

Title	Ecological importance of cross-feeding of the intermediate metabolite 1,2-propanediol between bacterial gut symbionts
Authors	Cheng, Christopher C.;Duar, Rebecca M.;Lin, Xiaoxi;Perez-Munoz, Maria Elisa;Tollenaar, Stephanie;Oh, Jee-Hwan;van Pijkeren, Jan-Peter;Li, Fuyong;van Sinderen, Douwe;Gänzle, Michael G.;Walter, Jens
Publication date	2020-04-10
Original Citation	Cheng, C. C., Duar, R. M., Lin, X., Perez-Munoz, M. E., Tollenaar, S., Oh, J.-H., van Pijkeren, J.-P., Li, F., van Sinderen, D., Gänzle, M. G. and Walter, J. (2020) 'Ecological importance of cross-feeding of the intermediate metabolite 1,2-propanediol between bacterial gut symbionts', Applied and Environmental Microbiology. doi: 10.1128/AEM.00190-20
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1128/AEM.00190-20
Rights	Open access - http://creativecommons.org/licenses/by/4.0/ © 2020, American Society for Microbiology.
Download date	2026-09-23 08:49:57
Item downloaded from	https://hdl.handle.net/10468/9878

1 **Ecological importance of cross-feeding of the intermediate metabolite 1,2-**
2 **propanediol between bacterial gut symbionts**

3

4 Christopher C. Cheng^{a*}, Rebecca M. Duar^{b,c*}, Xiaoxi Lin^b, Maria Elisa Perez-Munoz^b,
5 Stephanie Tollenaar^b, Jee-Hwan Oh^d, Jan-Peter van Pijkeren^d, Fuyong Li^b, Douwe van
6 Sinderen^e, Michael G. Gänzle^b, and Jens Walter^{a,b,d,e,f,#}

7

8 ^aDepartment of Biological Sciences, University of Alberta, Edmonton, Canada

9 ^bDepartment of Agricultural, Food & Nutritional Science, University of Alberta,
10 Edmonton, Canada

11 ^cEvolve Biosystems, Inc, Davis, California, United States

12 ^dDepartment of Food Science, University of Wisconsin-Madison, Madison, United States

13 ^eSchool of Microbiology & APC Microbiome Institute, University College Cork, Cork,
14 Ireland

15 ^fDepartment of Medicine, University College Cork, Cork, Ireland

16 Running Head: Cross-feeding of gut symbionts through 1,2-propanediol

17 * Equal contribution. Author order was determined by the amount of contribution to the
18 experimental work.

19 #Address correspondence to Jens Walter, jenswalter@ucc.ie

20 **Abstract**

21 Cross-feeding based on the metabolite 1,2-propanediol has been proposed to
22 have an important role in the establishment of trophic interactions among gut symbionts,
23 but its ecological importance has not been empirically established. Here, we show that
24 *in vitro* growth of *Lactobacillus reuteri* ATCC PTA 6475 is enhanced through 1,2-
25 propanediol produced by *Bifidobacterium breve* UCC2003 and *Escherichia coli* MG1655
26 from the metabolization of fucose and rhamnose, respectively. Work with isogenic
27 mutants showed that the trophic interaction is dependent on the *pduCDE* operon in *L.*
28 *reuteri*, which encodes for the ability to use 1,2-propanediol, and the L-fucose permease
29 (*fucP*) gene in *B. breve*, which is required for 1,2-propanediol formation from fucose.
30 Experiments in gnotobiotic mice revealed that, although the *pduCDE* operon bestows a
31 fitness burden on *L. reuteri* ATCC PTA 6475 in the mouse digestive tract, the ecological
32 performance of the strain was enhanced in the presence of *B. breve* UCC2003 and the
33 mucus-degrading species *Bifidobacterium bifidum*. The use of the respective *pduCDE*
34 and *fucP* mutants of *L. reuteri* and *B. breve* in the mouse experiments indicated that the
35 trophic interaction was specifically based on 1,2-propanediol. Overall, our work
36 established the ecological importance of cross-feeding relationships based on 1,2-
37 propanediol for the fitness of a bacterial symbiont in the vertebrate gut.

38

39

40 **Importance**

41 Through experiments in gnotobiotic mice that employed isogenic mutants of
42 bacterial strains that produce (*Bifidobacterium breve*) and utilize (*Lactobacillus reuteri*)
43 1,2-propanediol, this study provides mechanistic insight into the ecological ramifications
44 of a trophic interaction between gut symbionts. The findings improve our understanding
45 on how cross-feeding influences the competitive fitness of *L. reuteri* in the vertebrate gut
46 and revealed a putative selective force that shaped the evolution of the species. The
47 findings are relevant as they provide a basis to design rational microbial-based
48 strategies to modulate gut ecosystems, which could employ mixtures of bacterial strains
49 that establish trophic interactions or a personalized approach based on the ability of a
50 resident microbiota to provide resources for the incoming microbe.

51

52 Introduction

53 The gut microbiota is a complex microbial community whose members form
54 interdependent trophic relationships that determine the ecology of bacterial populations
55 and their interplay with the host (1). One such interaction involves the exchange of
56 products between microbes, otherwise known as cross-feeding (2). Cross-feeding of
57 nutrients is central to the fermentative degradation of non-digestible dietary fibres and
58 host-derived substrates, such as mucin or human milk oligosaccharides (HMO) (3-10).
59 For example, glycans released from the partial degradation of HMO are utilized by other
60 bifidobacterial species (11), and certain bifidobacteria and *Akkermansia muciniphila*
61 liberate glycans from host mucins that are utilized by other inhabitants of the gut (6, 12).
62 Similarly, *Ruminococcus bromii* releases carbohydrates from resistant starch
63 fermentations into the gut environment (13). These interactions ultimately lead to the
64 formation of dynamic metabolic networks essential in the ecology of the gut microbiome
65 and the production of short chain fatty acids (SCFA), which are a key ecosystem service
66 that benefits the host (14, 15).

67 Trophic interactions can also result from the exchange of metabolic end-products
68 derived from fermentation processes. Hydrogenotrophic microbes utilize the molecular
69 hydrogen produced by several fermentative organisms, a process important to maintain
70 energy flux (16). In addition, cross-feeding based on intermediary metabolites such as
71 lactate, acetate, succinate, and 1,2-propanediol is important for the production of SCFA
72 (5). In the gut, 1,2-propanediol results from the microbial fermentation of deoxyhexose
73 sugars that originate from the hydrolyzation of dietary fibre, fucosylated HMOs, and host
74 mucins, such as rhamnose and fucose (17, 18). Several gut commensal bacteria (i.e.

75 *Bacteroides thetaiotaomicron*, *Roseburia inulinivorans*, *Escherichia coli*, and
76 *Bifidobacterium breve*) can produce 1,2-propanediol from the fermentation of either
77 rhamnose or fucose (3, 5, 17, 18). Other bacteria such as *Eubacterium hallii* and
78 *Lactobacillus reuteri* do not utilize fucose or rhamnose directly but metabolize 1,2-
79 propanediol (19-21) and convert it to propanol and propionate (21, 22). Cross-feeding of
80 1,2-propanediol between *Eu. hallii* and *Bifidobacterium* species based on metabolism of
81 mucin and HMO has been studied *in vitro* (3, 4, 6). Although cross-feeding based on
82 1,2-propanediol is considered important in gut ecosystems (5) and has been shown to
83 confer a fitness advantage *in vivo* for pathogens such as *Salmonella* spp. (23), its
84 ecological importance for bacterial gut symbionts, and the underlying mechanisms by
85 which it impacts bacterial performance in the gut, have not been empirically established
86 *in vivo* in a tractable experimental model.

87 In both *Eu. hallii* and *L. reuteri*, 1,2-propanediol is metabolized through the
88 cobalamin-dependent glycerol/diol dehydratase encoded in the *pduCDE* operon, found
89 in the *pdu-cbi-cob-hem* gene (*pdu*) cluster (19-21). The glycerol/dehydratase converts
90 1,2-propanediol to propionaldehyde and further to propanol and propionate and have
91 been shown to increase growth rates for *L. reuteri in vitro* (19, 24). In *L. reuteri*, the *pdu*
92 cluster is observed predominantly in two *L. reuteri* phylogenetic lineages that are
93 dominated by strains from human, herbivore, and chicken origin, but rarer in strains
94 from other hosts such as mice and rats (25). This distribution suggests that the cluster
95 constitutes an adaptation to the characteristics of the gastrointestinal tract of specific
96 hosts (26, 27). In the murine forestomach, which is densely colonized by *L. reuteri* (25),
97 fermentable mono- and disaccharides are in ample supply (28). In contrast, distal

98 portions of the human intestinal tract are characterized by low concentrations of mono-
99 and disaccharides. The *pdu* cluster may therefore constitute a colonization factor in the
100 distal regions of the gut, to take advantage of 1,2-propanediol produced by other
101 microbes (19, 25).

102 Most studies on diol-metabolism in *L. reuteri* have focused on the synthesis of the
103 antimicrobial intermediate β -hydroxypropionaldehyde (reuterin) from glycerol, which is
104 also mediated by the *pdu* operon (29). It remains unclear whether *L. reuteri* uses the
105 *pdu* operon to engage in cross-feeding interrelationships with other gut bacterial species
106 that produce 1,2-propanediol in its gastrointestinal habitat, and how this interaction
107 contributes to its ecological competitiveness. It was therefore the aim of this study to
108 characterize the ecological importance of 1,2-propanediol-cross-feeding between *L.*
109 *reuteri* and the 1,2-propanediol producers *Bifidobacterium breve* and *Escherichia coli*,
110 both *in vitro* and in the digestive tract of mice.

111

112 Results

113 1,2-propanediol enhances growth of *L. reuteri* *in vitro*

114 We first sought to confirm the importance of 1,2-propanediol metabolism for *L.*
115 *reuteri*. *L. reuteri* ATCC PTA 6475 (referred to as *L. reuteri* PTA 6475) growth *in vitro*.
116 The presence of 1,2-propanediol in the medium containing glucose improved growth
117 rates and increased the final cell density of the strain when compared to the growth
118 solely on glucose or to growth of a *pduCDE* deletion mutant (referred to as *L. reuteri*
119 Δ *pduCDE*) (Fig. 1A). Neither *L. reuteri* PTA 6475 nor *L. reuteri* Δ *pduCDE* were able to

120 use 1,2-propanediol as the sole carbon source for growth (Fig. 1A). To confirm that the
121 enhanced growth of *L. reuteri* PTA 6475 was due to 1,2-propanediol metabolism, the
122 metabolic end-products in the supernatant were measured using HPLC. As shown in
123 Figure 1B and C, *L. reuteri* PTA 6475 but not *L. reuteri* $\Delta pduCDE$ converted 1,2-
124 propanediol to propanol and low concentrations of propionate. For *L. reuteri* PTA 6475
125 but not *L. reuteri* $\Delta pduCDE$, utilization of 1,2-propanediol resulted in acetate production
126 and decreased production of ethanol (Fig. 1D & E; Fig. S1A & B). These findings
127 confirm that *L. reuteri* PTA 6475 is able to disproportionate 1,2-propanediol to propanol
128 and propionate (22). However, 1,2-propanediol is not used as sole substrate but co-
129 metabolized with glucose. Predominant production of propanol demonstrates that the
130 reducing branch of the propanediol pathway is preferred over the oxidizing branch to
131 regenerate electron acceptors, thus enhancing acetate formation and ATP production,
132 and therefore growth (30).

133 **Interspecies cross-feeding of 1-2-propanediol enhances growth of *L. reuteri* in** 134 ***vitro***

135 We developed an experimental system to study cross-feeding between *L. reuteri*
136 and gut bacteria that produce 1,2-propanediol. We chose *B. breve* and *E. coli* which are
137 known to produce 1,2-propanediol from fucose and rhamnose, respectively, which are
138 substrates not utilized by *L. reuteri*. Since growth rates and growth conditions differ
139 among *L. reuteri*, *B. breve*, and *E. coli* are, cross-feeding was not studied in co-culture.
140 Instead, *B. breve* and *E. coli* were first grown under their respective optimal conditions
141 and on the specific substrates that result in the production of 1,2-propanediol. Spent
142 supernatant obtained from these fermentations was then supplemented with glucose

143 and half-strength mMRS (conditioned media; see Materials and Methods) and used for
144 analyzing growth kinetics and metabolite production of *L. reuteri* strains (Table 1).
145 Conditioned media from an isogenic mutant of *B. breve* with an insertion mutation in
146 *fucP* (encoding the L-fucose transporter) that is unable to metabolize fucose into 1,2-
147 propanediol served as a control (Table 1).

148 *B. breve* UCC2003 and its *fucP* mutant *B. breve* UCC2003-*fucP* were grown in a
149 medium containing cellobiose with and without fucose. Fucose does not support the
150 growth of *B. breve* UCC2003 yet is co-utilized with cellobiose to produce 1,2-
151 propanediol. Importantly, neither fucose or cellobiose are metabolized by *L. reuteri* (20).
152 In the fucose/cellobiose-containing medium, *B. breve* UCC2003 and *B. breve*
153 UCC2003-*fucP* reached similar cell density after 24 hours of growth (Fig. S2). *L. reuteri*
154 PTA 6475 reached a significantly higher OD₆₀₀ and showed elevated growth rate when
155 grown in conditioned medium with supernatant of *B. breve* UCC2003 grown with fucose
156 and cellobiose when compared to *L. reuteri* Δ *pduCDE* or *L. reuteri* PTA 6475 grown in
157 conditioned medium of *B. breve* UCC2003 grown in the absence of fucose (Fig. 2A).
158 The growth advantage of *L. reuteri* PTA 6475 was not observed in conditioned media
159 from the *B. breve* UCC2003-*fucP*, even if grown in the presence of fucose (Fig. 2B).
160 HPLC analysis confirmed the presence of 1,2-propanediol in the conditioned medium of
161 *B. breve* UCC2003 but not *B. breve* UCC2003-*fucP* grown with fucose (Fig. S3A), and
162 showed that enhanced growth of *L. reuteri* PTA 6475 was linked to the conversion of
163 1,2-propanediol to propanol, which was not detected in *L. reuteri* Δ *pduCDE* cultures
164 (Fig. 2C & D). Propionate, acetate, and ethanol could not be quantified as unknown
165 compounds in the conditioned media interfered with the metabolite analysis. Fucose

166 and cellobiose did not alter the growth kinetics of the *L. reuteri* strains when grown in
167 glucose (Fig. S4A), and they did not serve as growth substrates on their own (Fig. S4B),
168 confirming that the enhanced growth in *L. reuteri* PTA 6475 was not due to a direct
169 effect of residual concentrations of these sugars in the spend supernatant.

170 1,2-propanediol results also from the fermentation of rhamnose by *E. coli*
171 MG1655 (Fig S3B). Cross-feeding experiments revealed that *L. reuteri* PTA 6475 had a
172 higher growth rate and reached a significantly higher cell density when grown in
173 conditioned medium of *E. coli* grown on rhamnose when compared to *L. reuteri*
174 $\Delta pduCDE$, or *L. reuteri* PTA 6475 grown in conditioned medium from *E. coli* that did not
175 contain rhamnose (Fig. 3A). Importantly, growth experiments of *L. reuteri* strains in
176 media with rhamnose with or without glucose confirmed that rhamnose could neither be
177 used as a carbon source nor alter growth (Fig. S4C & D). Metabolite analysis of
178 fermentations conducted in the conditioned medium of *E. coli* grown on rhamnose
179 revealed that *L. reuteri* PTA 6475, and not *L. reuteri* $\Delta pduCDE$, could metabolize 1,2-
180 propanediol produced by *E. coli* and form propanol, propionate, and acetate (Fig. 3B-D;
181 Fig. S5). Metabolite interference in the conditioned media did not allow the
182 quantification of ethanol by HPLC.

183 Together, these findings demonstrate that *L. reuteri* PTA 6475 utilizes 1,2-
184 propanediol produced by *B. breve* and *E. coli* from the fermentation of deoxyhexose
185 sugars as an electron acceptor, enhancing its growth capabilities.

186

187 **Importance of 1,2-propanediol cross-feeding in the gastrointestinal tract**

188 The ecological relevance of cross-feeding based on 1,2-propanediol in the
189 gastrointestinal tract was investigated with a series of colonization experiments in
190 gnotobiotic mice (Fig 4; Table S1 & S2). Germ-free Swiss-Webster mice (6-16 weeks
191 old; male and female) were housed in individually ventilated cages (groups of 2-3). A
192 fat-free diet was used in order to avoid possible confounding affects from the hydrolysis
193 of dietary triglyceride fats, which is a source of glycerol (also utilized via the *pdu* cluster
194 and enhances growth). Fecal cell numbers of colonizing strains were determined by
195 selective plating (see Materials and Methods).

196 As described earlier, *B. breve* produces 1,2-propanediol from fucose. Host
197 mucins are an intrinsic source of fucose in the gastrointestinal tract, but *B. breve* does
198 not possess glycosidases required for mucin degradation (9). 1,2-Propanediol cross-
199 feeding between *B. breve* and *L. reuteri* was therefore studied in triple-species
200 associated mouse in the presence of the mucinolytic strain *B. bifidum* PRL2010, which
201 is capable of degrading mucin and releasing fucose without producing 1,2-propanediol
202 (Fig. 4A; Table S1) (9). We first tested wild-type and mutant *L. reuteri* strains separately.
203 Mice were gavaged with an inoculum that contained *B. bifidum* PRL2010, *B. breve*
204 UCC2003 or its *fucP* mutant, and either *L. reuteri* PTA 6475 or *L. reuteri* $\Delta pduCDE$
205 (Table S1, inocula A-D). In these experiments, the two *Bifidobacterium* strains formed
206 stable populations of $\sim 10^8$ - 10^9 CFU/g (Fig. S6A-H), while the *L. reuteri* strains colonized
207 at $\sim 10^6$ - 10^8 CFU/g (Fig. S7A). When colonizing with wild-type *B. breve* UCC2003, *L.*
208 *reuteri* PTA 6475 formed higher populations than *L. reuteri* $\Delta pduCDE$ over the duration
209 of the experiment. Contrary, fecal cell numbers of *L. reuteri* PTA6475 were
210 consistently lower than *L. reuteri* $\Delta pduCDE$ when colonizing with *B. breve* UCC2003-

211 fucP (but differences did not reach statistical significance due to the high variation
212 between mice) (Fig. S7A). However, normalized ratios (see Materials and Methods)
213 between fecal cell numbers of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ were
214 significantly higher in mice colonized with *B. breve* UCC2003 as compared to mice
215 colonized with *B. breve* UCC2003-fucP (Fig. 5A). Interestingly, while the ratio between
216 *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ was greater than 1 when the *L. reuteri*
217 strains were co-colonized with *B. breve* UCC2003, it was substantially lower than 1
218 when co-colonized with *B. breve* UCC2003-fucP (Fig. 5A). These finding suggests that
219 the *pdu* cluster confers a fitness burden on *L. reuteri* unless 1,2-propanediol is provided,
220 under which the cluster becomes advantageous.

221 In a parallel set of experiments, we tested the importance of 1,2-propanediol
222 cross-feeding in gnotobiotic mice that were gavaged with *B. bifidum* PRL2010, either *B.*
223 *breve* UCC2003 or *B. breve* UCC2003-fucP, and both *L. reuteri* PTA 6475 and *L. reuteri*
224 $\Delta pduCDE$ (wild-type and mutant) in direct competition (Table S1, inocula E & F). In
225 agreement with the experiments with only one *L. reuteri* strain, *Bifidobacterium* species
226 formed stable populations that were comparable among groups ($\sim 10^8$ - 10^9 CFU/g; Fig.
227 S6I-L). *L. reuteri* formed stable populations ($\sim 10^6$ - 10^8 CFU/g), and concordant with the
228 experiments using single strains of *L. reuteri*, cell counts of *L. reuteri* PTA 6475 were
229 higher than the mutant in mice colonized with *B. breve* UCC2003 as compared to the
230 *B. breve* UCC2003-fucP (but differences were not statistically significant) (Fig. S7B).
231 However, significant differences were observed between the relative proportions of the
232 two *L. reuteri* strains. Specifically, *L. reuteri* PTA 6475 reached significantly higher
233 proportions (>75 %) in mice colonized with *B. breve* UCC2003 as compared to *B. breve*

234 UCC2003-fucP (Fig. 5B). Interestingly, *L. reuteri* $\Delta pduCDE$ reached ~75 % of the total
235 *Lactobacillus* population in mice colonized with *B. breve* UCC2003-fucP (Fig. 5B),
236 supporting the notion that the *pdu* cluster is a fitness burden to *L. reuteri* unless 1,2-
237 propanediol is provided. Overall, these observations demonstrated that *B. breve*
238 UCC2003 can provide 1,2-propanediol as the result of a trophic chain from the
239 degradation of mucin by *B. bifidum* PRL2010 that facilitates colonization of *L. reuteri* in
240 the gastrointestinal tract (9).

241 A set of dual-associated gnotobiotic mouse experiments were also conducted to
242 test if the production of 1,2-propanediol, from the metabolism of rhamnose by *E. coli*,
243 influences the fitness of *L. reuteri* in the gastrointestinal tract. Mice were colonized
244 either with *E. coli* and *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ alone or in
245 competition. Rhamnose was provided to mice in the drinking water (Fig. 4B; Table S2).
246 Stable populations of *E. coli* were reached in all mice ($\sim 10^9$ CFU/g), and the provision of
247 rhamnose led to an ~10-fold increase in fecal cell numbers of *E. coli* (Fig. S8). Contrary
248 to the findings with gnotobiotic mice colonized by bifidobacteria, *L. reuteri* $\Delta pduCDE$
249 colonized with higher cell numbers and outcompeted *L. reuteri* PTA 6475 under all
250 conditions tested (Fig. 5C & D). This was indicated by ratios of less than 1 between *L.*
251 *reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in mice colonized with single *L. reuteri* strains
252 (Fig. 5C) and an enrichment of the mutant to ~60 % of the total *Lactobacillus* population
253 in competition experiments (Fig. 5D). The latter results resample those obtained with
254 the triple-species *Bifidobacterium* experiments in mice colonized with *B. breve*
255 UCC2003-fucP (Fig. 5), confirming the fitness burden of the *pdu* cluster.

256

257 **Discussion**

258 Cross-feeding between members of the gut microbiota based on 1,2-propanediol is
259 considered an important ecological process that shapes the gut microbiome and its
260 metabolism. However, conclusions have so far been derived from in vitro experiments
261 and metagenomic predictions. In this study, we demonstrate that *L. reuteri* engages in
262 trophic interactions with bacteria that are common in the human gut and provide 1,2-
263 propanediol, which is used predominantly to regenerate reduced metabolic cofactors.
264 Using isogenic mutants in both the bacterium that produces 1,2-propanediol and *L.*
265 *reuteri*, we ensured that this cross-feeding interaction is in fact based on this metabolic
266 intermediate. We further established that the *pduCDE* genes constitute a fitness burden
267 for *L. reuteri* in the gut unless 1,2-propanediol is provided, which makes the cluster
268 ecologically advantageous. Our findings therefore provide insight into both the
269 ecological role of the *pdu* cluster in *L. reuteri* and how it evolved.

270 Our results demonstrate that *in vitro*, *L. reuteri* can obtain a growth advantage by
271 cross-feeding from 1,2-propanediol derived from the fermentation of fucose and
272 rhamnose by *B. breve* and *E. coli*, respectively. These findings extend previous work
273 showing that *pdu* cluster-containing *L. reuteri* strains grow at a faster rate and to a
274 higher cell yield in the presence of glycerol or 1,2-propanediol (19). Contrary to findings
275 with *L. reuteri* DSM 20016, which produces propanol and propionate in equimolar
276 concentrations through disproportionation when utilizing 1,2-propanediol as a sole
277 substrate (22), *L. reuteri* PTA 6475 produces propanol in excess over propionate
278 (Figure 1B) and enhances acetate formation (Fig. 1D) when utilizing 1,2-propanediol
279 together with glucose. These metabolic patterns suggest that when *L. reuteri* PTA 6475

grows with glucose, 1,2-propanediol functions mainly as an electron acceptor, allowing the conversion of acetyl-phosphate to acetate and to generate an extra ATP (30). Apart from enhancing bacterial growth, 1,2-propanediol metabolism by *L. reuteri* can therefore contribute to SCFA formation in the gut, which might have health implications as propionate and acetate impact host physiology, e.g. by contributing to gluconeogenesis in the liver, reducing cholesterol, and promoting satiety (5, 18).

Our experiments in gnotobiotic mice provide empirical evidence for the ecological relevance of 1,2-propanediol cross-feeding for the ecological performance of a gut symbiont in the gastrointestinal tract. The use of the respective *pduCDE* and *fucP* mutants of *L. reuteri* and *B. breve* in the mouse experiments indicated that this trophic interaction was specifically based on 1,2-propanediol produced through the fermentation of fucose. Since *B. breve* cannot degrade mucins, and the mouse feed was devoid of fucose, the findings suggests that *L. reuteri* benefits from 1,2-propanediol resulting from interspecies trophic interactions between bifidobacteria, in which *B. bifidum* PRL2010 degrades host mucin and provides fucose for *B. breve* to produce 1,2-propanediol (9). The hydrolysis of mucin is suggested to play a key role in the facilitation of bacterial species in the gut microbiota and have been demonstrated *in vitro* (7, 31, 32) and with bacterial pathogens *in vivo* (e.g. *Salmonella* spp. and *Clostridium difficile*) (23, 33). Our data suggests that it also plays a role in cross-feeding interactions among symbionts or commensals, ultimately conferring a fitness advantage to *L. reuteri* in the murine gut through the provision of 1,2-propanediol.

Although our experiments demonstrated 1,2-propanediol cross-feeding between bifidobacteria and *L. reuteri*, equivalent findings were not observed in mouse

303 experiments with *L. reuteri* and *E. coli*. This observation can potentially be attributed to
304 a phenomenon called carbon catabolite repression or the “all-or-none” effect in *E. coli*,
305 in which a hierarchy-based regulatory system controls the sequential uptake of carbon
306 sources (34). The mouse diet was highly saturated with glucose (34.4 % w/w) and, *in*
307 *vitro*, the presence of glucose suppresses the uptake of other carbohydrates in *E. coli*
308 (35), which is in agreement with our finding that *E. coli* MG1655 did not produce 1,2-
309 propanediol from rhamnose while in the presence of glucose (Fig. S3B). Hence, it is
310 possible that the uptake and metabolism of rhamnose into 1,2-propanediol by *E. coli*
311 was suppressed in the murine gut.

312 Interestingly, the mouse experiments consistently revealed a fitness burden of
313 the *pduCDE* genes when 1,2-propanediol was absent. Fitness trade-offs are well
314 understood in the evolution of antibiotic resistance in bacteria, where resistance genes
315 are costly and lead to a reduction of growth (36), and are therefore often lost in the
316 absence of antibiotic pressure (37). Our findings indicate that genes that facilitate cross-
317 feeding interactions are also subjected to fitness trade-offs in that they are only
318 beneficial when the metabolite is provided. Such trade-offs have also been shown in
319 cross-feeding based on the exchange of carbohydrates. *Bacteroides ovatus* possesses
320 an enzyme system dedicated to the digestion of polysaccharides that does not directly
321 benefit itself, but rather cooperative members of the gut microbiota through reciprocal
322 cross-feeding. This enzyme system is energetically unfavorable and in the absence of a
323 reciprocating species, a knock-out mutant strain can outcompete the enzyme-encoding
324 wild-type (38). In *L. reuteri*, the fitness burden of the *pdu* genes provides a possible
325 explanation for the evolution of the *pdu* cluster (26). Although likely ancestral to

326 currently known lineages of *L. reuteri*, there is evidence of a deletion of the *pdu* cluster
327 from most rodent strains (25, 26). Growth substrates are abundant in the forestomach
328 and bacteria providing 1,2-propanediol are absent (25, 26), which might have led to loss
329 of the *pdu* cluster as it was evolutionary advantageous, in agreement with the Black
330 Queen Hypothesis (39). In the human distal gut, 1,2-propanediol is likely provided
331 through other microbiota members, which may explain why the *pdu* cluster is conserved
332 among these strains (26, 27).

333 Although cross-feeding based on 1,2-propanediol is only one of many trophic
334 interactions that establish interactive networks within the gut microbiota, this study
335 provides important information as it establishes the importance and consequences of
336 cross-feeding for the ecological performance of the involved members. Such knowledge
337 has repercussions for our understanding on the ecological and evolutionary forces that
338 shape gut ecosystems and determine how they function (38, 40-42). In addition, an
339 understanding of mutualistic interactions has important implications as it can be
340 translated into improved microbial-based strategies to modulate gut microbiomes (i.e.
341 probiotics). A challenge encountered in the field of probiotics is that gut ecosystems are
342 homeostatic, resilient to change, and thus difficult to modulate, and most probiotics do
343 not persist or change the resident community (43-46). One solution to this problem is
344 the adoption of an ecological framework for probiotic applications (44). A consideration
345 of the mutualistic and facilitative interactions between community members can be used
346 for designing probiotic strain mixtures or personalized probiotic applications with the
347 goal to achieve a more successful long-term persistence, which might be beneficial for
348 certain applications. For example, 1,2-propanediol cross-feeding could be considered in

349 generating probiotic products by pairing *L. reuteri* with *Bifidobacterium* species that
350 release fucose from the degradation of host-derived substrates and convert it into 1,2-
351 propanediol (9, 12). Additionally, bifidobacteria are more prevalent in the infant gut (47,
352 48) and some strains only partly utilize HMOs, releasing fucose, possibly forming an
353 effective synergistic combination with *L. reuteri* (3, 32, 40). Furthermore, cross-feeding
354 of 1,2-propanediol derived from gut symbionts and *S. enterica* serovar Typhimurium has
355 been demonstrated to play a role in promoting pathogen expansion in the gut (23). *L.*
356 *reuteri* could play a therapeutic role in excluding pathogenic *Salmonella* during
357 gastroenteritis, by directly competing for the intermediary metabolite. Overall, this
358 information could not only be used to formulate probiotic mixtures and synbiotic
359 products, but potentially personalize probiotic applications based on the baseline
360 microbiome (43).

361

362 **Materials and Methods**

363 **Bacterial strains and culture conditions**

364 Bacterial strains used in this study are listed in Table 2. *L. reuteri* strains were grown
365 in de Man, Rogosa, and Sharpe (MRS) medium (Difco) in an anaerobic chamber (gas
366 mix of 5 % CO₂, 5 % H₂, and 90 % N₂). *B. bifidum* PRL2010, *B. breve* UCC2003, and *B.*
367 *breve* UCC2003-fucP were grown anaerobically in MRS medium supplemented with
368 0.05 % L-cysteine. *E. coli* were grown in Luria-Bertani (LB) broth with agitation. All
369 incubations were performed at 37 °C.

370 **Evaluation of the impact of 1,2-propanediol on *L. reuteri* ATCC PTA 6475 growth**

371 Overnight cultures of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ were inoculated at
372 1 % into 15 ml of half-strength mMRS (49) containing 25mM glucose alone, 50 mM of
373 1,2-propanediol (Sigma-Aldrich) alone, or 25mM glucose plus 50mM of 1,2-propanediol.
374 Growth of cell cultures were monitored based on OD₆₀₀ with a spectrophotometer every
375 3 h over the span of 12 h. One mL samples were collected for HPLC analysis every 3 h.
376 All experiments were performed in triplicate at 37 °C under anaerobic conditions.

377 ***In vitro* production of 1,2-propanediol and cross-feeding assay development**

378 Pre-cultures of *B. breve* strains and *E. coli* were prepared as follows. Full-strength
379 mMRS supplemented with 30 mM cellobiose $\pm$ 30 mM L-fucose were inoculated with 1
380 % of overnight cultures of *B. breve* UCC2003 or *B. breve* UCC2003-fucP. Full-strength
381 mMRS containing 25 mM of glucose or 30 mM of L-rhamnose were inoculated with 1 %
382 of overnight *E. coli* MG1655 cultures. These fermentations were conducted under
383 anaerobic conditions for 24 h at 37 °C. Conditioned media were prepared from pre-
384 cultures as follows. Cells were removed from pre-cultures by centrifugation (5000 x *g* for
385 10 minutes) after which the supernatant was collected. The supernatant was
386 supplemented with half-strength mMRS from scratch (50 % w/v; mMRS dry reagents)
387 and glucose (25 mM; dry reagent) to the supernatant. These were further adjusted to
388 pH 6.6 and filter sterilized (0.22 μ m), stored at 4 °C and used within 48 h. Conditioned
389 media are described in Table 1. For growth experiments, conditioned media were
390 inoculated with *L. reuteri* strains (1 % inoculation). Growth was monitored for 12 h by
391 measuring OD₆₀₀ with a spectrophotometer and 1 mL samples for HPLC analysis were
392 collected every 3 h. All experiments were performed in triplicate under anaerobic
393 conditions at 37 °C.

394 **Experiments in gnotobiotic mice**

395 All animal experiments were performed with the approval of the Animal Care and
396 Use Committee (ACUC) of the University of Alberta (AUP 00002764). Germ-free Swiss-
397 Webster mice (6-16 weeks of age, male and female) were bred and maintained in the
398 Health Sciences Laboratory Animals Services (HSLAS) Facility at the University of
399 Alberta. Mice were randomly selected and moved from a flexible-film isolator and
400 housed in sterile, individually ventilated, positive-pressured biocontainment cages for
401 the duration of the experiments (IsoCage P Biocontainment; Tecniplast). To avoid
402 possible confounding effects of glycerol, which is also utilized by the *pdu* cluster-
403 encoded diol/glycerol dehydratase (50) , an irradiated fat-free diet (34.4 % glucose and
404 34.4 % cornstarch; Teklad TD.180765) was used in order to minimize possible
405 interference from the hydrolysis of triglyceride fats. After transfer to the biocontainment
406 cages, mice were fed with the new diet for 3 d before colonization with the bacteria.

407 To study the cross-feeding of 1,2-propanediol in the gastrointestinal tract, groups of
408 mice ($n=5$), 2 to 3 mice per cage, were assigned to receive either *Bifidobacterium-L.*
409 *reuteri* triple-species mixtures (Table S1) or *E. coli-L. reuteri* double-species mixtures
410 (Table S2). To test for cross-feeding of 1,2-propanediol produced from mucin derived
411 fucose, mice were gavaged with *Bifidobacterium-L. reuteri* triple-species mixtures
412 containing *B. bifidum* PRL2010, either *B. breve* UCC2003 or *B. breve* UCC2003-fucP,
413 and either *L. reuteri* PTA 6475 or *L. reuteri* $\Delta pduCDE$ (single *L. reuteri* strains), or both
414 strains in competition (Table S1). To test for cross-feeding of dietary rhamnose, we
415 gavaged mice with *E. coli-L. reuteri* double-species mixtures containing *E. coli* and *L.*
416 *reuteri* PTA 6475 or *L. reuteri* $\Delta pduCDE$ (single *L. reuteri* strains), or both strains in

417 competition (Table S2). Rhamnose was provided in the drinking water (2 % w/v). Each
418 mouse was gavaged with 200 μ L of the corresponding bacterial cell mixtures containing
419 $\sim 10^8$ viable cells of each strain. Fecal pellets were collected from individual mice 1, 3, 5,
420 and 7 days after inoculation and plated. Selective plating was used to enumerate
421 bacterial cells in fecal samples as follows: Modified Rogosa agar plates were used to
422 quantify *L. reuteri* strains (51). *L. reuteri* PTA 6475 and *L. reuteri* Δ pduCDE were
423 differentiated using a reuterin hydrazone detection assay (52). MacConkey agar was
424 used for quantifying *E. coli*. *Bifidobacterium* were selected using *Bifidobacterium*
425 selective iodoacetate mupirocin (BSIM) agar as previously described (48). *B. bifidum*
426 PRL2010 and *B. breve* strains were differentiated based on colony morphology.

427 **Metabolite analysis of post-fermentation**

428 1,2-Propanediol, propanol, propionate, acetate, and ethanol were measured using
429 HPLC. A BioRad Aminex HPX-87H column (300 mm x 7.8 mm) and a refractive index
430 detector was used (HPLC-RI). Samples taken from fermentations were mixed with 70 %
431 HClO₄ (0.005 % v/v), stored at 4 °C overnight to precipitate proteins, centrifuged (18,800
432 x g for 5 minutes), filtered (0.22 μ m), and stored at -20 °C before injection into HPLC.
433 Ten μ L were injected and eluted with 5 mM H₂SO₄ at a flow rate of 0.4 ml/min at 70 °C.
434 1,2-Propanediol, propanol, propionate, acetate, and ethanol were quantified using
435 external standards.

436 **Statistical analysis**

437 Statistical significance between *L. reuteri* growth curves were determined by two-
438 way analysis of variance (ANOVA) with Bonferroni multiple comparisons test ($\alpha = 0.05$).

439 An unpaired two-tailed Student's *t*-test was used to analyze significance between *B.*
440 *breve* growth in mMRS supplemented with cellobiose ± fucose.

441 Comparisons between *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ CFU recovered
442 from fecal samples over the duration of the gnotobiotic mice experiments were
443 performed by unpaired two-tailed Student's *t*-test. Tests were conducted between *L.*
444 *reuteri* strains that were associated with (i) *B. breve* UCC2003 or (ii) *B. breve* UCC2003-
445 fucPin the triple-species experiments and *E. coli*-*L. reuteri* double-species experiments
446 with rhamnose (iii) present or (iv) absent.

447 For the gnotobiotic mice experiments inoculated with the 'single *L. reuteri* strain'
448 mixtures (Table S1 & 2), CFU of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ recovered
449 from mouse feces were used to produce normalized ratios. Ratios were generated
450 using the formula (equation 1) below, where a_n is a CFU value for *L. reuteri* PTA 6475 -
451 from a single mouse - used in the comparison, b_n is the CFU value of *L. reuteri*
452 $\Delta pduCDE$ from each mouse in the group, and n_b is the total population of mice
453 inoculated with the mutant strain used in the experiment.

$$Normalized\ ratio = \frac{a_n}{\sum \left(\frac{(b_1 + b_2 + \dots + b_n)}{n_b} \right)} \quad (1)$$

454
455 The formula was used to generate sets of ratios for the following comparisons from
456 the 'Single *L. reuteri* strain' mice experiments: (i) *B. breve* UCC2003 vs *B. breve*
457 UCC2003-fucP and (ii) *E. coli*-*L. reuteri* double-associated mice in the presence vs the

458 absence of rhamnose. Statistical significance between the sets of ratios were
459 determined by Mann-Whitney U test (P value < 0.05).

460 Fisher's exact test was used to determine statistical significance between *L. reuteri*
461 population frequencies from murine groups inoculated with '*L. reuteri* strains in
462 competition' mixtures (P value < 0.05). This was performed between groups of mice
463 from either: (i) *Bifidobacterium-L. reuteri* triple-species associations including *B. breve*
464 UCC2003 vs *B. breve* UCC2003-fucP(ii) *E. coli-L. reuteri* double-species associations
465 with murine diet supplemented with rhamnose vs without rhamnose. Statistical analyses
466 were performed using GraphPad Prism 6.07.

467

468 **Acknowledgements**

469 JW acknowledges support from the Campus Alberta Innovation Program (CAIP) and the
470 Natural Sciences and Engineering Research Council of Canada (NSERC) for a
471 Discovery grant. We thank Gautam Gaur for his technical advice concerning HPLC use
472 and Nicole Coursen for her assistance throughout the mouse experiments. We also
473 thank Dr. Marco Ventura (University of Parma, Parma, Italy) for generously providing us
474 the *B. bifidum* PRL2010 strain.

475

476 **Author contributions**

477 C.C.C. designed and conducted the experiments, collected and analyzed data,
478 and wrote the manuscript. R.M.D. contributed to conceiving the project, the

479 development of experimental approaches, supervision, intellectual leadership, and
480 wrote the manuscript. X.B.L contributed to experimental designs and edited the
481 manuscript. M.E.P.M contributed to the design of animal experiments, and contributed
482 to the manuscript through editing and generation of figures. S.T. was responsible for
483 animal husbandry and oversaw animal experiments. J.H.O. and J.P.V. generated the
484 mutant *L. reuteri* ATCC PTA 6475 $\Delta pduCDE$ strains and contributed to manuscript
485 editing. F.L. contributed to supervision and manuscript editing. D.V. provided the
486 *Bifidobacterium bifidum* PRL2010, *Bifidobacterium breve* UCC2003, *Bifidobacterium*
487 *breve* UCC2003-fucP strains, contributed to conceiving animal experiments, and gave
488 technical and conceptual advice. M.G.G. oversaw analytical analysis and data
489 interpretation, and contributed to supervision and manuscript editing. J.W. conceived
490 project, contributed to the conceptualization of the experiments, supervised data
491 analysis and interpretation, and wrote the manuscript.

492

493

494 Table 1. Media used for *in vitro* cross-feeding experiments

Fermenting Strain	Deoxyhexose Sugar	Other carbohydrates	Abbreviation	Purpose
<i>B. breve</i> UCC2003	N/a	30 mM Cellobiose	BM (C)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
	30 mM Fucose	30 mM Cellobiose	BM (CF)	For the study of effect of 1,2-propanediol produced from Fucose fermentation on <i>L. reuteri</i>
<i>B. breve</i> UCC2003-fucP	N/a	30 mM Cellobiose	B-fucP M (C)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
	30 mM Fucose	30 mM Cellobiose	B-fucP M (CF)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
<i>E. coli</i> MG1655	N/a	25 mM Glucose	EM (G)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
	30 mM Rhamnose	N/a	EM (R)	For the study of effect of 1,2-propanediol produced from Rhamnose fermentation on <i>L. reuteri</i>

495 N/a: not applicable

496

497

498

499 Table 2. Strains used in this study

Species (strain code)	Origin	Relevant features	Reference
<i>Lactobacillus reuteri</i> ATCC PTA 6475	Breast Milk	1,2-propanediol utilizer with complete <i>pdu</i> cluster	BioGaia; (53)
<i>L. reuteri</i> Δ pduCDE	Isogenic mutant of PTA 6475	Deletion mutant of Glycerol/diol dehydratase genes (<i>pduCDE</i>)	(53)
<i>Escherichia coli</i> MG1655	Lab-derived strain	L-rhamnose utilizer, 1,2-propanediol producer	The Coli Genetic Stock Center (CGSC)
<i>Bifidobacterium bifidum</i> PRL2010	Infant stool	Mucin degrader, L-fucose producer	(32)
<i>Bifidobacterium breve</i> UCC2003	Infant stool	L-fucose utilizer, 1,2-propanediol producer	(54)
<i>Bifidobacterium breve</i> UCC2003-fucP	Isogenic mutant of UCC2003	Insertion mutant of the L-fucose transporter gene (<i>fucP</i>)	(9)

500

501

502 **Figures legends**

503 **Figure 1.** Impact of 1,2-propanediol on growth and metabolism of *L. reuteri* PTA 6475
504 and *L. reuteri* $\Delta pduCDE$. (A) *L. reuteri* strains were grown in half-strength mMRS
505 supplemented with either glucose (Glc; 25 mM), 1,2-propanediol (50 mM), or a mixture
506 of the two. Asterisks indicate a significant difference (Two-way ANOVA; $p < 0.001$) in
507 growth of *L. reuteri* PTA 6475 on glucose plus 1,2-propanediol compared to the other
508 conditions. (B-C) Utilization of 1,2-propanediol and production of propanol and
509 propionate by (B) *L. reuteri* PTA 6475 and (C) *L. reuteri* $\Delta pduCDE$ during growth on
510 glucose in the presence of 1,2-propanediol. (D-E) Production of (D) acetate and (E)
511 ethanol by the two strains during growth on glucose in the presence of 1,2-propanediol.

512
513 **Figure 2.** Growth and metabolites of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in
514 conditioned media of *B. breve* UCC2003 and *B. breve* UCC2003-fucP grown with
515 cellobiose alone or with the addition of fucose. Growth curves of (A) *L. reuteri* strains in
516 *B. breve* UCC2003 conditioned media and (B) *L. reuteri* in *B. breve* UCC2003-fucP
517 conditioned media. Asterisks indicate a significant difference (Two-way ANOVA; $p <$
518 0.001) in growth of *L. reuteri* PTA 6475 grown in *B. breve* UCC2003 conditioned
519 medium that had fermented cellobiose and fucose together compared to growth of *L.*
520 *reuteri* PTA 6475 in *B. breve* UCC2003 conditioned medium without fucose or to *L.*
521 *reuteri* $\Delta pduCDE$ grown in all conditioned media derived from *B. breve* UCC2003. (C-D)
522 Utilization of *B. breve* derived 1,2-propanediol present in the conditioned media and
523 production of propanol in cultures of (C) *L. reuteri* PTA 6475 and (D) *L. reuteri*
524 $\Delta pduCDE$ grown in the conditioned medium of *B. breve* UCC2003 grown in the

525 presence of fucose. Propionate, acetate, and ethanol concentrations could not be
526 determined due to interference of unknown compounds in the medium. Abbreviations:
527 BM, *B. breve* UCC2003 conditioned media; B-fucP M, *B. breve* UCC2003-fucP
528 conditioned media; (C), pre-culture fermentations of cellobiose only; (CF), pre-culture
529 fermentations of cellobiose with added fucose (See Table 1 for more details about
530 media used in the study).

531

532 **Figure 3.** Growth curves and metabolites of *L. reuteri* PTA 6475 and *L. reuteri*
533 $\Delta pduCDE$ in conditioned media of *E. coli* grown with glucose or rhamnose. (A) Growth
534 of *L. reuteri* strains. Asterisks indicate a significant difference (Two-way ANOVA; $p <$
535 0.01) in growth of *L. reuteri* PTA 6475 grown in *E. coli* conditioned medium that had
536 fermented rhamnose compared to *E. coli* conditioned medium that fermented glucose
537 and to growth of *L. reuteri* $\Delta pduCDE$ in both *E. coli* conditioned media fermentations of
538 either glucose or rhamnose. (B-C) Utilization of *E. coli*-derived 1,2-propanediol, and
539 production of propanol and propionate by (B) *L. reuteri* PTA 6475 and (C) *L. reuteri*
540 $\Delta pduCDE$ grown in conditioned medium from *E. coli* grown with rhamnose. (D)
541 Comparison of acetate production by the two strains grown in conditioned medium from
542 *E. coli* grown with rhamnose. Ethanol concentrations could not be determined due to
543 interference of an unknown compound in the medium. Abbreviations: EM, *E. coli*
544 conditioned media; (G), pre-culture fermentation of glucose only by *E. coli*; (R), pre-
545 culture fermentation of rhamnose only by *E. coli*. (See Table 1 for more details about
546 media used in the study).

547

548 **Figure 4.** Graphical illustration of hypothesized trophic interactions of 1,2-propanediol
549 in gnotobiotic mice. (A) In triple-species associated gnotobiotic mice (colonized by *B.*
550 *bifidum*, *B. breve*, and *L. reuteri*), *B. bifidum* liberates fucose from the degradation of
551 host mucin, which is metabolized by *B. breve* UCC2003 producing 1,2-propanediol, that
552 is subsequently utilized by *L. reuteri* PTA 6475. (B) In dual-species (*E. coli* and *L.*
553 *reuteri*) associated mice whose diet has been supplemented with rhamnose added
554 through the drinking water, *E. coli* metabolizes rhamnose producing 1,2-propanediol
555 that is subsequently utilized by *L. reuteri* PTA 6475.

556
557 **Figure 5.** Populations of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in the
558 gastrointestinal tract of triple-species and double-species associated gnotobiotic mice.
559 (A) Normalized ratios between *L. reuteri* PTA 6475 to *L. reuteri* $\Delta pduCDE$ obtained from
560 *Bifidobacterium*-*L. reuteri* triple-species associated gnotobiotic mice in which
561 colonization by *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ was tested separately. “+”
562 indicates mice colonized with *B. breve* UCC2003 and “-” indicates mice colonized with
563 *B. breve* UCC2003-fucP. (B) Percent CFU for *L. reuteri* PTA 6475 and *L. reuteri*
564 $\Delta pduCDE$ as measured in triple-species associated gnotobiotic mice in which the two *L.*
565 *reuteri* strains were tested in competition. (C) Normalized ratios between *L. reuteri* PTA
566 6475 and *L. reuteri* $\Delta pduCDE$ in *E. coli*-*L. reuteri* double-species associated gnotobiotic
567 mice in which colonization of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ was tested
568 separately. (D) Percent CFU for *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ mutant in
569 double-species associated gnotobiotic mice in which the two *L. reuteri* strains were
570 tested in competition. “+” indicates the presence of rhamnose (Rha) in the diet, while “-”

571 indicates absence of rhamnose in the diet. Statistical significance for ratios and percent
572 abundance (% CFU) was determined using Mann-Whitney test and Fisher's exact test,
573 respectively.

574

575 **Table S1.** Strains used in experiments with *Bifidobacterium-L. reuteri* triple-species
576 associated gnotobiotic mice.

577

578 **Table S2.** Strains used in experiments with *E. coli-L. reuteri* double-species associated
579 mice.

580

581 **Figure S1.** Concentrations of (A) acetate and (B) ethanol in media after growth of *L.*
582 *reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ on glucose (Glc) in the presence or absence
583 of 1,2-propanediol.

584

585 **Figure S2.** Growth of *B. breve* UCC2003 and *B. breve* UCC2003-fucP in mMRS
586 supplemented with cellobiose (30 mM; Cell), fucose (30 mM; Fuc), cellobiose and
587 fucose, or with no carbon source (No Carb) after 24 hours.

588

589 **Figure S3.** Total 1,2-propanediol concentrations in media after growth with (A) *B. breve*
590 UCC2003 and *B. breve* UCC2003-fucP after fermentation of cellobiose (Cell) and
591 cellobiose plus fucose (Cell + Fuc). (B) 1,2-propanediol concentrations in *E. coli* cultures
592 after growth with glucose (Glc), rhamnose (Rha), or glucose and rhamnose (Glc + Rha).

593

594 **Figure S4.** Growth of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in the presence of
595 deoxyhexose sugars. *L. reuteri* strains do not utilize (A & B) cellobiose (Cell), fucose
596 (Fuc), or (C & D) rhamnose (Rha) as growth substrates or electron acceptors when
597 cultured with glucose (Glc).

598

599 **Figure S5.** Acetate production of *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in the
600 conditioned media of *E. coli* fermentation of glucose and rhamnose. In the symbol
601 labels, *E. coli* conditioned media is abbreviated as EM, followed by fermentation of
602 glucose by *E. coli* as indicated with (G), and rhamnose with (R) (See Table 1 for more
603 details about media used in the study).

604

605 **Figure S6.** Quantification of *Bifidobacterium* strains in fecal samples from triple-species
606 associated gnotobiotic mice. Fecal CFU of *B. bifidum* PRL2010 recovered from of
607 gnotobiotic mice inoculated with (A) *B. breve* UCC2003 and *L. reuteri* PTA 6475 or (B)
608 *B. breve* UCC2003 and *L. reuteri* $\Delta pduCDE$. Fecal CFU of *B. bifidum* PRL2010 from
609 gnotobiotic mice inoculated with (C) *B. breve* UCC2003-fucP and *L. reuteri* PTA 6475 or
610 (D) *B. breve* UCC2003-fucP and *L. reuteri* $\Delta pduCDE$. Fecal CFU of *B. breve* UCC2003
611 from gnotobiotic mice inoculated with (E) *B. bifidum* PRL2010 and *L. reuteri* PTA 6475
612 or (F) *B. bifidum* PRL2010 and *L. reuteri* $\Delta pduCDE$. Fecal CFU of *B. breve* UCC2003-
613 fucP from gnotobiotic mice inoculated with (G) *B. bifidum* PRL2010 and *L. reuteri* PTA
614 6475 or (H) *B. bifidum* PRL2010 and *L. reuteri* $\Delta pduCDE$. Fecal CFU of *B.*
615 *bifidum* PRL2010 from gnotobiotic mice inoculated with *L. reuteri* PTA 6475 and *L.*

616 *reuteri* $\Delta pduCDE$ in competition as well as with (I) *B. breve* UCC2003 or (J) *B.*
617 *breve* UCC2003-fucP. (K) Fecal CFU of *B. breve* UCC2003 from gnotobiotic mice
618 inoculated with *B. bifidum* PRL2010 and *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in
619 competition. (L) Fecal CFU of *B. breve* UCC2003-fucP from gnotobiotic mice inoculated
620 with *B. bifidum* PRL2010 and *L. reuteri* PTA 6475 and *L. reuteri* $\Delta pduCDE$ in
621 competition.

622 **Figure S7.** Quantification of *L. reuteri* strains from triple-species and double-species
623 associated gnotobiotic mice experiments. (A-B) CFU of *L. reuteri* recovered from feces
624 of the *Bifidobacterium* triple-species gnotobiotic mice experiments containing either (A)
625 single *L. reuteri* strains or (B) *L. reuteri* strains in competition. “+” indicates mice
626 colonized with *B. breve* UCC2003 and “-” indicates mice colonized with the *fucP* mutant
627 of *B. breve* UCC2003. (C-D) CFU of *L. reuteri* recovered from feces of *E. coli* double-
628 species gnotobiotic mice experiments from either (C) single *L. reuteri* strains or (D) *L.*
629 *reuteri* strains in competition. “+” indicates the presence of rhamnose (Rha) in the diet,
630 while “-” indicates absence of rhamnose in the diet.

631

632 **Figure S8.** Quantification of *E. coli* from double-species associated gnotobiotic mice
633 experiments. CFU of *E. coli* recovered from ‘single *L. reuteri* strain’ bacterial mixtures in
634 the (A) presence of rhamnose in the diet, (B) absence of rhamnose in the diet, and from
635 (C) *L. reuteri* in competition where rhamnose was either supplemented into the mouse
636 diet or not.

637

638

639 **References**

- 640 1. Thursby E, Juge N. 2017. Introduction to the human gut microbiota. *Biochem J*
641 474:1823-1836. 10.1042/BCJ20160510.
- 642 2. D'Souza G, Shitut S, Preussger D, Yousif G, Waschina S, Kost C. 2018. Ecology and
643 evolution of metabolic cross-feeding interactions in bacteria. *Natural product reports*
644 35:455-488. 10.1039/C8NP00009C.
- 645 3. Bunesova V, Lacroix C, Schwab C. 2018. Mucin Cross-Feeding of Infant
646 bifidobacteria and *Eubacterium hallii*. *Microb Ecol* 75:228-238. 10.1007/s00248-017-
647 1037-4.
- 648 4. Schwab C, Ruscheweyh H, Bunesova V, Pham VT, Beerenwinkel N, Lacroix C.
649 2017. Trophic interactions of infant bifidobacteria and *Eubacterium hallii* during L-fucose
650 and fucosyllactose degradation. *Front Microbiol* 8:95. 10.3389/fmicb.2017.00095.
- 651 5. Louis P, Flint HJ. 2017. Formation of propionate and butyrate by the human colonic
652 microbiota. *Environ Microbiol* 19:29-41. 10.1111/1462-2920.13589.
- 653 6. Belzer C, Chia LW, Aalvink S, Chamlagain B, Piironen V, Knol J. 2017. Microbial
654 metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin
655 B12 production by intestinal symbionts. *mBio* 8(5): e00770-17 10.1128/mBio.00770-17.

- 656 7. Martens EC, Chiang HC, Gordon JI. 2008. Mucosal glycan foraging enhances fitness
657 and transmission of a saccharolytic human gut bacterial symbiont. *Cell Host Microbe*
658 4:447-457. 10.1016/j.chom.2008.09.007.
- 659 8. Egan M, O'Connell Motherway M, Ventura M, van Sinderen D. 2014. Metabolism of
660 sialic acid by *Bifidobacterium breve* UCC2003. *Appl and Environ Microbiol* 80:4414-
661 4426. 10.1128/AEM.01114-14.
- 662 9. Egan M, O'Connell Motherway M, Kilcoyne M, Kane M, Joshi L, Ventura M, van
663 Sinderen D. 2014. Cross-feeding by *Bifidobacterium breve* UCC2003 during co-
664 cultivation with *Bifidobacterium bifidum* PRL2010 in a mucin-based medium. *BMC*
665 *Microbiol* 14:282. 10.1186/s12866-014-0282-7
- 666 10. Duranti S, Lugli GA, Milani C, James K, Mancabelli L, Turrone F, Alessandri G,
667 Mangifesta M, Mancino W, Ossiprandi MC, Iori A, Rota C, Gargano G, Bernasconi S, Di
668 Pierro F, van Sinderen D, Ventura M. 2019. *Bifidobacterium bifidum* and the infant gut
669 microbiota: an intriguing case of microbe-host co-evolution. *Enviro Microbiol* 21
670 (10):3686-3695. 10.1111/1462-2920.14705.
- 671 11. Gotoh A, Katoh T, Sakanaka M, Ling Y, Yamada C, Asakuma S, Urashima T,
672 Tomabechi Y, Katayama-Ikegami A, Kurihara S, Yamamoto K, Harata G, He F, Hirose
673 J, Kitaoka M, Okuda S, Katayama T. 2018. Sharing of human milk oligosaccharides
674 degradants within bifidobacterial communities in faecal cultures supplemented with
675 *Bifidobacterium bifidum*. *Sci Rep* 8:13958-14. 10.1038/s41598-018-32080-3.

- 676 12. Tailford LE, Crost EH, Kavanaugh D, Juge N. 2015. Mucin glycan foraging in the
677 human gut microbiome. *Front Genet* 6:81. 10.3389/fgene.2015.00081.
- 678 13. Ze X, Duncan SH, Louis P, Flint HJ. 2012. *Ruminococcus bromii* is a keystone
679 species for the degradation of resistant starch in the human colon. *ISME J* 6:1535-1543.
680 10.1038/ismej.2012.4.
- 681 14. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. 2012. Microbial degradation of
682 complex carbohydrates in the gut. *Gut Microbes* 3:289-306. 10.4161/gmic.19897.
- 683 15. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. 2016. From dietary fiber
684 to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 165:1332-
685 1345. 10.1016/j.cell.2016.05.041.
- 686 16. Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. 2019. Hydrogen
687 cross-feeders of the human gastrointestinal tract. *Gut Microbes* 10:270-288.
688 10.1080/19490976.2018.1546522.
- 689 17. Saxena RK, Anand P, Saran S, Isar J, Agarwal L. 2010. Microbial production and
690 applications of 1,2-propanediol. *Indian J Microbiol* 50:2-11. 10.1007/s12088-010-0017-
691 x.
- 692 18. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP,
693 Flint HJ, Louis P. 2014. Phylogenetic distribution of three pathways for propionate
694 production within the human gut microbiota. *ISME J* 8:1323-1335.
695 10.1038/ismej.2014.14.

- 696 19. Rattanaprasert M, Roos S, Hutkins RW, Walter J. 2014. Quantitative evaluation of
697 synbiotic strategies to improve persistence and metabolic activity of *Lactobacillus reuteri*
698 DSM 17938 in the human gastrointestinal tract. J Funct Foods 10:85-94.
699 10.1016/j.jff.2014.05.017.
- 700 20. Schwab C, Gänzle M. 2011. Lactic acid bacteria fermentation of human milk
701 oligosaccharide components, human milk oligosaccharides and
702 galactooligosaccharides. FEMS Microbiol Lett 315:141-148. 10.1111/j.1574-
703 6968.2010.02185.x.
- 704 21. Engels C, Ruscheweyh H, Beerenwinkel N, Lacroix C, Schwab C. 2016. The
705 common gut microbe *Eubacterium hallii* also contributes to intestinal propionate
706 formation. Front Microbiol 7:713. 10.3389/fmicb.2016.00713.
- 707 22. Sriramulu DD, Liang M, Hernandez-Romero D, Raux-Deery E, Lünsdorf H, Parsons
708 JB, Warren MJ, Prentice MB. 2008. *Lactobacillus reuteri* DSM 20016 produces
709 cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-
710 propanediol by disproportionation. Journal of Bacteriology 190:4559-4567.
711 10.1128/JB.01535-07. J Bacteriol 190:4559-4567. 10.1128/JB.01535-07.
- 712 23. Faber F, Thiennimitr P, Spiga L, Byndloss MX, Litvak Y, Lawhon S, Andrews-
713 Polymenis HL, Winter SE, Bäumler AJ. 2017. Respiration of microbiota-derived 1,2-
714 propanediol drives *Salmonella* expansion during colitis. PLoS Pathog 13:1. e1006129.
715 10.1371/journal.ppat.1006129.

- 716 24. Chen L, Hatti-Kaul R. 2017. Exploring *Lactobacillus reuteri* DSM20016 as a
717 biocatalyst for transformation of longer chain 1,2-diols: Limits with microcompartment.
718 PloS One 12:9. e0185734. 10.1371/journal.pone.0185734.
- 719 25. Walter J, Britton RA, Roos S. 2011. Host-microbial symbiosis in the vertebrate
720 gastrointestinal tract and the *Lactobacillus reuteri* paradigm. Proc Natl Acad Sci USA
721 108:4645.
- 722 26. Frese SA, Benson AK, Tannock GW, Loach DM, Kim J, Zhang M, Oh PL, Heng
723 NCK, Patil PB, Juge N, Mackenzie DA, Pearson BM, Lapidus A, Dalin E, Tice H,
724 Goltsman E, Land M, Hauser L, Ivanova N, Kyrpides NC, Walter J. 2011. The evolution
725 of host specialization in the vertebrate gut symbiont *Lactobacillus reuteri*. PLoS Genet
726 7:2. e1001314. 10.1371/journal.pgen.1001314.
- 727 27. Oh PL, Benson AK, Peterson DA, Patil PB, Moriyama EN, Roos S, Walter J. 2010.
728 Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven
729 evolution. ISME J 4:377-387. 10.1038/ismej.2009.123.
- 730 28. Tannock GW, Wilson CM, Loach D, Cook GM, Eason J, O'Toole PW, Holtrop G,
731 Lawley B. 2012. Resource partitioning in relation to cohabitation of *Lactobacillus*
732 species in the mouse forestomach. ISME J 6:927-938. 10.1038/ismej.2011.161.
- 733 29. Cleusix V, Lacroix C, Vollenweider S, Duboux M, Le Blay G. 2007. Inhibitory activity
734 spectrum of reuterin produced by *Lactobacillus reuteri* against intestinal bacteria. BMC
735 Microbiol 7:101. 10.1186/1471-2180-7-101.

- 736 30. Gänzle MG. 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in
737 food fermentations and food spoilage. *Curr Opin Food Sci* 2:106-117.
738 10.1016/j.cofs.2015.03.001.
- 739 31. Turrone F, Milani C, Duranti S, Mahony J, van Sinderen D, Ventura M. 2018. Glycan
740 utilization and cross-feeding activities by bifidobacteria. *Trends Microbiol* 26:339-350.
741 10.1016/j.tim.2017.10.001.
- 742 32. Turrone F, Bottacini F, Foroni E, Mulder I, Kim J, Zomer A, Sánchez B, Bidossi A,
743 Ferrarini A, Giubellini V, Delledonne M, Henrissat B, Coutinho P, Oggioni M, Fitzgerald
744 GF, Mills D, Margolles A, Kelly D, van Sinderen D, Ventura M, Klaenhammer TR. 2010.
745 Genome analysis of *Bifidobacterium bifidum* PRL2010 reveals metabolic pathways for
746 host-derived glycan foraging. *Proc Natl Acad Sci USA* 107:19514-19519.
747 10.1073/pnas.1011100107.
- 748 33. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu
749 N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL. 2013. Microbiota-liberated
750 host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* 502:96-99.
751 10.1038/nature12503.
- 752 34. Kremling A, Geiselman J, Ropers D, de Jong H. 2014. Understanding carbon
753 catabolite repression in *Escherichia coli* using quantitative models. *Trends Microbiol*
754 23:99-109. 10.1016/j.tim.2014.11.002.

- 755 35. Aidelberg G, Towbin BD, Rothschild D, Dekel E, Bren A, Alon U. 2014. Hierarchy of
756 non-glucose sugars in *Escherichia coli*. BMC Syst Biol 8:133. 10.1186/s12918-014-
757 0133-z.
- 758 36. Basra P, Alsaadi A, Bernal-Astrain G, O'Sullivan ML, Hazlett B, Clarke LM,
759 Schoenrock A, Pitre S, Wong A. 2018. Fitness tradeoffs of antibiotic resistance in
760 extraintestinal pathogenic *Escherichia coli*. Genome Biol and Evol 10:667-679.
761 10.1093/gbe/evy030.
- 762 37. Andersson DI, Hughes D. 2010. Antibiotic resistance and its cost: is it possible to
763 reverse resistance? Nat Rev Microbiol 8:260-271. 10.1038/nrmicro2319.
- 764 38. Rakoff-Nahoum S, Foster KR, Comstock LE. 2016. The evolution of cooperation
765 within the gut microbiota. Nature 533:255-259. 10.1038/nature17626.
- 766 39. Smith NW, Shorten PR, Altermann E, Roy NC, McNabb WC. 2019. The
767 classification and evolution of bacterial cross-feeding. Front Ecol and Evol 7:153.
768 10.3389/fevo.2019.00153.
- 769 40. O'Connell Motherway M, O'Brien F, O'Driscoll T, Casey PG, Shanahan F, van
770 Sinderen D. 2018. Carbohydrate syntrophy enhances the establishment of
771 *Bifidobacterium breve* UCC2003 in the neonatal gut. Sci Rep 8:10627-10.
772 10.1038/s41598-018-29034-0.

- 773 41. Coyte KZ, Rakoff-Nahoum S. 2019. Understanding competition and cooperation
774 within the mammalian gut microbiome. *Curr Biol* 29:11. R538-R544.
775 10.1016/j.cub.2019.04.017.
- 776 42. Rakoff-Nahoum S, Coyne M, Comstock L. 2014. An ecological network of
777 polysaccharide utilization among human intestinal symbionts. *Curr Biol* 24:40-49.
778 10.1016/j.cub.2013.10.077.
- 779 43. Maldonado-Gómez M, Martínez I, Bottacini F, O'Callaghan A, Ventura M,
780 van Sinderen D, Hillmann B, Vangay P, Knights D, Hutkins R, Walter J. 2016. Stable
781 engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on
782 individualized features of the resident microbiome. *Cell Host Microbe* 20:515-526.
783 10.1016/j.chom.2016.09.001.
- 784 44. Walter J, Maldonado-Gómez MX, Martínez I. 2018. To engraft or not to engraft: an
785 ecological framework for gut microbiome modulation with live microbes. *Curr Opin*
786 *Biotechnol* 49:129-139. 10.1016/j.copbio.2017.08.008.
- 787 45. Khalesi S, Bellissimo N, Vandelandotte C, Williams S, Stanley D, Irwin C. 2018. A
788 review of probiotic supplementation in healthy adults: helpful or hype? *Eur J Clin Nutr*
789 73:24-37. 10.1038/s41430-018-0135-9.
- 790 46. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S,
791 Kotler E, Zur M, Regev-Lehavi D, Brik RB, Federici S, Cohen Y, Linevsky R, Rothschild
792 D, Moor AE, Ben-Moshe S, Harmelin A, Itzkovitz S, Maharshak N, Shibolet O, Shapiro
793 H, Pevsner-Fischer M, Sharon I, Halpern Z, Segal E, Elinav E. 2018. Personalized gut

794 mucosal colonization resistance to empiric probiotics is associated with unique host and
795 microbiome features. Cell 174:1388-1405.e21. 10.1016/j.cell.2018.08.041.

796 47. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, Armanini F, Truong DT,
797 Manara S, Zolfo M, Beghini F, Bertorelli R, De Sanctis V, Bariletti I, Canto R, Clementi
798 R, Cologna M, Crifò T, Cusumano G, Gottardi S, Innamorati C, Masè C, Postai D, Savoi
799 D, Duranti S, Lugli GA, Mancabelli L, Turrone F, Ferrario C, Milani C, Mangifesta M,
800 Anzalone R, Viappiani A, Yassour M, Vlamakis H, Xavier R, Collado CM, Koren O,
801 Tateo S, Soffiati M, Pedrotti A, Ventura M, Huttenhower C, Bork P, Segata N. 2018.
802 Mother-to-Infant microbial transmission from different body sites shapes the developing
803 infant gut microbiome. Cell Host Microbe 24:133-145.e5. 10.1016/j.chom.2018.06.005.

804 48. Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, Tassell ML,
805 Miller MJ, Jin Y, German JB, Lebrilla CB, Mills DA. 2015. Maternal fucosyltransferase 2
806 status affects the gut bifidobacterial communities of breastfed infants. Microbiome 3:13.
807 10.1186/s40168-015-0071-z.

808 49. Stolz P, Vogel RF, Hammes WP. 1995. Utilization of electron acceptors by
809 *Lactobacilli* isolated from sourdough. Zeitschrift für Lebensmittel-Untersuchung und -
810 Forschung 201:402-410. 10.1007/BF01192742.

811 50. Talarico TL, Axelsson LT, Novotny J, Fuizat M, Walter J. 1990. Utilization of glycerol
812 as a hydrogen acceptor by *Lactobacillus reuteri*: purification of 1,3-propanediol:NAD⁺
813 oxidoreductase. Appl Environ Microbiol. 56(4):943-948.

- 814 51. Duar RM, Frese SA, Lin XB, Fernando SC, Burkey TE, Tasseva G, Peterson DA,
815 Blom J, Wenzel CQ, Szymanski CM, Walter J. 2017. Experimental evaluation of host
816 adaptation of *Lactobacillus reuteri* to different vertebrate species. Appl Environ Microbiol
817 83:(12) e00132-17.
- 818 52. Rosander A, Connolly E, Roos S. 2008. Removal of antibiotic resistance gene-
819 carrying plasmids from *Lactobacillus reuteri* ATCC 55730 and characterization of the
820 resulting daughter strain, *L. reuteri* DSM 17938. Appl and Environl Microbiol 74:6032-
821 6040. 10.1128/AEM.00991-08.
- 822 53. Zhang S, Oh J, Alexander LM, Özçam M, van Pijkeren J. 2018. D-Alanyl-D-alanine
823 ligase as a broad-host-range counterselection marker in vancomycin-resistant lactic
824 acid bacteria. J Bacteriol 200:13. 10.1128/JB.00607-17.
- 825 54. Mazé A, O'Connell-Motherway M, Fitzgerald GF, Deutscher J, van Sinderen D.
826 2007. Identification and characterization of a fructose phosphotransferase system in
827 *Bifidobacterium breve* UCC2003. Appl and Environ Microbiol 73:545-553.
828 10.1128/AEM.01496-06.









