

1 **Formation of propionate and butyrate by the human colonic microbiota**

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10 Running title: Propionate and butyrate producing gut microbes

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12 *Originality-Significance statement:* In recent years there has been a tendency to rely on sequence data to
13 assign function to microorganisms, however, this has at times led to miss-annotations and the wrong
14 conclusions being drawn. This manuscript pulls together the current knowledge on butyrate and propionate
15 metabolism in the human gut by taking account of biochemical studies performed on microorganisms in
16 addition to sequence information. In particular the areas on 1,2-propanediol metabolism and protein
17 metabolism in the gut have, to our knowledge, not been comprehensively reviewed in the context of gut
18 microbiology.

19

20 **Summary**

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22 **The human gut microbiota ferments dietary non-digestible carbohydrates into short-chain fatty acids**
23 **(SCFA). These microbial products are utilized by the host and propionate and butyrate in particular exert**
24 **a range of health-promoting functions. Here we provide an overview of the metabolic pathways utilized**
25 **by gut microbes to produce these two SCFA from dietary carbohydrates and from amino acids resulting**
26 **from protein breakdown. This overview emphasizes the important role played by cross-feeding of**
27 **intermediary metabolites (in particular lactate, succinate and 1,2-propanediol) between different gut**
28 **bacteria. The ecophysiology, including growth requirements and responses to environmental factors, of**
29 **major propionate and butyrate producing bacteria are discussed in relation to dietary modulation of**
30 **these metabolites. A detailed understanding of SCFA metabolism by the gut microbiota is necessary to**
31 **underpin effective strategies to optimize SCFA supply to the host.**

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34 **Introduction**

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36 Short chain fatty acids (SCFA) are the major metabolic products of anaerobic fermentation by microbial
37 communities that colonize the mammalian gut, typically reaching total concentrations of 50-200 mM in the
38 human large intestine. They are taken up efficiently by the gut mucosa and have important impacts upon
39 host physiology as sources of energy, as regulators of gene expression and as signaling molecules that are
40 recognized by specific receptors (Morrison & Preston, 2016; Koh *et al.*, 2016). New mechanisms by which
41 SCFA regulate immune cell development and suppress inflammation have been uncovered recently (Louis
42 *et al.*, 2014; Richards *et al.*, 2016). It is apparent however that the three major SCFA, acetate, propionate
43 and butyrate, differ considerably in their potential effects upon host physiology. First, they differ in their
44 fate and tissue distribution, with butyrate being used preferentially as an energy source by the gut mucosa,
45 propionate contributing to gluconeogenesis in the liver and acetate achieving the highest systemic
46 concentrations in blood (Morrison & Preston, 2016). Second, there are differences in interactions with host

47 proteins (eg. inhibition of histone deacetylases by butyrate and propionate) and receptors (Bolognini *et al.*,
48 2016). This makes it particularly relevant to consider the microbial origin of these major fermentation
49 products and the potential for changes in diet and gut physiology to affect their relative production rates
50 and concentrations. This brief review will focus on butyrate and propionate as these two acids are most
51 often considered to benefit health, including protection against colorectal cancer in the case of butyrate
52 and promotion of satiety and reduction in cholesterol in the case of propionate (Morrison & Preston, 2016).
53 Acetate is a net fermentation product for most gut anaerobes that is also produced by reductive
54 acetogenesis, and almost invariably achieves the highest concentrations among the SCFA in the gut lumen.
55 In contrast, propionate and butyrate are produced by distinct subsets of gut bacteria. We consider here
56 what is currently known about the phylogenetic distribution of pathways leading to the formation of these
57 two SCFA within the human colonic microbiota and the potential for diverse dietary and environmental
58 factors to differentially modulate their production. Some fermentation products, including lactate,
59 succinate and 1,2-propanediol, do not usually accumulate to high levels in the human colon of healthy
60 adults, as they can also serve as substrates for other bacteria, including propionate and butyrate producers.
61 As the microbial metabolism of these compounds is intricately linked to the degradation of the main
62 dietary substrates, it will be discussed together with propionate and butyrate formation from
63 carbohydrates and proteins, respectively.

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66 **Pathways and bacterial groups contributing to butyrate formation from carbohydrates**

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68 Butyrate is produced from carbohydrates via glycolysis from the combination of two molecules of acetyl-
69 CoA to form acetoacetyl-CoA, followed by stepwise reduction to butyryl-CoA. Two different pathways are
70 known for the final step in butyrate formation from butyryl-CoA, which proceeds either via butyryl-
71 CoA:acetate CoA-transferase or via phosphotransbutyrylase and butyrate kinase (Louis & Flint, 2009) (Fig.
72 1). Butyrate-producing species are found interspersed with butyrate non-producing species in the two
73 predominant families of human colonic Firmicutes, *Ruminococcaceae* and *Lachnospiraceae*, as well as in

74 other Firmicutes families, including *Erysipelotrichaceae* and *Clostridiaceae* (Barcenilla *et al.*, 2000; Louis *et*
75 *al.*, 2004). We will briefly consider the characteristics of butyrate-producers that belong to the two most
76 abundant families of Firmicutes. We should note that, as summarized in Table 1, many dominant human
77 colonic Firmicutes (eg. *Blautia* spp., *Eubacterium eligens*, *Ruminococcus* spp.) lack the ability to form
78 butyrate from carbohydrates.

79 ***Ruminococcaceae.*** *Faecalibacterium prausnitzii*, one of the most abundant species present in the
80 healthy human microbiota, produces butyrate via butyryl-CoA:acetate CoA-transferase with net
81 consumption of acetate, and acetate stimulates its growth on carbohydrate energy sources (Duncan *et al.*,
82 2002). While *F. prausnitzii* strains are obligate anaerobes, they also show growth stimulation by low
83 concentrations of oxygen in the presence of riboflavin and reduced compounds such as cysteine or
84 glutathione (Khan *et al.*, 2012). It is hypothesized that this ability may provide a niche for the bacterium to
85 thrive in the proximity of the colonic wall, where oxygen is diffusing in from the bloodstream. Oxygen
86 consumption is accompanied by a decrease in butyrate formation (Khan *et al.*, 2012). *F. prausnitzii* isolates
87 show limited ability to utilize dietary polysaccharides such as starch and hemicellulose for growth, but
88 some strains utilize inulin and pectin derivatives and the ability to utilize uronic acids is widespread (Lopez-
89 Siles *et al.*, 2012). *F. prausnitzii* is depleted in inflammatory bowel disease patients, especially Crohn's
90 disease, and evidence that it has anti-inflammatory action has attracted interest in this species as a
91 potential therapeutic (Quévrain *et al.*, 2016). Similarly *Butyricicoccus pullicaecorum* is also reported to be
92 less abundant in inflammatory bowel disease patients, and might also have therapeutic potential (Eeckhaut
93 *et al.*, 2013). Butyrate production has been reported for other Ruminococcaceae (Table 1), but rather little
94 is known about most of these organisms.

95 ***Lachnospiraceae.*** *Eubacterium rectale* and the closely related *Roseburia* species constitute a major
96 group of butyrate-producing Firmicutes that share the butyryl-CoA:acetate CoA-transferase route for
97 butyrate production and the same genomic organization of their butyrate synthetic genes from acetyl-CoA
98 to butyryl-CoA (Louis & Flint, 2009). In some *Roseburia* strains, particularly at mildly acidic pH, butyrate is
99 almost the sole fermentation acid produced, with net consumption of acetate typically accompanying the
100 formation of butyrate (Kettle *et al.*, 2015). Other strains and species produce formate and lactate in

101 addition to butyrate (Louis & Flint, 2009). Genome analysis reveals that there is considerable capacity
102 within this group to utilize diet-derived polysaccharides including starch, arabinoxylan and inulin, that
103 varies substantially between strains and species (Sheridan *et al.*, 2016).

104 Butyrate-producing Lachnospiraceae show considerable divergence in their phylogeny, gene
105 organization and physiology (Louis & Flint, 2009) (Table 1). Other Lachnospiraceae that possess the butyryl-
106 CoA:acetate CoA-transferase gene include *Eubacterium hallii*, *Anaerostipes hadrus*, *Coprococcus catus*, un-
107 characterised species related to isolates SS3/4 and M62/1, and some uncultured organisms (Louis *et al.*,
108 2010; Reichardt *et al.*, 2014). Two species of *Coprococcus*, in common with many *Clostridium* species that
109 belong to other families of Firmicutes, use the butyrate kinase rather than CoA-transferase enzyme for the
110 final step in butyrate formation (Louis *et al.*, 2004; Louis & Flint, 2009). *E. rectale* and *E. hallii* are among the
111 10 most abundant species reported in the human faecal microbiota (Qin *et al.*, 2010; Walker *et al.*, 2011)
112 (Table 1) and together accounted for 44% of butyryl CoA:acetate CoA-transferase sequences amplified from
113 faecal samples of 10 healthy volunteers (Louis *et al.*, 2010).

114 Lactate can be produced from carbohydrates by many different gut bacteria (Duncan *et al.*, 2004).
115 *In vitro* incubations of ¹³C lactate with human intestinal microbiota show that the label is recovered in
116 acetate, propionate and butyrate. The proportions of these products can vary widely, however, with acidic
117 pH favouring butyrate (Belenguer *et al.*, 2007). In addition, there is considerable inter-individual variation in
118 the fate of ¹³C lactate, which is assumed to reflect differences in the relative abundance of lactate-utilising
119 species within the microbiota (Bourriaud *et al.*, 2005; Morrison *et al.*, 2006). Certain Lachnospiraceae have
120 the ability to grow in the presence of lactate and acetate to produce butyrate, showing an overall net
121 stoichiometry of 4 mols of lactate and 2 mols of acetate producing 3 mols of butyrate (Duncan *et al.*, 2004).
122 These include the abundant species *A. hadrus*, which uses only D-lactate (Allen-Vercoe *et al.*, 2012) and *E.*
123 *hallii*, which is able to utilize both lactate isomers (Duncan *et al.*, 2004; Muñoz-Tamayo *et al.*, 2011). Lactate
124 oxidation to pyruvate by direct reduction of NAD⁺ is energetically unfavourable. Anaerobic lactate utilisers
125 carry a lactate dehydrogenase that operates in complex with an electron-transferring flavoprotein that
126 couples the endergonic NAD⁺ reduction to ferredoxin oxidation, in a process called electron confurcation
127 (Weghoff *et al.*, 2014).

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130 **Pathways and bacterial groups contributing to propionate formation from carbohydrates**

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132 Two pathways are known for the formation of propionate from sugar fermentation by gut bacteria. Most
133 hexose and pentose sugars are processed through the succinate pathway (Fig.2) whereas the deoxy sugars
134 fucose and rhamnose are metabolized by the propanediol pathway (Fig.3).

135 The succinate pathway is found mainly in Bacteroidetes and in the Negativicutes class of Firmicutes
136 (Reichardt *et al.*, 2014). It is the major route for propionate formation from dietary carbohydrates driven by
137 the abundant Bacteroidetes, and relative Bacteroidetes abundance was found to correlate with relative
138 faecal propionate levels in human volunteers (Salonen *et al.*, 2014). Succinate is a precursor of propionate,
139 but can accumulate in cultures of *Bacteroides* spp. under growth conditions where PEP carboxykinase is
140 repressed, eg. at high pCO₂ and high dilution rates (Caspari & Macy, 1983). Conversion of succinate to
141 propionate also requires vitamin B₁₂ and succinate has been shown to accumulate in B₁₂-depleted cultures
142 of *Prevotella ruminicola* (Strobel 1992). Some species of Bacteroidetes, notably *Prevotella copri*, apparently
143 produce succinate rather than propionate as their main fermentation product and succinate accumulation
144 has been reported particularly in the rat gut (De Vadder *et al.*, 2016). The succinate pathway is known to be
145 present in some Ruminococcaceae, such as *Ruminococcus flavefaciens*, which also produces succinate
146 rather than propionate as the end product (Macfarlane & Gibson, 1997). On the other hand, some human
147 colonic bacteria belonging to the Negativicutes class of Firmicutes (eg. *Phascolarctobacterium*
148 *succinatutens*; Watanabe *et al.*, 2012), have the ability to convert succinate to propionate (Flint *et al.*, 2014;
149 Reichardt *et al.*, 2014). This activity may explain why succinate accumulation is infrequently reported for
150 human faecal samples, although 3 of the 14 overweight human volunteers in one recent dietary study
151 showed elevated faecal succinate concentrations (>30 mM) in samples from a non-starch polysaccharide-
152 supplemented diet (reported in Salonen *et al.*, 2014, Supplementary information). Other Negativicutes
153 bacteria convert lactate to propionate either via the succinate pathway (eg. *Veillonella* spp.) or via the

154 acrylate pathway (*Megasphaera elsdenii*) (Reichardt *et al.*, 2014) (Fig. 2). The acrylate pathway has also
155 been shown to operate recently in a species of Lachnospiraceae, *Coproccoccus catus* (Reichardt *et al.*, 2014).

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157 Formation of propionate and propanol from the deoxy sugars rhamnose and fucose via the
158 propanediol pathway has been demonstrated in dominant gut commensal bacteria belonging to the
159 Lachnospiraceae, including *Roseburia inulinivorans* and *Blautia* species (Scott *et al.*, 2006; Reichardt *et al.*,
160 2014) (Table 1, Fig. 3). Metabolism of rhamnose and fucose via this pathway has also been reported for
161 *Salmonella* and *Listeria* species (Xue *et al.*, 2008). Other bacteria, including *Bacteroides* species, *Escherichia*
162 *coli* and *Anaerostipes rhamnosivorans*, are able to degrade deoxy sugars via the propanediol pathway, but
163 produce the pathway intermediate 1,2-propanediol as the final product (Saxena *et al.*, 2010; Rodionova *et*
164 *al.*, 2013; Bui *et al.*, 2014). 1,2-propanediol can also be produced from other sugars via the glycolysis
165 intermediate dihydroxyacetone-phosphate and methylglyoxal by microbes including *Escherichia coli*,
166 *Clostridium sphenoides* and the yeast *Saccharomyces cerevisiae* (Bennett & San, 2001; Saxena *et al.*, 2010).
167 Methylglyoxal is further metabolised to 1,2-propanediol either via lactaldehyde or via hydroxyacetone (Fig.
168 3). In *C. sphenoides* it has been shown that 1,2-propanediol formation via dihydroxyacetone-phosphate
169 operates under phosphate limitation and it remains to be established whether it plays a major role in the
170 gut environment. A third pathway for 1,2-propanediol production via lactaldehyde operates from lactate in
171 *Lactobacillus buchneri*. The pathway has been elucidated in a strain isolated from maize silage (Gänzle,
172 2015), but this species has also been detected in the human gut (Mikelsaar *et al.*, 2016).

173 *E. hallii* and *Lactobacillus reuteri*, although unable to grow on fucose or rhamnose, are nevertheless
174 able to utilise 1,2-propanediol to produce propionate and propanol (Gänzle, 2015; Engels *et al.*, 2016) (Fig.
175 3). Furthermore, metagenomic mining for dehydratases has indicated that further gut anaerobes, including
176 *Flavonifractor plautii*, *Intestinimonas butyriproducens* and *Veillonella* spp. may also be able to produce
177 propionate from this substrate (Engels *et al.*, 2016). Thus, cross-feeding of the intermediate 1,2-
178 propanediol between different bacteria may play an important role in the production of propionate from
179 deoxy sugars. The conversion of 1,2-propanediol to propionate, which is dependent on vitamin B₁₂, takes
180 place in polyhedral bodies, microcompartments that sequester the toxic pathway intermediate

181 propionaldehyde (Chowdhury *et al.*, 2014). Interestingly, glycerol is converted to 1,3-propanediol and 3-
182 hydroxypropionate in *L. reuteri* and *E. hallii* by the same dehydratase that acts on 1,2-propanediol (Gänzle,
183 2015; Engels *et al.*, 2016) indicating that glycerol utilization may be the primary function of this enzyme in
184 these species. It is also worth noting that the pathway intermediate 3-hydroxypropionaldehyde, also known
185 as reuterin, is a potent antimicrobial compound (Gänzle, 2015).

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189 **Butyrate and propionate formation from proteins and amino acids**

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191 Propionate and butyrate are also formed as products from peptide and amino acid fermentation (Fig. 1 &
192 2), although the numbers of amino acid-fermenting bacteria have been estimated to constitute less than
193 1% of the large intestinal microbiota (Smith & Macfarlane, 1998; Dai *et al.*, 2011). It is estimated that the
194 colon receives approximately 13 g of protein and peptides per day, and large amounts of soluble protein
195 and peptides were found in intestinal contents of sudden death victims (Smith & Macfarlane, 1998).

196 Peptides seem to be preferred over free amino acids by gut bacteria. Low gut pH and the presence of
197 carbohydrates reduces peptide and amino acid fermentation *in vitro*, which helps to explain why microbial
198 amino acid fermentation is higher in the distal than the proximal colon contents (Smith & Macfarlane,
199 1998). Amino acid fermentation leads to the production of potentially harmful metabolites (for example
200 phenolic and indolic compounds, amines, ammonia) in addition to branched-chain fatty acids (BCFA) and
201 SCFA (Smith & Macfarlane, 1997; Dai *et al.*, 2011).

202 *In vitro* incubations of faecal slurries with individual amino acids showed that propionate was
203 produced mainly from aspartate, alanine, threonine and methionine, whereas butyrate was a major
204 fermentation product from glutamate, lysine, histidine, cysteine, serine and methionine (Smith &
205 Macfarlane, 1997). While several Bacteroidetes have major roles in proteolysis and in propionate formation
206 from peptides (Macfarlane & Macfarlane, 1995), certain Firmicutes species also show high activity on
207 amino acids, notably *Intestinimonas* AF211, which ferments glucose and lysine to butyrate via distinct

208 pathways (Bui *et al.*, 2015) (Fig. 1). Several different pathways exist for glutamate degradation in butyrate-
209 producing bacteria, which have mainly been studied in *Clostridium* species not originating from gut
210 environments (Barker, 1981; Buckel, 2001). However, there is genomic and metagenomic evidence that
211 they are also present in some gut bacteria (Potrykus *et al.*, 2008; Vital *et al.*, 2014). The glutamate
212 degradation pathways enter the main butyrate pathway either via pyruvate (3-methylaspartate pathway;
213 *Clostridium limosum*, *Fusobacterium* spp.) or crotonyl-CoA (4-aminobutyrate pathway, discussed in more
214 detail below, and 2-hydroxyglutarate pathway, found in different Firmicutes including *Acidaminococcus*
215 *fermentans*, *Clostridium sporosphaeroides*, *Clostridium symbiosum*, *Fusobacterium* spp. and
216 *Peptostreptococcus asaccharolyticus*) (Fig. 1). Some bacteria belonging to the Acidaminococcaceae also
217 degrade glutamate via the 3-methylaspartate pathway, but produce propionate rather than butyrate from
218 the intermediate pyruvate (Buckel, 2001) (Fig. 2).

219 Glutamate degradation to 4-aminobutyrate (gamma-aminobutyrate, GABA) is carried out under
220 acid stress to maintain intracellular pH homeostasis in a number of gut bacteria (Feehily & Karatzas, 2013),
221 and a bacterial isolate exclusively growing on GABA has recently been found
222 (<http://www.abstractsonline.com/pp8/#!/4060/presentation/18619>). As GABA also acts as a
223 neurotransmitter, the abundance of microbes involved in the production or consumption of GABA may
224 influence mood and behaviour. The pathway for GABA degradation is shared with succinate degradation via
225 succinate semialdehyde and 4-hydroxybutyrate (Fig. 1), and butyrate production from succinate via this
226 pathway has been demonstrated in *Porphyromonas gingivalis* and *Clostridioides difficile* (Ferreya *et al.*,
227 2014; Yoshida *et al.*, 2016).

228 The fermentation routes of other amino acids are less well understood. Histidine is converted to
229 glutamate (Potrykus *et al.*, 2008; Kanehisa *et al.*, 2016), which is in agreement with high levels of butyrate
230 being formed from histidine by faecal microbiota (Smith & Macfarlane, 1997). Alanine, serine and cysteine
231 are broken down to pyruvate (Potrykus *et al.*, 2008; Carbonero *et al.*, 2012), thus product formation
232 depends on the bacterium utilizing those amino acids and their corresponding fermentative pathways. For
233 example, in *Clostridium propionicum*, alanine fermentation leads to the production of propionate via
234 pyruvate, lactate and the acrylate pathway (Buckel, 2001) (Fig. 2). Threonine and methionine are converted

235 to 2-oxobutyrate, which leads to propionate formation (Fig. 2) (Barker, 1981; Smith & Macfarlane, 1997;
236 Kanehisa *et al.*, 2016). Several routes for the breakdown of aspartate exist, via alanine, threonine,
237 oxaloacetate or fumarate (Smith & Macfarlane, 1997; Kanehisa *et al.*, 2016) (Fig. 2), which accounts for the
238 fact that it is mainly converted to propionate in *in vitro* incubations.

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241 **Role of CoA-transferases in SCFA metabolism**

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243 Propionate and butyrate can be generated from their respective CoA thioesters either by transfer of the
244 CoA-moiety onto another metabolite, or by conversion via propionyl-phosphate or butyryl-phosphate. The
245 second (kinase) route leads to the generation of ATP, but the CoA-transferase route also conserves the
246 energy of the CoA bond in the newly formed CoA-derivative of the co-substrate. Acetate is a common co-
247 substrate in CoA-transferase reactions, and the high acetate concentrations in the large intestine provide a
248 possible explanation for the prevalence of the butyryl-CoA:acetate CoA-transferase route in gut microbes
249 (Louis *et al.*, 2004) (see also section on pH below). Bacteria often carry multiple different CoA-transferases
250 in their genomes, with *Intestinimonas* AF211 encoding at least 14 such enzymes (Bui *et al.*, 2015). It can be
251 difficult to pin-point which gene is responsible for SCFA formation, especially as CoA-transferases tend to
252 have broad substrate specificity. For example, the purified butyryl-CoA:acetate CoA-transferase (*butCoAT*
253 gene product) from *Roseburia hominis* has a similar affinity for butyryl-CoA and propionyl-CoA although the
254 enzyme is clearly responsible for butyrate formation in this species (Charrier *et al.*, 2006) (Table 2). Gene
255 expression evidence in *Intestinimonas* AF211 suggested that the enzyme AtoD-A, responsible for butyryl
256 CoA:acetoacetate CoA-transferase activity, plays a key role in conversion of lysine to butyrate, while the
257 *ButCoA* gene product mediated the final step in butyrate formation from glucose (Bui *et al.*, 2015). In
258 *Clostridium aminobutyricum*, a CoA-transferase that acts on 4-hydroxybutyrate and butyryl-CoA links the
259 final step of butyrate production to the formation of 4-hydroxybutyryl-CoA further up in the glutamate
260 fermentation pathway (Buckel, 2001). Similarly, *C. propionicum* links the formation of lactoyl-CoA in the
261 acrylate pathway to propionate formation via a CoA-transferase (Buckel, 2001). There are also instances

262 where different CoA-transferases appear to have evolved for the same enzymatic reaction. Thus, bacteria
263 belonging to the Erysipelotrichaceae do not carry a gene closely related to the butyryl-CoA:acetate CoA-
264 transferase identified in other Firmicutes. Instead, a gene more closely related to propionate CoA-
265 transferases is thought to be responsible for butyrate formation in these organisms (Eeckhaut *et al.*, 2011).

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268 **Impact of the gut environment**

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270 **pH.** Gut pH has a major impact on competition between different groups of bacteria within the microbial
271 community. In pH-controlled *in vitro* continuous culture experiments with soluble polysaccharide provided
272 as the main energy source, mildly acidic pH has been shown to curtail the growth of *Bacteroides* spp.
273 relative to Firmicutes and Actinobacteria (Walker *et al.*, 2005; Chung *et al.*, 2016). This is because human
274 colonic *Bacteroides* spp. are generally less able than many dominant Firmicutes to tolerate the presence of
275 short chain fatty acids at pH 5.5 (Duncan *et al.*, 2009). This selective inhibition and the resulting shift in
276 community composition has the consequence of limiting propionate formation and enhancing butyrate
277 production by the community at pH values around 5.5 compared with 6.5-6.8 (Walker *et al.*, 2005; Chung *et*
278 *al.*, 2016). The impact of pH shifts upon experimentally observed butyrate and propionate concentrations
279 has been successfully modelled mathematically, based on the differing tolerance to low pH of the major
280 bacterial functional groups that comprise the human colonic microbiota (Kettle *et al.*, 2015).

281 For bacteria that use the butyryl-CoA:acetate CoA-transferase route, acetate consumption and
282 butyrate production are reported to increase at mildly acidic pH compared with near neutral pH (Kettle *et*
283 *al.*, 2015). Although conversion of glucose to butyrate, 2 CO₂ and 2 H₂ can occur with no net uptake of
284 acetate (Gottschalk, 1979), net acetate uptake is typically observed for species of *Roseburia* and *F.*
285 *prausnitzii*. Theoretical stoichiometries involving net acetate uptake are shown in Fig. 4A, which also
286 assumes that some of the reducing power that is generated drives proton export, increasing the ATP yield
287 per glucose fermented (Buckel & Thauer, 2013). Incorporation of exogenous acetate via the CoA-
288 transferase reaction results in some loss of ATP production via acetyl-phosphate, but this is more than

289 compensated by the additional ATP formed from proton export, giving a potential maximum of 4 ATP
290 formed per glucose metabolized when 2 mols of acetate are taken in for each mol of glucose fermented.
291 Interestingly, Fig. 4B shows that the predictions from these stoichiometries (based on the generalised
292 equation shown in Fig. 4A) fit experimental data for the impact of pH on metabolites produced by *F.*
293 *prausnitzii* and two *Roseburia* spp. in anaerobic batch culture (Kettle *et al.*, 2015). Thus low pH (5.5) tends
294 to increase acetate uptake and butyrate production while near neutral pH (6.7) has the opposite effect. It
295 seems possible that the increased ATP gain associated with net acetate uptake helps to compensate for the
296 effects of low pH and might account for the reliance in the CoA-transferase route for butyrate formation in
297 these bacteria.

298 **Growth requirements.** It has been show in a rodent model that limitation of dietary iron intake can
299 dramatically decrease the production of both butyrate and propionate as lactobacilli and Proteobacteria
300 are favoured (Dostal *et al.*, 2012). Populations of *Roseburia*-related butyrate producers appear particularly
301 sensitive to iron availability, while in pure cultures of *R. intestinalis* butyrate production was favoured at
302 high iron concentrations with a switch to lactate production under iron-deficient conditions (Dostal *et al.*,
303 2015). It remains to be established whether other growth factors also have a major impact on SCFA
304 formation.

305 **Intestinal gases.** SCFA formation is also likely to be affected by differences in oxygen concentration
306 in different regions and micro-compartments of the gut due to differences in oxygen sensitivity and
307 metabolic capacity between microbes, as exemplified by the peculiar relationship of *F. prausnitzii* with
308 oxygen (discussed above). Furthermore, the abundance of microbes consuming hydrogen and thereby
309 influencing the hydrogen partial pressure in the gut also influences SCFA formation, as this affects the
310 overall balance of fermentation products formed (Macfarlane & Macfarlane, 2003; Wolf *et al.*, 2016).

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313 **Concluding remarks**

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315 Huge advances have been made in recent years in our understanding of SCFA metabolism in the human
316 gut, and many of the dominant propionate- and butyrate-producing bacteria are available in culture,
317 enabling detailed investigations into their metabolism. Recent work has emphasized that butyrate and
318 propionate can arise from fermentation both of amino acids and of carbohydrates, but the relative
319 contributions of protein and carbohydrate fermentation *in vivo* over the wide range of 'normal' human
320 dietary intakes is not yet clear. We know that high protein, low carbohydrate weight loss diets lead to a
321 disproportionate decrease in butyrate among total faecal SCFA, together with an increased proportion of
322 branched chain fatty acids that are wholly derived from branched chain amino acids and therefore provide
323 an indicator of protein fermentation (Duncan *et al.*, 2007, Russell *et al.*, 2011). This suggests strongly that
324 butyrate production is mainly determined by the supply of non-digestible carbohydrates, rather than by
325 protein fermentation. This may however reflect the particular ecology of butyrate-producing bacteria, as
326 discussed above. In the case of propionate, on the other hand, the major producers of propionate from
327 dietary carbohydrates, the Bacteroidetes, are also important peptide fermenters and the propionate
328 proportion among faecal SCFA was not decreased by such low carbohydrate diets (Duncan *et al.*, 2007). It is
329 also clear that compounds normally regarded as intermediates (eg. succinate, lactate) may accumulate in
330 certain individuals or in particular conditions. This makes it important also to consider the impacts of these
331 metabolites on the host, as for example in the case of succinate which it is suggested may provide health
332 benefits (De Vadder *et al.*, 2016). Lactate is detected as a major fermentation product in breast-fed infants
333 whose microbiota is dominated by *Bifidobacterium* spp. In adults, however, lactate accumulation is
334 associated with dysbiosis, eg, in severe colitis (Hove *et al.*, 1994), that may result in part from a lack of
335 lactate-utilizing bacteria (Belenguer *et al.*, 2007).

336 The ever-increasing availability of genomic and metagenomics sequences is a highly useful resource
337 to foster our understanding of microbial metabolism in the gut, but care has to be taken with assigning
338 function to genes by sequence analysis, which should ideally be complemented by evidence from genetic or
339 enzymatic studies. A renewed interest in isolation and study of gut bacteria (Walker *et al.*, 2014, Browne *et*
340 *al.*, 2016) together with novel systems for gene transfer and knockout on the horizon will enable a
341 thorough understanding of the different members of the microbial community. This will benefit *in vitro* and

342 *in vivo* microbial community-based studies to foster our understanding of the different ecological niches of
343 the community members, how they interact with each other and how we can modulate the system by
344 dietary means to optimize SCFA production. The fact that, in general, different phylogenetic groups of
345 bacteria are responsible for butyrate and propionate production suggests that there may be scope for
346 differentially manipulating their production by the gut microbiota.

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353

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355

356

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546 **Figure legends**

547

548 **Fig. 1.** Microbial pathways for butyrate formation from carbohydrates, organic acids, glutamate and lysine
549 in gut communities. Carbohydrate fermentation to pyruvate via glycolysis is shown in green, butyrate
550 formation from acetyl-CoA in black, amino acid fermentation pathways in blue (intermediates after which
551 the different glutamate pathways are named are highlighted), and lactate and succinate fermentation in
552 purple and pink, respectively. See main text for key enzymes and bacteria harbouring the different
553 pathways. Redox reactions which involve electron carriers are indicated by [H]. CoA-transferase-mediated
554 reactions are indicated by ●. As indicated, co-substrates other than acetate may operate in CoA-transferase
555 reactions in some bacteria (for further detail see main text). CoA, coenzyme A; P, bound phosphate; Pi,
556 inorganic phosphate; PEP, phosphoenolpyruvate; (B₁₂), enzyme dependent on vitamin B₁₂. Dotted line
557 indicates that several intermediate steps are involved.

558

559 **Fig. 2.** Microbial pathways for propionate formation from carbohydrates, organic acids and amino acids. As
560 indicated, amino acids capable of conversion to pyruvate can also give rise to butyrate (Fig. 1).
561 Carbohydrate fermentation to pyruvate via glycolysis is shown in green, propionate formation via the
562 succinate pathway in black, amino acid fermentation pathways in blue, and acrylate pathway for lactate
563 utilisation in purple. See main text for key enzymes and bacteria harbouring the different pathways. Redox
564 reactions which involve electron carriers are indicated by [H]. CoA-transferase-mediated reactions are
565 indicated by ● (*may be performed by a CoA-transferase or CoA-ligase reaction). Propionate formation

566 from propionyl-CoA in the succinate pathway may involve either a CoA-transferase or phosphate
567 propanoyltransferase/propionate kinase reaction. CoA, coenzyme A; PEP, phosphoenolpyruvate; (B₁₂),
568 dependent on vitamin B₁₂. Dotted lines indicate that several intermediate steps are involved.

569

570 **Fig. 3.** Microbial pathways for propionate formation via 1,2-propanediol. Carriage of the different pathways
571 in gut microbes is indicated by colour. Redox reactions which involve electron carriers are indicated by [H].
572 Propionate formation from propionyl-CoA may involve either a CoA-transferase or phosphate
573 propanoyltransferase/propionate kinase reaction (indicated by a dashed line). Grey hexagon indicates that
574 the reaction is carried out in polyhedral bodies to sequester toxic intermediate propionaldehyde. CoA,
575 coenzyme A; P, bound phosphate; (B₁₂), dependent on vitamin B₁₂. Dotted line indicates that several
576 intermediate steps are involved.

577

578 **Fig. 4.** Butyrate production in bacteria that use the butyryl-CoA:acetate CoA-transferase route. A: General
579 equation for the relationship between acetate consumption and butyrate production, assuming no lactate
580 or formate are produced (modified from Louis & Flint 2009 and Kettle et al. 2015). Etf, electron-transferring
581 flavoprotein; Fd, ferredoxin; P, bound phosphate; Pi, inorganic phosphate. B: Alternative stoichiometries
582 for butyrate production based on A. Experimental data (coloured symbols) refer to *R. intestinalis* L1-82, *R.*
583 *hominis* A2-183 and *F. prausnitzii* A2-165 grown at three different initial pH values (5.5, 6.2, 6.7) (Kettle et
584 al 2015 and Sylvia Duncan, personal communication).

585

586 **Table 1.** Capabilities for butyrate and propionate production among dominant bacterial species detected in
 587 faecal samples of human subjects (Qin *et al.*, 2010; Zhernakova *et al.*, 2016)

Phylum (family)	species	Butyrate ¹	Propionate ²
Bacteroidetes (Bacteroidaceae)	<i>Bacteroides uniformis</i>	-	+ (Suc)
	<i>Bacteroides vulgatus</i>	-	+ (Suc)
Bacteroidetes (Prevotellaceae)	<i>Prevotella copri</i>	-	+ (Suc)
Bacteroidetes (Rikenellaceae)	<i>Alistipes putredinis</i>	-	+ (Suc)
Firmicutes (Lachnospiraceae)	<i>Eubacterium rectale</i>	+ (CoAT)	-
	<i>Roseburia inulinivorans</i>	+ (CoAT)	+ (Pdu)
	<i>Roseburia intestinalis</i>	+ (CoAT)	-
	<i>Dorea longicatena</i>	-	-
	<i>Eubacterium hallii</i>	+ (CoAT)	+ (Pdu)
	<i>Anaerostipes hadrus</i>	+ (CoAT)	-
	<i>Ruminococcus torques</i>	-	-
	<i>Coprococcus eutactus</i>	+ (ButK)	-
	<i>Blautia obeum</i>	-	+ (Pdu)
	<i>Dorea formicigenerans</i>	-	-
	<i>Coprococcus catus</i>	+ (CoAT)	+ (Acr)
Firmicutes (Ruminococcaceae)	<i>Faecalibacterium prausnitzii</i>	+ (CoAT)	-
	<i>Subdoligranulum variabile</i>	+ (ButK)	-
	<i>Ruminococcus bromii</i>	-	-
	<i>Eubacterium siraeum</i>	-	-
Firmicutes (Veillonellaceae)	<i>Dialister invisus</i>	-	+ (Suc)
Firmicutes (Acidaminococcaceae)	<i>Phascolarctobacterium succinatutens</i>	-	+ (Suc)
Firmicutes (Erysipelotrichaceae)	<i>Eubacterium bifforme</i> ³	+ (CoAT)	-
Actinobacteria (Bifidobacteriaceae)	<i>Bifidobacterium adolescentis</i>	-	-

	<i>Bifidobacterium longum</i>	-	-
Actinobacteria (Coriobacteriaceae)	<i>Collinsella aerofaciens</i>	-	-
Verrucomicrobia (Verrucomicrobiaceae)	<i>Akkermansia muciniphila</i>	-	+ (Suc)

588

589 ¹-, absent; +, present; ButK, butyrate kinase route; CoAT, butyryl-CoA:acetate CoA-transferase route.

590 ²-, absent; +, present; Acr, acrylate pathway; Pdu, 1,2-propanediol pathway; Suc, succinate pathway

591 (succinate may be the major product formed instead of propionate in some species and/or under some

592 growth conditions).

593 ³Reclassified as *Holdemanella bififormis* (De Maesschalck *et al.*, 2014). CoA-transferase route is proposed

594 based on closely related butyrate producers within the Erysipelotrichaceae (see also main text, section on

595 CoA-transferases).

596

597

598 **Table 2.** Activity of the butyryl-CoA:acetate CoA-transferase of *Roseburia hominis* A2-183 (purified
 599 recombinant *butCoAT* gene product expressed in *Escherichia coli* (Charrier *et al.*, 2006)).
 600

	K_m [mM]	V_{max} [$\mu\text{mol}/\text{min}/\text{mg}$ protein]	Inhibition by competition with acetate [%] ¹
acetate	6.4		
butyryl CoA	0.098	112	
propionyl CoA	0.099	51	
Butyrate			75
Propionate			70
Isobutyrate			56
Valerate			28

601 ¹No significant inhibition was found for caproate, 3-hydroxybutyrate, 4-hydroxybutyrate, 4-aminobutyrate,
 602 lactate, acetoacetate and succinate.

603

Fig. 1

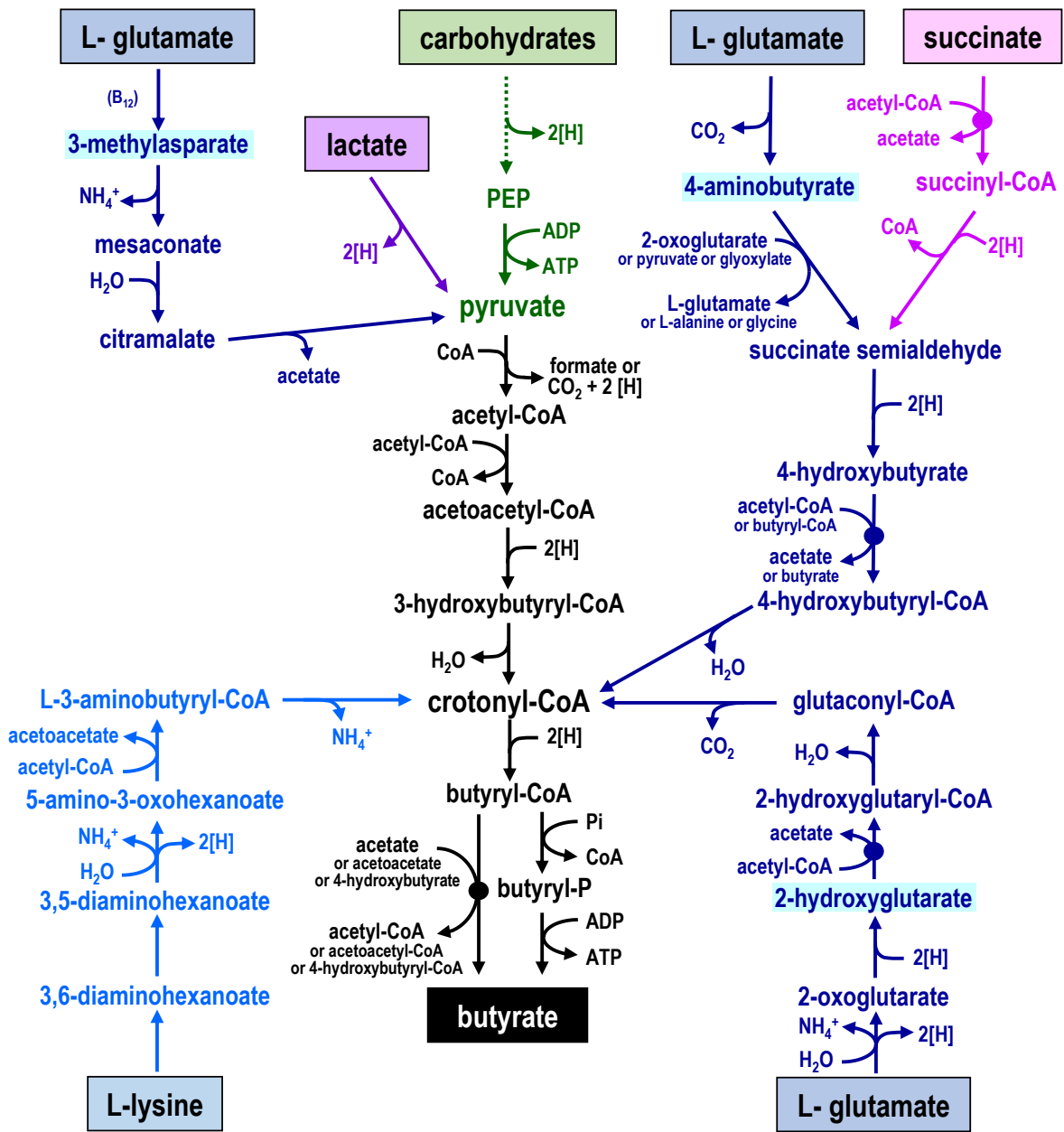


Fig. 2

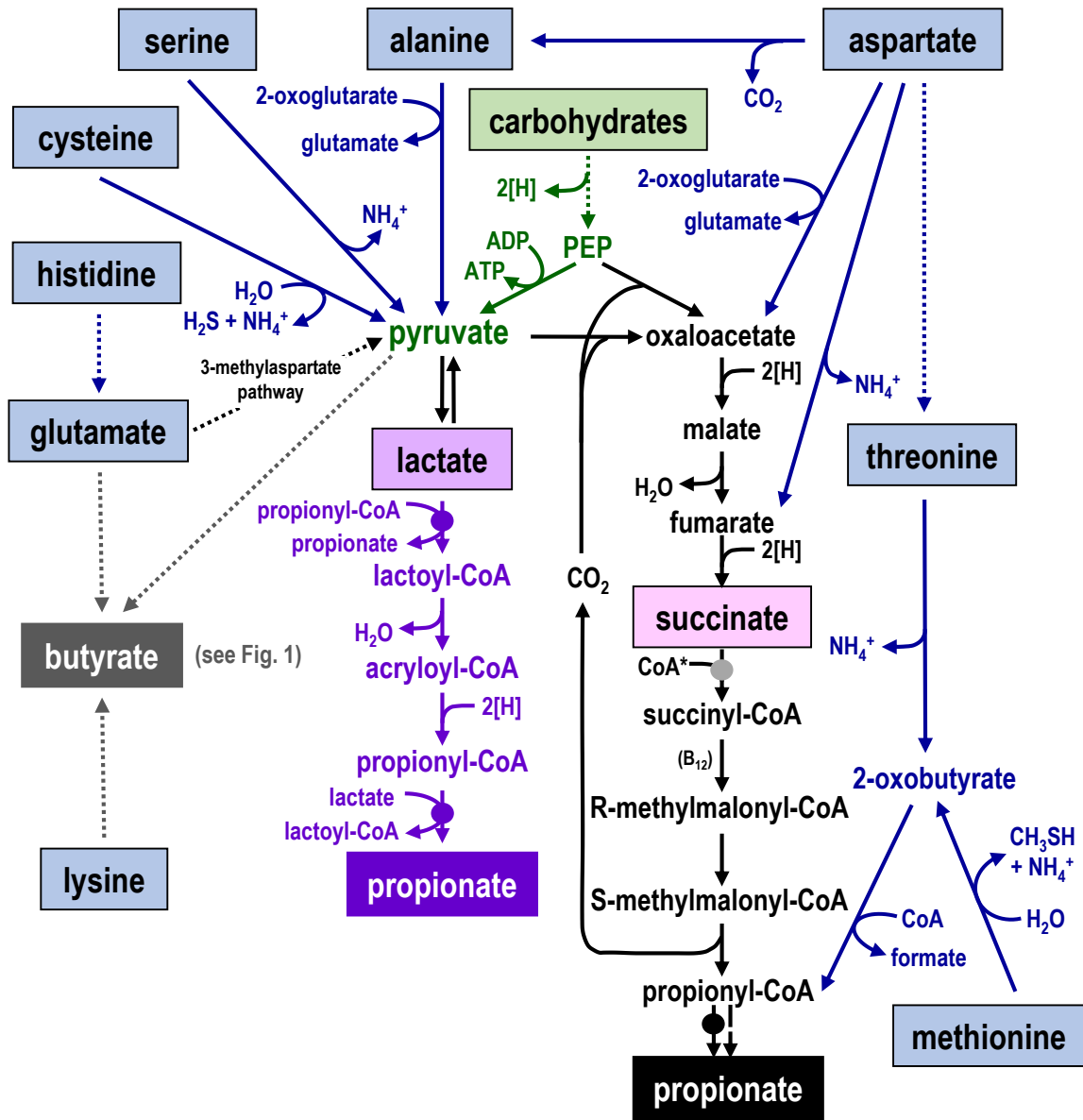
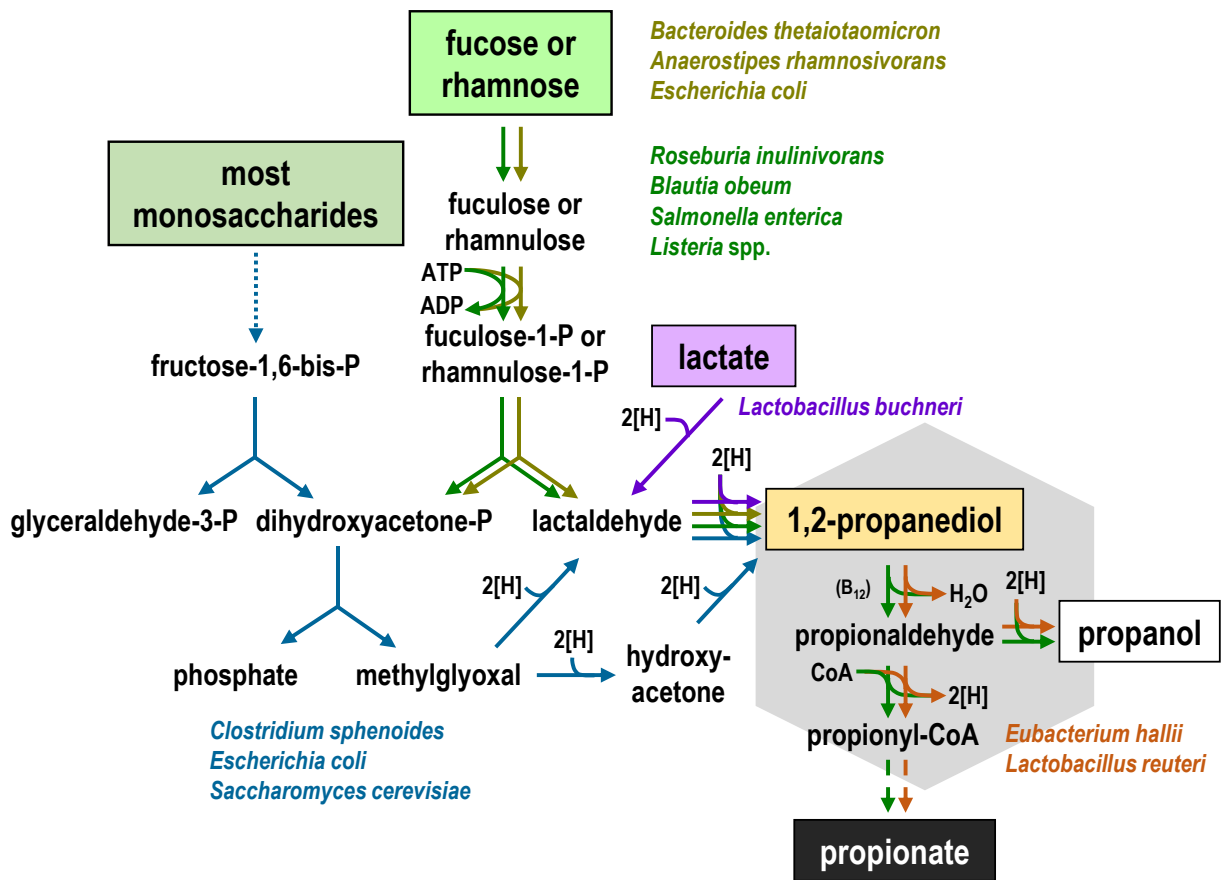


Fig. 3



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Fig. 4

