Juge, N. (2015) The pan-genome of *Lactobacillus reuteri* strains
518 originating from the pig gastrointestinal tract. *BMC Genomics* 16, 1023.
519 Yang, Y., Galle, S., Le, M. H. A., Zijlstra, R. T., and Gänzle, M. G. (2015a). Feed
520 fermentation with reuteran- and levan-producing *Lactobacillus reuteri* reduces colonization

521 of weanling pigs by enterotoxigenic *Escherichia coli*. *Appl Environ Microbiol* **81**, 5743–
522 5752.
523 Yang, Y., Zhao, X., Le, M. H. A., Zijlstra, R. T., and Gänzle, M. G. (2015b) Reutericyclin
524 producing *Lactobacillus reuteri* modulates development of fecal microbiota in weanling pigs.
525 *Front Microbiol* **6**, 762.
526 Zheng, J., Ruan, L., Sun, M., and Gänzle, M. G. (2015a) A genomic view of lactobacilli and
527 pediococci demonstrates that phylogeny matches ecology and physiology. *Appl Environ*
528 *Microbiol* **81**, 7233–7243.
529 Zheng, J., Zhao, X., Lin, X. B., and Gänzle, M. G. (2015b) Comparative genomics
530 *Lactobacillus reuteri* from sourdough reveals adaptation of an intestinal symbiont to food
531 fermentations. *Sci Rep* **5**, 18234.
532

533 **Figure legends**

534 **Figure 1**. Quantification of probiotic lactobacilli in stomach, ileal, cecal, and colonic digesta.
535 Animals received diets containing *L. casei* K9-1 (dark gray bars) and *L. fermentum* K9-2 (light
536 gray bars) in freeze-dried from (**hatched bars**, Diet C), or through fermentation (Diet D), *L.*
537 *reuteri* TMW1.656 (Diet E) or *L. reuteri* TMW1.656Δ*rtcN* (white bars, Diet F). Digesta were
538 sampled at sacrifice after 3 weeks of feeding. Probiotic organisms were detected with strain
539 specific primers to quantify *L. casei* K9-1 (**dark gray bars**); *L. fermentum* K9-2 (**light gray**
540 **bars**); *L. reuteri* TMW1.656 (**black bars**), and *L. reuteri* TMW1.656Δ*rtcN* (**white bars**). Data
541 are presented as mean (n=8) ± standard deviations of 8 replicate observations. One-way
542 ANOVA was performed to assess differences of gene copy numbers between different strains
543 in the same region of gut. Gene copy numbers of different strain in the same compartment of
544 the intestine are significantly ($P < 0.05$) different if bars do not share a common lower case
545 superscript. Gene copy numbers of strains in colonic digesta are marked with an asterisk if they
546 were lower ($P < 0.05$) in comparison to gene copy numbers of the same strain in stomach
547 digesta.

548 **Figure 2**. Gene copy numbers of *L. reuteri* group (black bar), *L. salivarius* group (gray bar), *L.*
549 *delbrueckii* group (white bar) in digesta obtained from the stomach (**Panel A**) and the colon
550 (**Panel B**) of piglets. Digesta were sampled at sacrifice after 3 weeks of feeding. The capital
551 letter codes at X-axis indicate respective diet: Diet A, control; Diet B, acidified control; Diet
552 C, *L. casei* K9-1 and *L. fermentum* K9-2 in freeze-dried form; Diet D, *L. casei* K9-1 and *L.*
553 *fermentum* K9-2 in fermented wheat; Diet E, *L. reuteri* TMW1.656 in fermented wheat; Diet
554 F, *L. reuteri* TMW1.656Δ*rtcN* in fermented wheat. Data is expressed as mean ± standard

555 deviation of 8 individual piglets. The gene copy number was quantified using HRM-qPCR
556 method with a detection limit of 1×10^5 gene copies/ g of digesta. Significant differences
557 between gene copy numbers from animals fed different diets were assessed by one-way
558 ANOVA. Bars without a common superscript differ ($P < 0.05$); superscripts are not shown if
559 none of the values were different.

560 **Figure 3**. Relative abundance of the species *L. reuteri* (white bars), *L. salivarius* (white,
561 hatched bars), *L. amylovorans* (light gray bars), *L. gasseri / L. johnsonii* (dark gray bars), and
562 other members of the *L. delbrueckii* group (black bars) in feces of pigs during the first three
563 weeks post weaning. Sequencing of 16S rDNA tags allowed assignment of *Lactobacillus*
564 sequences at the level of the phylogenetic group (Table 4); the assignment of sequences to
565 specific *Lactobacillus* species was enabled by combination of rDNA sequence data with HRM-
566 qPCR and Sanger sequencing of PCR amplicons (Figure S4). Bars indicate the average
567 abundance of *Lactobacillus* species relative to total rDNA. Different colors represent different
568 species as indicated; letters indicate the different diet as follows. Diet A: control, Diet B:
569 acidified control, Diet C: *L. casei* K9-1 and *L. fermentum* K9-2 in freeze-dried form, Diet D:
570 *L. casei* K9-1 and *L. fermentum* K9-2 in fermented form, Diet E: *L. reuteri* TWM1.656, Diet
571 F: *L. reuteri* TMW1.656 Δ*rtcN*. Data without a common capital superscript differ ($P < 0.05$).

Table 1. Primers used in PCR amplification

Target	Primer	Sequence (5’-3’)	Product size (bp)	Tm (°C)	Reference
Lactobacillus complex[a]	Lab F/ R	AGCAGTAGGGAATCTTCCA / CACCGCTACACATGGAG	341	63	(Walter et al., 2001)
L. reuteri group	sg-Lreu F/ R	GAACGCAYTGGCCCAA / TCCATTGTGGCCGATCAGT	289	60	(Matsuda et al., 2009)
L. delbrueckii group	sg-Ldel F/R	GATGCATAGCCGAGTTGAGAGACTGAT / TAAAGGCCAGTTACTACCTCTATCC	197	60	(Matsuda et al., 2009)
L. salivarius group	sg-Lsal F/R	CACCGAATGCTTGCAYTCACC / GCCGCGGGTCCATCCAAAA	182	60	(Matsuda et al., 2009)
L. casei K9-1	K9-1F/R	GTTGGAGGATCGCGGATTAG / CGTCACCGGAAGTGATGTT	98	62	This study
L. fermentum K9-2	K9-2F/R	CCCACGAGATTGCCCATATT / GAAGATCCATTGCCGTTTCATTAG	111	62	This study
L. reuteri TMW1.656	WT F/R	ACCGGAACATAACAACACCTTA / GAGGTTCCACCGTCATCAAA	105	62	This study
L. reuteri TMW1.656△*rtcN*	rtcN F/R	ACGTTCTAGTAACACAAGTTGGA / TGTAGAGTGTGCTTGAGGAAAG	134	62	This study

[a] Lactic acid bacteria detected by these primers include *Lactobacillus* spp., *Pediococcus* spp., *Weissella* spp., and *Leuconostoc* spp.

Table 2. Gene copy number of the orally administered probiotic strains in fecal samples. Data are presented as average ± SD of 8 pigs per diet.

Microorganism and time (day)[a]	Log (gene copy#/g of wet feces) for the following diets[b]					
	Control	Acidified controls	*L. casei* / *L. fermentum* freeze-dried	*L. casei* / *L. fermentum* fermented	*L. reuteri* TMW1.656	*L. reuteri* TMW1.656Δ*rtcN*
Lactobacillus complex						
0	9.2±0.9	9.2±0.7	9.4±0.9	9.8±0.4	9.7±0.9	9.3±0.8
7	10.0±0.3	10.3±0.6	10.3±0.6	10.5±0.7	9.9±0.5	9.7±0.5
14	10.0±0.6	9.9±0.8	10.3±0.3	10.0±0.7	9.6±0.6	10.0±0.7
21	9.7±0.8	9.6±0.5	9.7±0.6	10.3±0.7	9.6±0.6	9.5±0.6
L. reuteri group						
0	8.7±1.4	8.1±1.2	8.8±1.8	9.2±1.2	8.6±1.6	9.4±1.4
7	10.1±0.5	10.0±0.8	10.4±0.2	9.6±0.7	9.8±1.3	9.4±1.5
14	10.2±0.4	10.4±0.4	10.3±0.4	10.1±0.5	9.8±0.6	9.7±0.8
21	10.2±0.5	9.9±0.4	10.0±0.3	10.3±0.5	9.5±0.5	9.6±0.7
L. casei K9-1						
0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
7	<5.0	<5.0	8.8±0.3	8.5±0.5	<5.0	<5.0
14	<5.0	<5.0	9.4±0.7^{A}	8.7±0.7^{B}	<5.0	<5.0
21	<5.0	<5.0	9.0±0.4	8.8±0.32	<5.0	<5.0
L. fermentum K9-2						
0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
7	<5.0	<5.0	5.9±0.7^{B}	6.8±1.1^{A}	<5.0	<5.0
14	<5.0	<5.0	6.4±0.6^{A}	6.7±0.5^{A}	5.6±0.6^{B}	<5.0
21	<5.0	<5.0	6.0±0.8	6.8±0.5	<5.0	<5.0
L. reuteri TMW1.656						
0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
7	<6.0	<6.0	<6.0	<6.0	9.8±0.7	<6.0
14	<6.0	<6.0	<6.0	<6.0	9.4±0.4	<6.0
21	<6.0	<6.0	<6.0	<6.0	9.3±0.2	<6.0
L. reuteri TMW1.656△*rtcN*						
0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
7	<6.0	<6.0	<6.0	7.2±0.8^{B}	<6.0	9.2±0.5^{A}
14	<6.0	<6.0	<6.0	<6.0	<6.0	8.9±0.7
21	<6.0	<6.0	<6.0	<6.0	<6.0	8.9±0.3

[a] Fecal samples were collected weekly in the 3-week animal experiment, at day 0, 7, 14 and 21.

[b] Gene copy number of respective probiotic strains was determined by qPCR assay. Data are represented as mean ± standard deviations of 8 animals. Data for the same strain in the same row without a common capital superscript differ ($P < 0.05$).

Table 3. Viable cell counts of probiotic strains in pig diets

Diet	Cell count in diet (log CFU/g)[a,b,c]				
	L. casei K9-1	*L. fermentum* K9-2	*L. reuteri* TMW1.656	*L. reuteri* TMW1.656△*rtcN*	Total
Control	n.d.[C]	n.d.	n.d.	n.d.	n.d.
Acidified control	n.d.	n.d.	n.d.	n.d.	n.d.
Freeze-dried *L. casei* and *L. fermentum*	7.5±0.4	7.3±0.5	n.d.	n.d.	7.7±0.4
Fermented *L. casei* and *L. fermentum*	8.1±0.5	7.7±0.4	n.d.	n.d.	8.2±0.5
L. reuteri TMW1.656	n.d.	n.d.	8.4±0.5	n.d.	8.4±0.5
L. reuteri TMW1.656△*rtcN*	n.d.	n.d.	n.d.	8.3±0.5	8.3±0.5

[a] Cell counts of respective strains.

[b] Data is represented as mean ± SD. The average was calculated on 25 samples for each diet collected daily over the 3-week animal trial.

[c] n.d., cell counts below the detection limit of 10^5 CFU/g.

Table 4. Abundance of rDNA corresponding to the *L. reuteri* group, *L. salivarius* group, *L. delbrueckii* group and *Lactobacillus* spp. relative to total bacterial rDNA in feces of piglets during the first 3 weeks post weaning. Data were determined by sequencing of 16S rDNA tags, and are represented as mean ± SD of 8 pigs. Within each row, means without common capital superscript differ ($P < 0.05$). Within each column, means without common lowercase superscript differ ($P < 0.05$).

Group / Collection day	Control	Acidified controls	*L. casei* / *L. fermentum* freeze-dried	*L. casei* / *L. fermentum* fermented	*L. reuteri* TMW1.656	*L. reuteri* TMW1.656Δ*rtcN*
L. reuteri group						
0	0.6±0.7 $^{B, b}$	2.1±3.2 ^{A}	1.7±2.0 $^{AB, b}$	0.7±1.0 $^{B, b}$	1.7±2.4 ^{AB}	0.6±0.76 $^{B, b}$
7	1.7±1.2 $^{AB, a}$	3.7±3.9 ^{AB}	2.5±1.6 $^{AB, ab}$	4.7±4.0 $^{A, a}$	1.6±1.3 ^{B}	2.1±2.29 $^{AB, ab}$
14	3.4±3.3 $^{A, a}$	2.7±1.8 ^{AB}	3.6±2.1 $^{A, a}$	2.3±1.2 $^{AB, a}$	1.1±0.5 ^{B}	2.1±1.68 $^{AB, a}$
21	1.6±0.6 $^{AB, a}$	2.5±2.4 ^{AB}	2.1±1.2 $^{AB, ab}$	2.7±1.4 $^{A, a}$	1.0±0.3 ^{B}	2.7±2.47 $^{AB, a}$
L. salivarius group						
0	0.05±0.06	0.1±0.2	0.04±0.06	0.03±0.1	0.08±0.1	0.02±0.04
7	0.03±0.05 ^{AB}	0.03±0.07 ^{AB}	0.08±0.1 ^{AB}	1.7±4.7 ^{A}	0.08±0.1 ^{AB}	0.01±0.02 ^{B}
14	0.02±0.02	0.02±0.03	0.03±0.07	0.03±0.04	0.01±0.01	0.01±0.02
21	0.05±0.10	0.03±0.07	0.01±0.01	0.04±0.05	0.00±0.00	0.01±0.01
L. delbrueckii group						
0	1.9±1.5 $^{B, b}$	2.9±3.3 $^{AB, b}$	3.0±2.6 $^{AB, b}$	1.9±1.7 $^{B, b}$	4.0±4.1 ^{A}	2.5±3.6 $^{AB, b}$
7	6.8±6.5 ^{a}	11.5±7.3 ^{a}	7.4±4.8 ^{a}	12.6±13.7 ^{a}	6.2±4.6	9.1±12.2 ^{a}
14	4.6±2.9 ^{a}	5.6±4.6 ^{ab}	6.0±2.7 ^{a}	5.9±4.1 ^{a}	4.2±2.0	9.1±6.7 ^{a}
21	5.5±3.5 ^{a}	8.8±4.0 ^{a}	5.3±2.0 ^{a}	6.0±3.3 ^{a}	7.1±3.7	6.8±3.7 ^{a}
Lactobacillus spp.						
0	2.5±1.6 $^{B, b}$	5.1±5.8 $^{A, b}$	4.8±4.4 $^{A, b}$	2.7±2.5 $^{B, b}$	5.8±6.2 ^{A}	3.1±4.3 $^{AB, b}$
7	8.5±7.5 $^{B, a}$	15.2±6.3 $^{A, a}$	10.0±5.0 $^{AB, a}$	19.1±20.5 $^{A, a}$	7.9±5.0 ^{B}	11.2±14.3 $^{AB, a}$
14	8.0±3.5 $^{AB, a}$	8.3±4.9 $^{AB, ab}$	9.6±3.7 $^{A, a}$	8.3±4.8 $^{AB, a}$	5.1±2.5 ^{B}	11.2±7.1 $^{A, a}$
21	7.1±3.6 ^{a}	11.30±5.31 ^{a}	7.5±2.5 ^{a}	8.7±3.1 ^{a}	8.0±3.8	9.6±5.4 ^{a}

Figure 1.

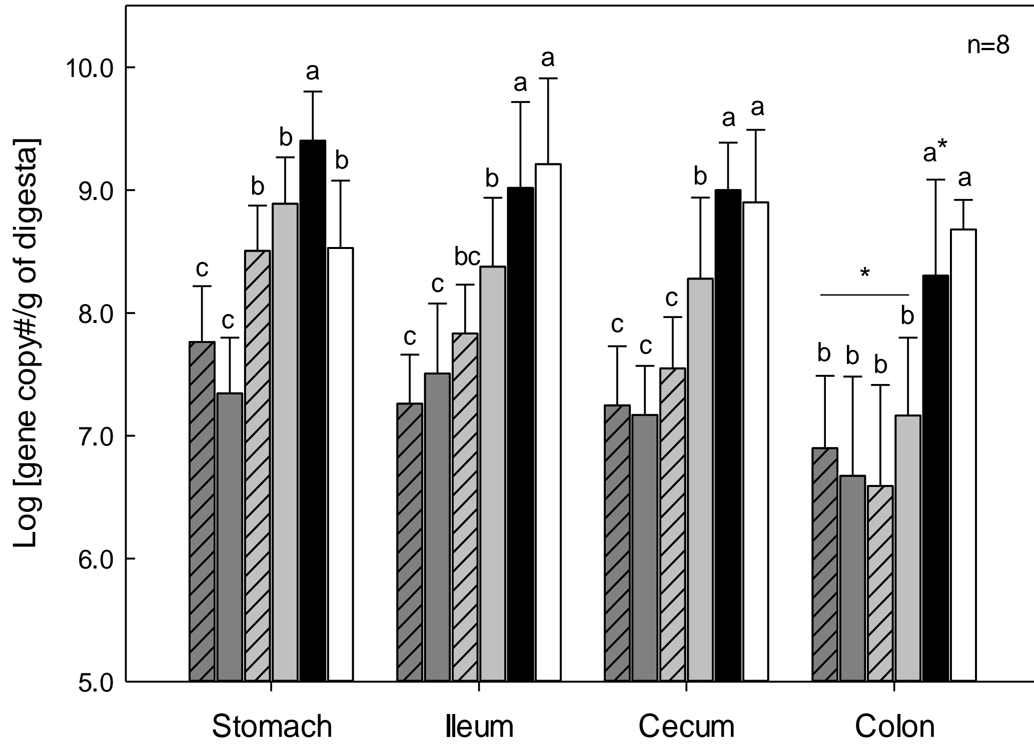


Figure 2.

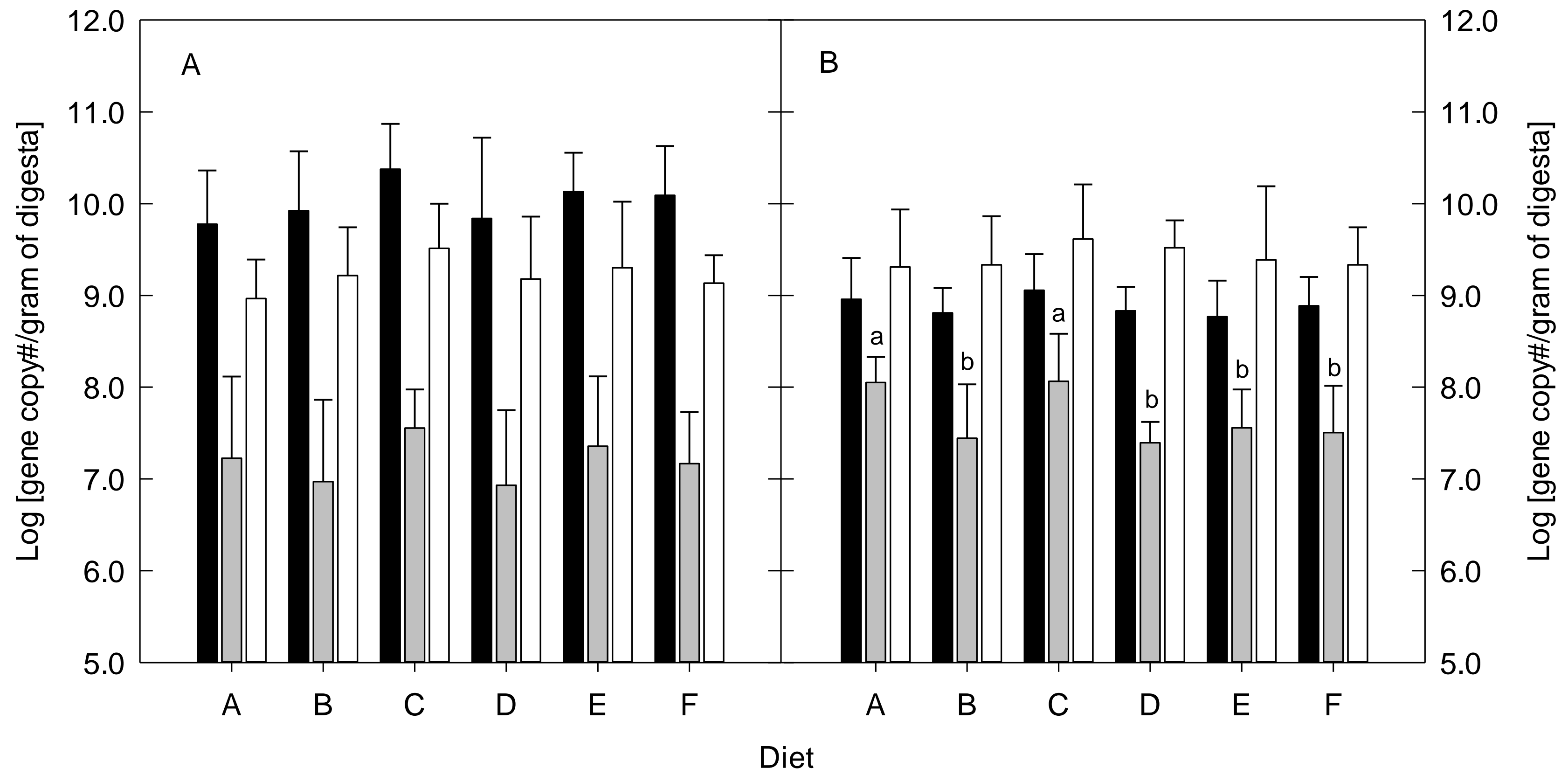
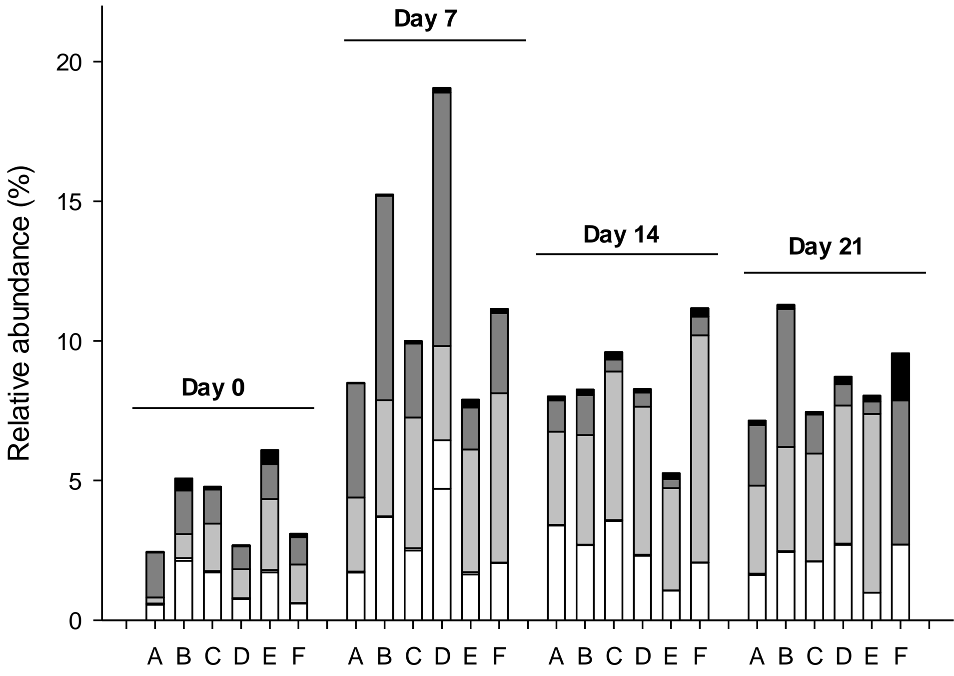


Figure 3.



Online supplementary material

Impact of probiotic *Lactobacillus* spp. on autochthonous lactobacilli in weaned piglets

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2) CanBiocin Inc., Edmonton, Canada.

Table S1. Ingredient composition of basal diets.

Table S2. Genomes used for multiple genome alignment

Table S3. Summary of studies reporting strain-specific identification or quantification methods

Figure S1. Overview on the animal experimental design. Animals were started on the experimental diets at day 1 after weaning.

Figure S2. Genome alignments of *L. casei* and *L. fermentum* for design of strain specific primers.

Figure S3. Visualization of gel electrophoresis for PCR amplicons obtained with genomic DNA of lactobacilli and strain-specific primers

Figure S4. First derivatives of melt curves of PCR products obtained from digesta microbiota DNA with *Lactobacillus* group-specific primers.

Figure S5. Principle Coordinate Analysis (PCoA) of fecal microbiota composition based on unweighted unifrac distance of rDNA sequences.

Table S1. Ingredient composition of basal diets.

Ingredient[a]	Composition (%)	
	Phase I[b]	Phase II[b]
Wheat, hard red spring	20.00	50.00
Corn	31.54	1.76
Lactose	15.00	10.00
Soybean meal	15.00	15.00
Brassica napus canola meal		2.50
Soy protein concentrate	3.00	2.50
Herring meal	6.00	2.50
Corn distillers dried grain with solubles		5.00
Canola oil	4.00	3.40
Limestone	1.15	1.10
Salt	0.50	0.50
Other vitamin and mineral ingredients	3.31	5.24
TiO_2	0.50	0.50

[a] Composition of basal diets fulfills the National Research Council (NRC) recommendations (2012) for pigs (5-11 kg body weight).

[b] Phase I was day 0 to 6 and Phase II was day 7 to 21.

Table S2. Genomes used for multiple genome alignment

Genome Accession	NCBI FTP site
Lactobacillus casei	
GCA_000014525.1_ASM1452v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/014/525/GCA_000014525.1_ASM1452v1
GCA_000019245.3_ASM1924v3	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/019/245/GCA_000019245.3_ASM1924v3
GCA_000026485.1_ASM2648v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/026/485/GCA_000026485.1_ASM2648v1
GCA_000194765.1_ASM19476v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/194/765/GCA_000194765.1_ASM19476v1
GCA_000194785.1_ASM19478v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/194/785/GCA_000194785.1_ASM19478v1
GCA_000309565.2_ASM30956v2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/565/GCA_000309565.2_ASM30956v2
GCA_000309585.1_ASM30958v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/585/GCA_000309585.1_ASM30958v1
GCA_000309605.1_ASM30960v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/605/GCA_000309605.1_ASM30960v1
GCA_000309625.1_ASM30962v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/625/GCA_000309625.1_ASM30962v1
GCA_000309645.1_ASM30964v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/645/GCA_000309645.1_ASM30964v1
GCA_000309665.1_ASM30966v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/665/GCA_000309665.1_ASM30966v1
GCA_000309685.1_ASM30968v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/685.GCA_000309685.1_ASM30968v1
GCA_000309705.1_ASM30970v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/705/GCA_000309705.1_ASM30970v1
GCA_000309725.1_ASM30972v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/725/GCA_000309725.1_ASM30972v1
GCA_000309745.1_ASM30974v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/745/GCA_000309745.1_ASM30974v1
GCA_000309765.1_ASM30976v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/765/GCA_000309765.1_ASM30976v1
GCA_000309785.1_ASM30978v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/309/785/GCA_000309785.1_ASM30978v1
GCA_000318035.1_ASM31803v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/318/035/GCA_000318035.1_ASM31803v1
GCA_000376145.1_ASM37614v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/376/145/GCA_000376145.1_ASM37614v1
GCA_000388095.2_LcY_assembly050913	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/388/095/GCA_000388095.2_LcY_assembly050913
GCA_000400585.1_LcA_0213	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/400/585/GCA_000400585.1_LcA_0213
GCA_000418515.1_ASM41851v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/418/515/GCA_000418515.1_ASM41851v1
GCA_000472345.1_ASM47234v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/472/345/GCA_000472345.1_ASM47234v1
GCA_000474615.1_Lcasei5b_2.0	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/474/615/GCA_000474615.1_Lcasei5b_2.0
GCA_000510825.1_ASM51082v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/510/825/GCA_000510825.1_ASM51082v1
GCA_000615205.1_ASM61520v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/615/205/GCA_000615205.1_ASM61520v1
GCA_000736295.3_L._casei_Hybrid_assembly	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/736/295/GCA_000736295.3_L._casei_Hybrid_assembly
GCA_000827145.1_ASM82714v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/827/145/GCA_000827145.1_ASM82714v1
GCA_000829055.1_ASM82905v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/829/055/GCA_000829055.1_ASM82905v1
GCA_001013375.1_ASM101337v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/013/375/GCA_001013375.1_ASM101337v1
GCA_001066565.1_ASM106656v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/066/565/GCA_001066565.1_ASM106656v1
GCA_001066695.1_ASM106669v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/066/695/GCA_001066695.1_ASM106669v1
GCA_001433735.1_ASM143373v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/433/735/GCA_001433735.1_ASM143373v1

Genome Accession	NCBI FTP site
Lactocbacillus fermentum	
GCA_000010145.1_ASM1014v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/010/145/GCA_000010145.1_ASM1014v1
GCA_000159215.1_ASM15921v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/159/215/GCA_000159215.1_ASM15921v1
GCA_000162395.1_ASM16239v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/162/395/GCA_000162395.1_ASM16239v1
GCA_000210515.1_ASM21051v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/210/515/GCA_000210515.1_ASM21051v1
GCA_000397165.1_ASM39716v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/397/165/GCA_000397165.1_ASM39716v1
GCA_000417005.1_ASM41700v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/417/005/GCA_000417005.1_ASM41700v1
GCA_000466785.3_ASM46678v3	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/466/785/GCA_000466785.3_ASM46678v3
GCA_000472265.1_LF1_1.0	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/472/265/GCA_000472265.1_LF1_1.0
GCA_000477515.1_Reference_Assembly	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/477/515/GCA_000477515.1_Reference_Assembly
GCA_000496435.1_LfermNB22_1.0	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/496/435/GCA_000496435.1_LfermNB22_1.0
GCA_000966835.1_ASM96683v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/966/835/GCA_000966835.1_ASM96683v1
GCA_001010185.1_ASM101018v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/010/185/GCA_001010185.1_ASM101018v1
GCA_001010245.1_ASM101024v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/010/245/GCA_001010245.1_ASM101024v1
GCA_001039735.1_LFE2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/039/735/GCA_001039735.1_LFE2
GCA_001077025.1_ASM107702v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/077/025/GCA_001077025.1_ASM107702v1
GCA_001297025.1_ASM129702v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/297/025/GCA_001297025.1_ASM129702v1
GCA_001297905.1_ASM129790v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/297/905/GCA_001297905.1_ASM129790v1
GCA_001368755.1_LF_newbler2.7	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/368/755/GCA_001368755.1_LF_newbler2.7
GCA_001436835.1_ASM143683v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/436/835/GCA_001436835.1_ASM143683v1

Table S3. Summary of studies reporting strain-specific identification or quantification methods

Strain	Culture independent (Yes/No)	Method	Identification/ quantification	Sample type	Ref.
L. reuteri DSM 16350	Yes	SSH & strain specific qPCR	Quantification	Chicken feed and intestine	(Sattler et al., 2014)
L. sobrius 001	Yes	Representational difference analysis (RDA) & strain-specific qPCR	Quantification	Pure culture mix	(Konstantinov et al., 2005)
L. rhamnosus GG	Yes	RAPD & strain-specific qPCR	Quantification	Human feces	(Ahlroos and Tynkkynen, 2009)
Lactococcus lactis subsp. *cremoris* FC	Yes	RAPD & strain-specific qPCR	Quantification	Human feces	(Maruo et al., 2006)
B. longum Y10, *B. infantis* Y1 and *B. breve* Y8	Yes	ITS (*in silico* comparison) & strain-specific qPCR	Quantification	Yogurt/ Human feces	(Vitali et al., 2003)
L. gasseri 4B2	No	Colony-multiplex PCR	Identification	Mouse feces	(Lucchini et al., 1998)
L. rhamnosus Lc 1/3	Yes	RAPD & PCR	Identification	Pure culture mix	(Tilsala-Timisjärvi and Alatossava, 1998)
L. paracasei LTH 2579	No	Subtraction hybridization & PCR	Quantification	Fermented sausage/ human feces	(Bunte et al., 2000)
L. paracasei IMPC2.1	Yes	f-AFLP & PCR	Identification	Pure culture mix	(Sisto et al., 2009)
L. rhamnosus 35	Yes	Subtractive hybridization & PCR	Identification	Pure culture mix	(Coudeyras et al., 2008)
L. gasseri K7	Yes	qPCR targeting bacteriocin gene	Quantification	Human feces	(Treven et al., 2013)
L. casei strain Shirota	Yes	RAPD & strain-specific qPCR	Quantification	Human feces	(Fujimoto et al., 2008)
L. reuteri TMW1.656	Yes	*in silico* comparison & strain-specific qPCR targeting RTC biosynthesis gene	Quantification	Pig intestine and feces	(Yang et al., 2015a)
Bifidobacterium longum AH1206	Yes	*in silico* comparison & strain-specific qPCR	Quantification	Human feces	(Maldonado-Gómez et al., 2016)
L. salivarius abp118	No	Selective medium for rifampicin resistance plasmid	Quantification	Mouse and pig intestine and feces	(Riboulet-Bisson et al., 2012)
L. reuteri ATCC PTA 6475 and *L. mucosae* FSL-04	No	RAPD typing	Quantification	Human feces	(Frese et al., 2012)
Bifidobacterium breve strain Yakult (BbrY)	No	RAPD & strain-specific qPCR	Quantification	Human feces	(Fujimoto et al., 2011)

Ahlroos, T., and Tynkkynen, S. (2009) Quantitative strain-specific detection of *Lactobacillus rhamnosus* GG in human faecal samples by real-time PCR. *J Appl Microbiol* **106,** 506–514.;

Bunte, C., Hertel, C., and Hammes, W. P. (2000) Monitoring and survival of *Lactobacillus paracasei* LTH 2579 in food and the human intestinal tract. *Syst Appl Microbiol* **23**, 260–266.

Coudeyras, S., Marchandin, H., Fajon, C., and Forestier, C. (2008) Taxonomic and strain-specific identification of the probiotic strain *Lactobacillus rhamnosus* 35 within the *Lactobacillus casei* group. *Appl Environ Microbiol* **74**, 2679–2689.

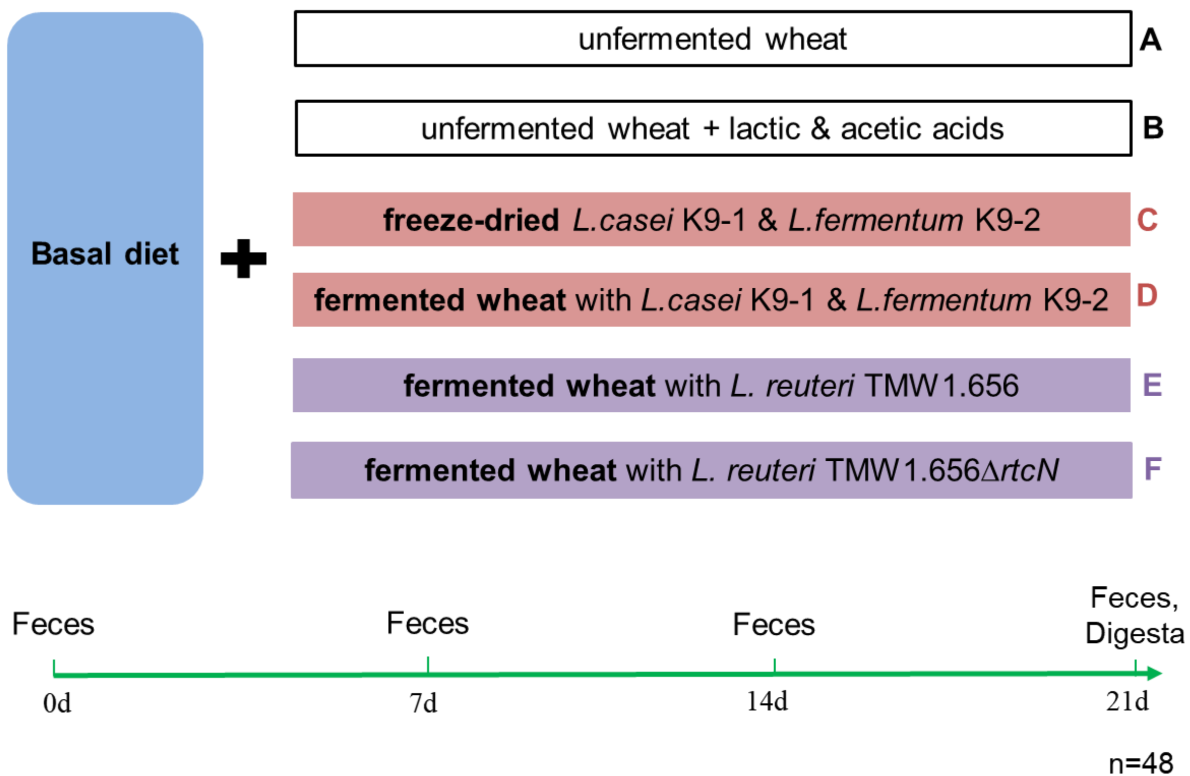
Lucchini, F., Kmet, V., Cesena, C., Coppi, L., Bottazzi, V., and Morelli, L. (1998) Specific detection of a probiotic *Lactobacillus* strain in faecal samples by using multiplex PCR. *FEMS Microbiol Lett* **158**, 273–278.

Maruo, T., Sakamoto, M., Toda, T., and Benno, Y. (2006) Monitoring the cell number of *Lactococcus lactis* subsp. *cremoris* FC in human feces by real-time PCR with strain-specific primers designed using the RAPD technique. *Int J Food Microbiol* 110, 69–76.

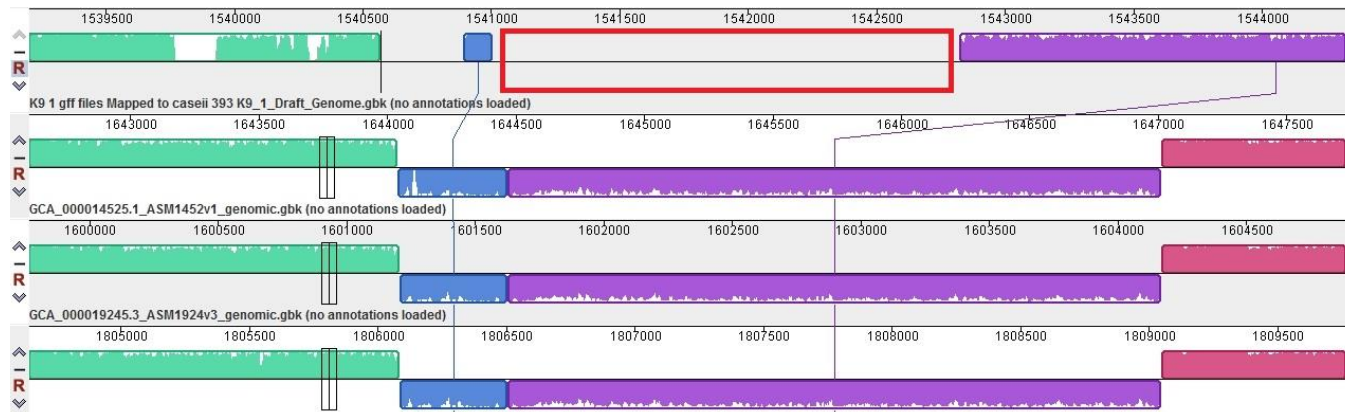
Sattler, V. A., Mohnl, M., and Klose, V. (2014) Development of a strain-specific real-time PCR assay for enumeration of a probiotic *Lactobacillus reuteri* in chicken feed and intestine. *PLoS One* **9**, e90208.

Tilsala-Timisjärvi, A., and Alatossava, T. (1998) Strain-specific identification of probiotic *Lactobacillus rhamnosus* with randomly amplified polymorphic DNA-derived PCR primers. *Appl Environ Microbiol* **64**, 4816–4819.

Figure S1. Overview on the animal experimental design. Eight animals per treatment group were started on the experimental diets at 21 days of age, one day after weaning; experimental diets were fed for 21 days.



(A)



(B)

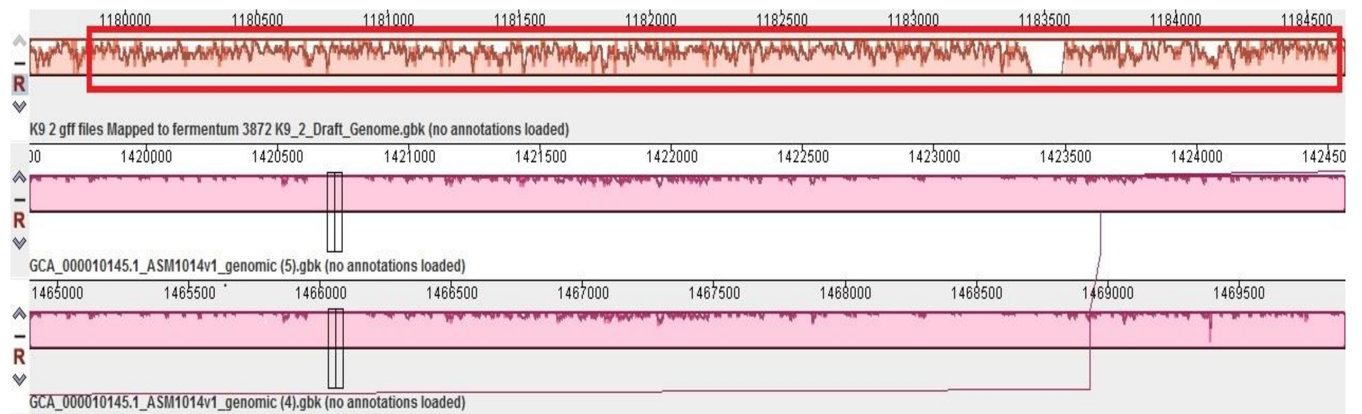
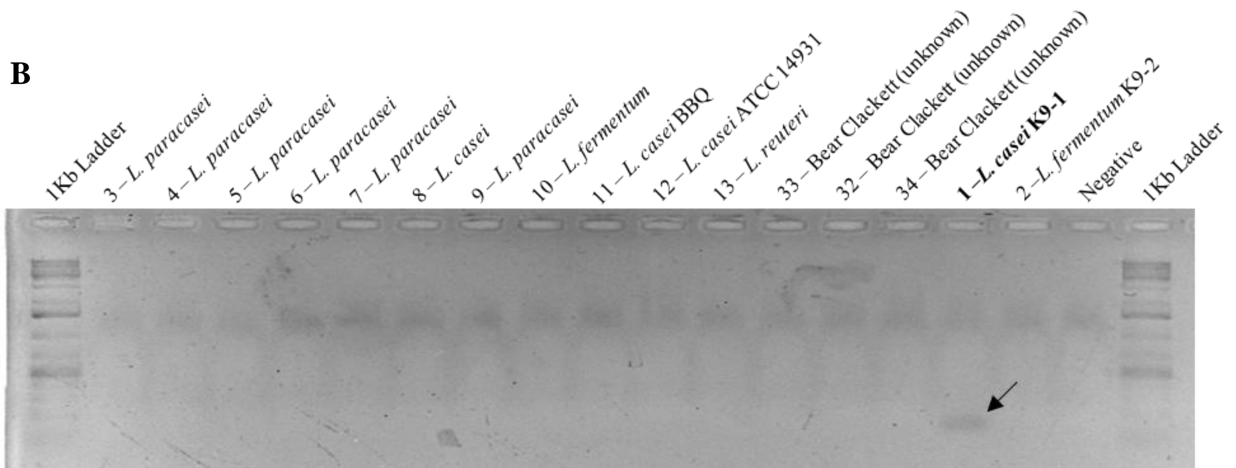


Figure S2. Genome alignments of *L. casei* and *L. fermentum* for design of strain specific primers. (**Panel A**) Genome alignment of *L. casei* K9-1 against genomes of *L. casei*. Shown is the comparison of an area of interest to the three of 33 strains that are most closely related to *L. casei* K9-1. The white area was selected as unique sequence region (highlighted by red box) for strain specific primer design. (**Panel B**) Genome alignment of *L. fermentum* K9-2 against genomes of *L. fermentum*. Shown is the comparison of an area of interest to the two of 19 strains that are most closely related to *L. fermentum* K9-2. The brown block was selected as unique sequence region (highlighted by red box) for strain specific primer design.



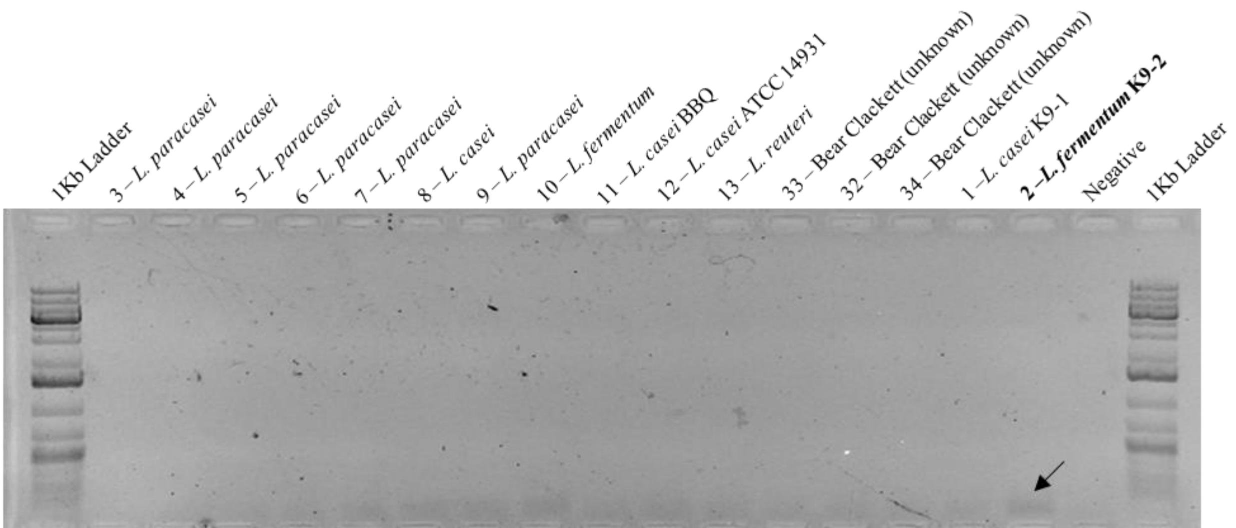
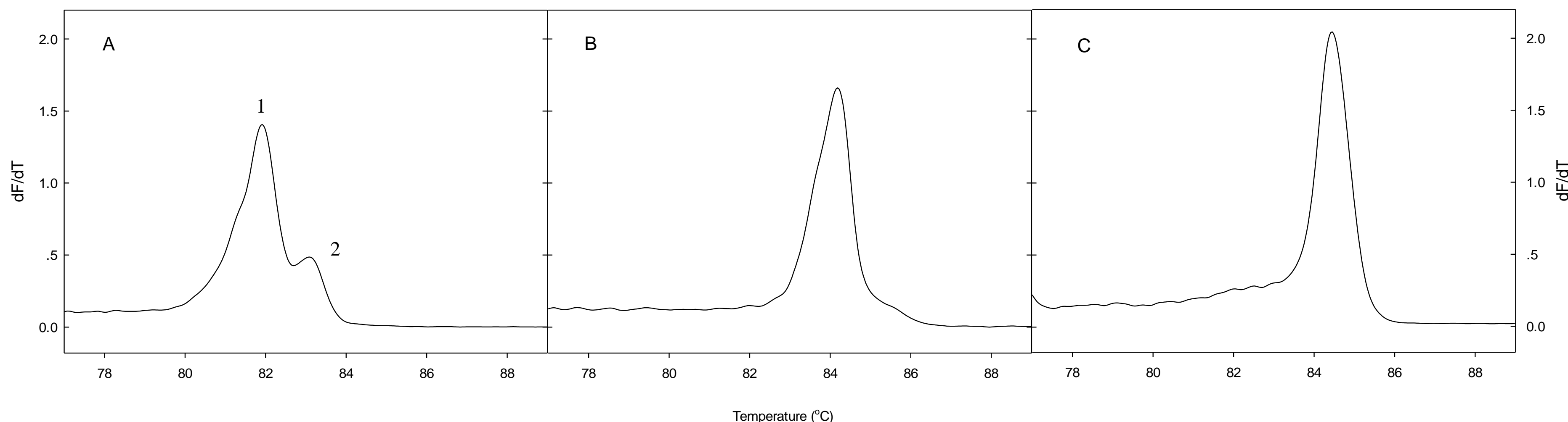


Figure S3. Visualization of gel electrophoresis for PCR amplicons obtained with genomic DNA of lactobacilli and strain-specific primers targeting *L. casei* K9-1 (A) and *L. fermentum* K9-2 (B)

4



5

6 **Figure S4**. First derivatives of melt curves of PCR products obtained from digesta microbiota DNA with *Lactobacillus* group-specific
7 primers. (**Panel A**) *L. salivarius* group primer. Based on the Sanger sequencing and Tm values of reference strains, two peaks shown
8 in above spectrum are presumably assigned to *L. salivarius* (Peak 1 as marked on the curve) and *L. ruminis* (Peak 2 as marked on the
9 curve). OTU's matching to *L. salivarius* was most abundant in 16S sequencing. Colon digesta of Piglet #10 were used for melt curves
10 shown. (**Panel B**) *L. reuteri* group primer. The peak shown in the above spectrum is assigned to *L. reuteri* matching the Tm value of
11 the reference strain. OTUs matching to *L. reuteri* were most abundant in 16S sequencing. Colonic digesta of Piglet #10 were used for
12 the melt curves shown. (**Panel C**) *L. delbrueckii* group primer. The peak shown in above spectrum was assigned to *L. amylovorus* or
13 *L. johnsonii* or *L. gasseri* based on estimated Tm value of respective species and fecal OTU composition. Colonic digesta of Piglet
14 #10 were used for melt curves.

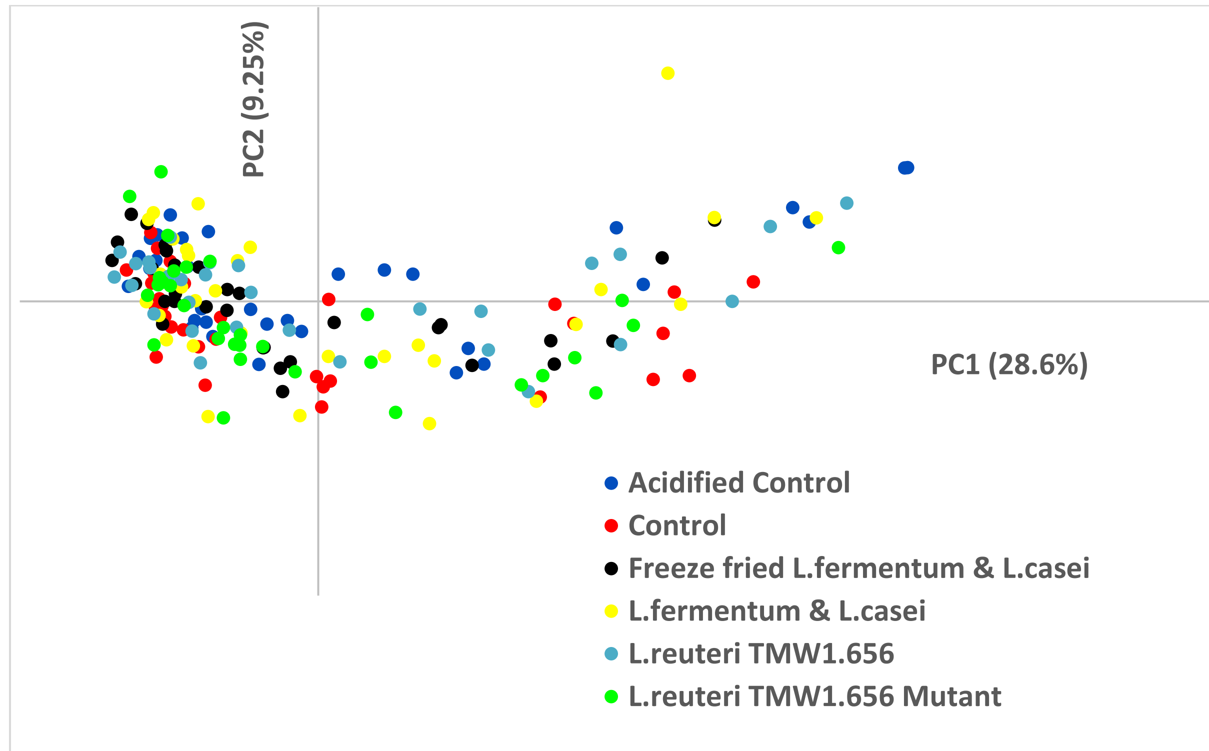
15

16 **Figure S5**. Principle Coordinate Analysis (PCoA) of fecal microbiota composition based on unweighted unifrac distance of rDNA

17 sequences (total of 6,647,893 sequences with an average length of 266 bp, corresponding to an average 34,805 sequences per sample).

18 No significant correlation (p = 0.122, R = 0.008) between diets and fecal microbial composition was detected by Analysis of

19 Similarities (ANOSIM)



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