Formation of propionate and butyrate by the human colonic microbiota

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Running title: Propionate and butyrate producing gut microbes

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

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

Introduction

Short chain fatty acids (SCFA) are the major metabolic products of anaerobic fermentation by microbial communities that colonize the mammalian gut, typically reaching total concentrations of 50-200 mM in the human large intestine. They are taken up efficiently by the gut mucosa and have important impacts upon host physiology as sources of energy, as regulators of gene expression and as signaling molecules that are recognized by specific receptors (Morrison & Preston, 2016; Koh et al., 2016). New mechanisms by which SCFA regulate immune cell development and suppress inflammation have been uncovered recently (Louis et al., 2014; Richards et al., 2016). It is apparent however that the three major SCFA, acetate, propionate and butyrate, differ considerably in their potential effects upon host physiology. First, they differ in their fate and tissue distribution, with butyrate being used preferentially as an energy source by the gut mucosa, propionate contributing to gluconeogenesis in the liver and acetate achieving the highest systemic concentrations in blood (Morrison & Preston, 2016). Second, there are differences in interactions with host
proteins (eg. inhibition of histone deacetylases by butyrate and propionate) and receptors (Bolognini et al., 2016). This makes it particularly relevant to consider the microbial origin of these major fermentation products and the potential for changes in diet and gut physiology to affect their relative production rates and concentrations. This brief review will focus on butyrate and propionate as these two acids are most often considered to benefit health, including protection against colorectal cancer in the case of butyrate and promotion of satiety and reduction in cholesterol in the case of propionate (Morrison & Preston, 2016).

Acetate is a net fermentation product for most gut anaerobes that is also produced by reductive acetogenesis, and almost invariably achieves the highest concentrations among the SCFA in the gut lumen. In contrast, propionate and butyrate are produced by distinct subsets of gut bacteria. We consider here what is currently known about the phylogenetic distribution of pathways leading to the formation of these two SCFA within the human colonic microbiota and the potential for diverse dietary and environmental factors to differentially modulate their production. Some fermentation products, including lactate, succinate and 1,2-propanediol, do not usually accumulate to high levels in the human colon of healthy adults, as they can also serve as substrates for other bacteria, including propionate and butyrate producers.

As the microbial metabolism of these compounds is intricately linked to the degradation of the main dietary substrates, it will be discussed together with propionate and butyrate formation from carbohydrates and proteins, respectively.

Pathways and bacterial groups contributing to butyrate formation from carbohydrates

Butyrate is produced from carbohydrates via glycolysis from the combination of two molecules of acetyl-CoA to form acetoacetyl-CoA, followed by stepwise reduction to butyryl-CoA. Two different pathways are known for the final step in butyrate formation from butyryl-CoA, which proceeds either via butyryl-CoA:acetate CoA-transferase or via phosphotransbutyrylase and butyrate kinase (Louis & Flint, 2009) (Fig. 1). Butyrate-producing species are found interspersed with butyrate non-producing species in the two predominant families of human colonic Firmicutes, Ruminococcaceae and Lachnospiraceae, as well as in
other Firmicutes families, including *Erysipelotrichaceae* and *Clostridiaceae* (Barcenilla et al., 2000; Louis et al., 2004). We will briefly consider the characteristics of butyrate-producers that belong to the two most abundant families of Firmicutes. We should note that, as summarized in Table 1, many dominant human colonic Firmicutes (eg. *Blautia* spp., *Eubacterium eligens*, *Ruminococcus* spp.) lack the ability to form butyrate from carbohydrates.

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

*Lachnospiraceae*. *Eubacterium rectale* and the closely related *Roseburia* species constitute a major group of butyrate-producing Firmicutes that share the butyryl-CoA:acetate CoA-transferase route for butyrate production and the same genomic organization of their butyrate synthetic genes from acetyl-CoA to butyryl-CoA (Louis & Flint, 2009). In some *Roseburia* strains, particularly at mildly acidic pH, butyrate is almost the sole fermentation acid produced, with net consumption of acetate typically accompanying the formation of butyrate (Kettle et al., 2015). Other strains and species produce formate and lactate in
addition to butyrate (Louis & Flint, 2009). Genome analysis reveals that there is considerable capacity within this group to utilize diet-derived polysaccharides including starch, arabinoxylan and inulin, that varies substantially between strains and species (Sheridan et al., 2016).

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

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

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

The succinate pathway is found mainly in Bacteroidetes and in the Negativicutes class of Firmicutes (Reichardt et al., 2014). It is the major route for propionate formation from dietary carbohydrates driven by the abundant Bacteroidetes, and relative Bacteroidetes abundance was found to correlate with relative faecal propionate levels in human volunteers (Salonen et al., 2014). Succinate is a precursor of propionate, but can accumulate in cultures of Bacteroides spp. under growth conditions where PEP carboxykinase is repressed, eg. at high pCO$_2$ and high dilution rates (Caspari & Macy, 1983). Conversion of succinate to propionate also requires vitamin B$_{12}$ and succinate has been shown to accumulate in B$_{12}$-depleted cultures of Prevotella ruminicola (Strobel 1992). Some species of Bacteroidetes, notably Prevotella copri, apparently produce succinate rather than propionate as their main fermentation product and succinate accumulation has been reported particularly in the rat gut (De Vadder et al., 2016). The succinate pathway is known to be present in some Ruminococcaceae, such as Ruminococcus flavefaciens, which also produces succinate rather than propionate as the end product (Macfarlane & Gibson, 1997). One the other hand, some human colonic bacteria belonging to the Negativicutes class of Firmicutes (eg. Phascolarctobacterium succinatutens; Watanabe et al., 2012), have the ability to convert succinate to propionate (Flint et al., 2014; Reichardt et al., 2014). This activity may explain why succinate accumulation is infrequently reported for human faecal samples, although 3 of the 14 overweight human volunteers in one recent dietary study showed elevated faecal succinate concentrations (>30 mM) in samples from a non-starch polysaccharide-supplemented diet (reported in Salonen et al., 2014, Supplementary information). Other Negativicutes bacteria convert lactate to propionate either via the succinate pathway (eg. Veillonella spp.) or via the
acrylate pathway (\textit{Megasphaera elsdenii}) (Reichardt et al., 2014) (Fig. 2). The acrylate pathway has also been shown to operate recently in a species of Lachnospiraceae, \textit{Coprococcus catus} (Reichardt et al., 2014).

Formation of propionate and propanol from the deoxy sugars rhamnose and fucose via the propanediol pathway has been demonstrated in dominant gut commensal bacteria belonging to the Lachnospiraceae, including \textit{Roseburia inulinivorans} and \textit{Blautia} species (Scott et al., 2006; Reichardt et al., 2014) (Table 1, Fig. 3). Metabolism of rhamnose and fucose via this pathway has also been reported for \textit{Salmonella} and \textit{Listeria} species (Xue et al., 2008). Other bacteria, including \textit{Bacteroides} species, \textit{Escherichia coli} and \textit{Anaerostipes rhamnosivorans}, are able to degrade deoxy sugars via the propanediol pathway, but produce the pathway intermediate 1,2-propanediol as the final product (Saxena et al., 2010; Rodionova et al., 2013; Bui et al., 2014). 1,2-propanediol can also be produced from other sugars via the glycolysis intermediate dihydroxyacetone-phosphate and methylglyoxal by microbes including \textit{Escherichia coli}, \textit{Clostridium sphenoides} and the yeast \textit{Saccharomyces cerevisiae} (Bennett & San, 2001; Saxena et al., 2010).

Methylglyoxal is further metabolised to 1,2-propanediol either via lactaldehyde or via hydroxyacetone (Fig. 3). In \textit{C. sphenoides} it has been shown that 1,2-propanediol formation via dihydroxyacetone-phosphate operates under phosphate limitation and it remains to be established whether it plays a major role in the gut environment. A third pathway for 1,2-propanediol production via lactaldehyde operates from lactate in \textit{Lactobacillus buchneri}. The pathway has been elucidated in a strain isolated from maize silage (Gänzle, 2015), but this species has also been detected in the human gut (Mikelsaar et al., 2016).

\textit{E. hallii} and \textit{Lactobacillus reuteri}, although unable to grow on fucose or rhamnose, are nevertheless able to utilise 1,2-propanediol to produce propionate and propanol (Gänzle, 2015; Engels et al., 2016) (Fig. 3). Furthermore, metagenomic mining for dehydratases has indicated that further gut anaerobes, including \textit{Flavonifractor plautii}, \textit{Intestinimonas butyriproducens} and \textit{Veillonella} spp. may also be able to produce propionate from this substrate (Engels et al., 2016). Thus, cross-feeding of the intermediate 1,2-propanediol between different bacteria may play an important role in the production of propionate from deoxy sugars. The conversion of 1,2-propanediol to propionate, which is dependent on vitamin B\textsubscript{12}, takes place in polyhedral bodies, microcompartments that sequester the toxic pathway intermediate...
Propionaldehyde (Chowdhury et al., 2014). Interestingly, glycerol is converted to 1,3-propanediol and 3-hydroxypropionate in L. reuteri and E. hallii by the same dehydratase that acts on 1,2-propanediol (Gänzle, 2015; Engels et al., 2016) indicating that glycerol utilization may be the primary function of this enzyme in these species. It is also worth noting that the pathway intermediate 3-hydroxypropionaldehyde, also known as reuterin, is a potent antimicrobial compound (Gänzle, 2015).

Butyrate and propionate formation from proteins and amino acids

Propionate and butyrate are also formed as products from peptide and amino acid fermentation (Fig. 1 & 2), although the numbers of amino acid-fermenting bacteria have been estimated to constitute less than 1% of the large intestinal microbiota (Smith & Macfarlane, 1998; Dai et al., 2011). It is estimated that the colon receives approximately 13 g of protein and peptides per day, and large amounts of soluble protein and peptides were found in intestinal contents of sudden death victims (Smith & Macfarlane, 1998). Peptides seem to be preferred over free amino acids by gut bacteria. Low gut pH and the presence of carbohydrates reduces peptide and amino acid fermentation in vitro, which helps to explain why microbial amino acid fermentation is higher in the distal than the proximal colon contents (Smith & Macfarlane, 1998). Amino acid fermentation leads to the production of potentially harmful metabolites (for example phenolic and indolic compounds, amines, ammonia) in addition to branched-chain fatty acids (BCFA) and SCFA (Smith & Macfarlane, 1997; Dai et al., 2011).

In vitro incubations of faecal slurries with individual amino acids showed that propionate was produced mainly from aspartate, alanine, threonine and methionine, whereas butyrate was a major fermentation product from glutamate, lysine, histidine, cysteine, serine and methionine (Smith & Macfarlane, 1997). While several Bacteroidetes have major roles in proteolysis and in propionate formation from peptides (Macfarlane & Macfarlane, 1995), certain Firmicutes species also show high activity on amino acids, notably Intestimonas AF211, which ferments glucose and lysine to butyrate via distinct
Several different pathways exist for glutamate degradation in butyrate-producing bacteria, which have mainly been studied in *Clostridium* species not originating from gut environments (Barker, 1981; Buckel, 2001). However, there is genomic and metagenomic evidence that they are also present in some gut bacteria (Potrykus et al., 2008; Vital et al., 2014). The glutamate degradation pathways enter the main butyrate pathway either via pyruvate (3-methylasparate pathway; *Clostridium limosum, Fusobacterium* spp.) or crotonyl-CoA (4-aminobutyrate pathway, discussed in more detail below, and 2-hydroxyglutarate pathway, found in different Firmicutes including *Acidaminococcus fermentans, Clostridium sporosphaeroides, Clostridium symbiosum, Fusobacterium* spp. and *Peptostreptococcus asaccharolyticus* (Fig. 1). Some bacteria belonging to the Acidaminococcaceae also degrade glutamate via the 3-methylasparate pathway, but produce propionate rather than butyrate from the intermediate pyruvate (Buckel, 2001) (Fig. 2).

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

The fermentation routes of other amino acids are less well understood. Histidine is converted to glutamate (Potrykus et al., 2008; Kanehisa et al., 2016), which is in agreement with high levels of butyrate being formed from histidine by faecal microbiota (Smith & Macfarlane, 1997). Alanine, serine and cysteine are broken down to pyruvate (Potrykus et al., 2008; Carbonero et al., 2012), thus product formation depends on the bacterium utilizing those amino acids and their corresponding fermentative pathways. For example, in *Clostridium propionicum*, alanine fermentation leads to the production of propionate via pyruvate, lactate and the acrylate pathway (Buckel, 2001) (Fig. 2). Threonine and methionine are converted...
to 2-oxobutyrate, which leads to propionate formation (Fig. 2) (Barker, 1981; Smith & Macfarlane, 1997; Kanehisa et al., 2016). Several routes for the breakdown of asparate exist, via alanine, threonine, oxaloacetate or fumarate (Smith & Macfarlane, 1997; Kanehisa et al., 2016) (Fig. 2), which accounts for the fact that it is mainly converted to propionate in *in vitro* incubations.

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

Propionate and butyrate can be generated from their respective CoA thioesters either by transfer of the CoA-moiety onto another metabolite, or by conversion via propionyl-phosphate or butyryl-phosphate. The second (kinase) route leads to the generation of ATP, but the CoA-transferase route also conserves the energy of the CoA bond in the newly formed CoA-derivative of the co-substrate. Acetate is a common co-substrate in CoA-transferase reactions, and the high acetate concentrations in the large intestine provide a possible explanation for the prevalence of the butyryl-CoA:acetate CoA-transferase route in gut microbes (Louis et al., 2004) (see also section on pH below). Bacteria often carry multiple different CoA-transferases in their genomes, with *Intestinimonas AF211* encoding at least 14 such enzymes (Bui et al., 2015). It can be difficult to pin-point which gene is responsible for SCFA formation, especially as CoA-transferases tend to have broad substrate specificity. For example, the purified butyryl-CoA:acetate CoA-transferase (*butCoAT* gene product) from *Roseburia hominis* has a similar affinity for butyryl-CoA and propionyl-CoA although the enzyme is clearly responsible for butyrate formation in this species (Charrier et al., 2006) (Table 2). Gene expression evidence in *Intestinimonas AF211* suggested that the enzyme AtoD-A, responsible for butyryl CoA:acetoacetate CoA-transferase activity, plays a key role in conversion of lysine to butyrate, while the ButCoA gene product mediated the final step in butyrate formation from glucose (Bui et al., 2015). In *Clostridium aminobutyricum*, a CoA-transferase that acts on 4-hydroxybutyrate and butyryl-CoA links the final step of butyrate production to the formation of 4-hydroxybutyryl-CoA further up in the glutamate fermentation pathway (Buckel, 2001). Similarly, *C. propionicum* links the formation of lactoyl-CoA in the acrylate pathway to propionate formation via a CoA-transferase (Buckel, 2001). There are also instances
where different CoA-transferases appear to have evolved for the same enzymatic reaction. Thus, bacteria belonging to the Erysipelotrichaceae do not carry a gene closely related to the butyryl-CoA:acetate CoA-transferase identified in other Firmicutes. Instead, a gene more closely related to propionate CoA-transferases is thought to be responsible for butyrate formation in these organisms (Eckhaut et al., 2011).

Impact of the gut environment

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

For bacteria that use the butyryl-CoA:acetate CoA-transferase route, acetate consumption and butyrate production are reported to increase at mildly acidic pH compared with near neutral pH (Kettle et al., 2015). Although conversion of glucose to butyrate, 2 CO₂ and 2 H₂ can occur with no net uptake of acetate (Gottschalk, 1979), net acetate uptake is typically observed for species of Roseburia and F. prausnitzii. Theoretical stoichiometries involving net acetate uptake are shown in Fig. 4A, which also assumes that some of the reducing power that is generated drives proton export, increasing the ATP yield per glucose fermented (Buckel & Thauer, 2013). Incorporation of exogenous acetate via the CoA-transferase reaction results in some loss of ATP production via acetyl-phosphate, but this is more than
compensated by the additional ATP formed from proton export, giving a potential maximum of 4 ATP formed per glucose metabolized when 2 mols of acetate are taken in for each mol of glucose fermented. Interestingly, Fig. 4B shows that the predictions from these stoichiometries (based on the generalised equation shown in Fig. 4A) fit experimental data for the impact of pH on metabolites produced by \textit{F. prausnitzii} and two \textit{Roseburia} spp. in anaerobic batch culture (Kettle \textit{et al.}, 2015). Thus low pH (5.5) tends to increase acetate uptake and butyrate production while near neutral pH (6.7) has the opposite effect. It seems possible that the increased ATP gain associated with net acetate uptake helps to compensate for the effects of low pH and might account for the reliance in the CoA-transferase route for butyrate formation in these bacteria.

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

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

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

The ever-increasing availability of genomic and metagenomics sequences is a highly useful resource to foster our understanding of microbial metabolism in the gut, but care has to be taken with assigning function to genes by sequence analysis, which should ideally be complemented by evidence from genetic or enzymatic studies. A renewed interest in isolation and study of gut bacteria (Walker et al., 2014, Browne et al., 2016) together with novel systems for gene transfer and knockout on the horizon will enable a thorough understanding of the different members of the microbial community. This will benefit in vitro and
in vivo microbial community-based studies to foster our understanding of the different ecological niches of the community members, how they interact with each other and how we can modulate the system by dietary means to optimize SCFA production. The fact that, in general, different phylogenetic groups of bacteria are responsible for butyrate and propionate production suggests that there may be scope for differentially manipulating their production by the gut microbiota.

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References


**Figure legends**

**Fig. 1.** Microbial pathways for butyrate formation from carbohydrates, organic acids, glutamate and lysine in gut communities. Carbohydrate fermentation to pyruvate via glycolysis is shown in green, butyrate formation from acetyl-CoA in black, amino acid fermentation pathways in blue (intermediates after which the different glutamate pathways are named are highlighted), and lactate and succinate fermentation in purple and pink, respectively. See main text for key enzymes and bacteria harbouring the different pathways. Redox reactions which involve electron carriers are indicated by [H]. CoA-transferase-mediated reactions are indicated by ●. As indicated, co-substrates other than acetate may operate in CoA-transferase reactions in some bacteria (for further detail see main text). CoA, coenzyme A; P, bound phosphate; Pi, inorganic phosphate; PEP, phosphoenolpyruvate; (B<sub>12</sub>), enzyme dependent on vitamin B<sub>12</sub>. Dotted line indicates that several intermediate steps are involved.

**Fig. 2.** Microbial pathways for propionate formation from carbohydrates, organic acids and amino acids. As indicated, amino acids capable of conversion to pyruvate can also give rise to butyrate (Fig. 1). Carbohydrate fermentation to pyruvate via glycolysis is shown in green, propionate formation via the succinate pathway in black, amino acid fermentation pathways in blue, and acrylate pathway for lactate utilisation in purple. See main text for key enzymes and bacteria harbouring the different pathways. Redox reactions which involve electron carriers are indicated by [H]. CoA-transferase-mediated reactions are indicated by ● (**may be performed by a CoA-transferase or CoA-ligase reaction). Propionate formation
from propionyl-CoA in the succinate pathway may involve either a CoA-transferase or phosphate propanoyltransferase/propionate kinase reaction. CoA, coenzyme A; PEP, phosphoenolpyruvate; (B_{12}), dependent on vitamin B_{12}. Dotted lines indicate that several intermediate steps are involved.

**Fig. 3.** Microbial pathways for propionate formation via 1,2-propanediol. Carriage of the different pathways in gut microbes is indicated by colour. Redox reactions which involve electron carriers are indicated by [H]. Propionate formation from propionyl-CoA may involve either a CoA-transferase or phosphate propanoyltransferase/propionate kinase reaction (indicated by a dashed line). Grey hexagon indicates that the reaction is carried out in polyhedral bodies to sequester toxic intermediate propionaldehyde. CoA, coenzyme A; P, bound phosphate; (B_{12}), dependent on vitamin B_{12}. Dotted line indicates that several intermediate steps are involved.

**Fig. 4.** Butyrate production in bacteria that use the butyryl-CoA:acetate CoA-transferase route. A: General equation for the relationship between acetate consumption and butyrate production, assuming no lactate or formate are produced (modified from Louis & Flint 2009 and Kettle et al. 2015). Etf, electron-transferring flavoprotein; Fd, ferredoxin; P, bound phosphate; Pi, inorganic phosphate. B: Alternative stoichiometries for butyrate production based on A. Experimental data (coloured symbols) refer to *R. intestinalis* L1-82, *R. hominis* A2-183 and *F. prausnitzii* A2-165 grown at three different initial pH values (5.5, 6.2, 6.7) (Kettle et al 2015 and Sylvia Duncan, personal communication).
Table 1. Capabilities for butyrate and propionate production among dominant bacterial species detected in faecal samples of human subjects (Qin et al., 2010; Zhernakova et al., 2016)

<table>
<thead>
<tr>
<th>Phylum (family)</th>
<th>species</th>
<th>Butyrate</th>
<th>Propionate</th>
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<tbody>
<tr>
<td>Bacteroidetes (Bacteroidaceae)</td>
<td><em>Bacteroides uniformis</em></td>
<td>-</td>
<td>+ (Suc)</td>
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<tr>
<td></td>
<td><em>Bacteroides vulgatus</em></td>
<td>-</td>
<td>+ (Suc)</td>
</tr>
<tr>
<td>Bacteroidetes (Prevotellaceae)</td>
<td><em>Prevotella copri</em></td>
<td>-</td>
<td>+ (Suc)</td>
</tr>
<tr>
<td>Bacteroidetes (Rikenellaceae)</td>
<td><em>Alistipes putredinis</em></td>
<td>-</td>
<td>+ (Suc)</td>
</tr>
<tr>
<td>Firmicutes (Lachnospiraceae)</td>
<td><em>Eubacterium rectale</em></td>
<td>+ (CoAT)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Roseburia inulinivorans</em></td>
<td>+ (CoAT)</td>
<td>+ (Pdu)</td>
</tr>
<tr>
<td></td>
<td><em>Roseburia intestinalis</em></td>
<td>+ (CoAT)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Dorea longicatena</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Eubacterium hallii</em></td>
<td>+ (CoAT)</td>
<td>+ (Pdu)</td>
</tr>
<tr>
<td></td>
<td><em>Anaerostipes hadrus</em></td>
<td>+ (CoAT)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Ruminococcus torques</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Coprococcus eutactus</em></td>
<td>+ (ButK)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Blaunia obeum</em></td>
<td>-</td>
<td>+ (Pdu)</td>
</tr>
<tr>
<td></td>
<td><em>Dorea formicigenerans</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Coprococcus catus</em></td>
<td>+ (CoAT)</td>
<td>+ (Acr)</td>
</tr>
<tr>
<td>Firmicutes (Ruminococcaceae)</td>
<td><em>Faecalibacterium prausnitzii</em></td>
<td>+ (CoAT)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Subdoligranulum variabile</em></td>
<td>+ (ButK)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Ruminococcus bromii</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Eubacterium siraeum</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Firmicutes (Veillonellaceae)</td>
<td><em>Dialister invisus</em></td>
<td>-</td>
<td>+ (Suc)</td>
</tr>
<tr>
<td>Firmicutes (Acidaminococcaceae)</td>
<td><em>Phascolarctobacterium succinatutens</em></td>
<td>-</td>
<td>+ (Suc)</td>
</tr>
<tr>
<td>Firmicutes (Erysipelotrichaceae)</td>
<td><em>Eubacterium biforme</em></td>
<td>+ (CoAT)</td>
<td>-</td>
</tr>
<tr>
<td>Actinobacteria (Bifidobacteriaceae)</td>
<td><em>Bifidobacterium adolescentis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bifidobacterium longum</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria (Coriobacteriaceae)</td>
<td>Collinsella aerofaciens</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>Akkermansia muciniphila</td>
<td>-</td>
<td>+ (Suc)</td>
</tr>
</tbody>
</table>

(Verrucomicrobiaceae)

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

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

(succinate may be the major product formed instead of propionate in some species and/or under some growth conditions).

Reclassified as Holdemanella biformis (De Maesschalck et al., 2014). CoA-transferase route is proposed based on closely related butyrate producers within the Erysipelotrichaceae (see also main text, section on CoA-transferases).
Table 2. Activity of the butyryl-CoA:acetate CoA-transferase of *Roseburia hominis* A2-183 (purified recombinant butCoAT gene product expressed in *Escherichia coli* (Charrier et al., 2006)).

<table>
<thead>
<tr>
<th></th>
<th>K&lt;sub&gt;m&lt;/sub&gt; [mM]</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; [µmol/min/mg protein]</th>
<th>Inhibition by competition with acetate [%]&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>butyryl CoA</td>
<td>0.098</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>propionyl CoA</td>
<td>0.099</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td></td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Isobutyrate</td>
<td></td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Valerate</td>
<td></td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

<sup>3</sup>No significant inhibition was found for caproate, 3-hydroxybutyrate, 4-hydroxybutyrate, 4-aminobutyrate, lactate, acetoacetate and succinate.
Fig. 2

- serine
- alanine
- aspartate
- cysteine
- histidine
- glutamate
- butyrate
- lysine
- carbohydrates
- pyruvate
- lactate
- succinate
- threonine
- propionate
- methionine

(see Fig. 1)
Fig. 3

Most monosaccharides

- Fucose or rhamnose

Fructose-1,6-bis-P

Glyceraldehyde-3-P, dihydroxyacetone-P, lactaldehyde

ATP, ADP, fuculose-1-P or rhamnulose-1-P

Lactate

1,2-propanediol

Propionate

Bacteroides thetaiotaomicron
Anaerostipes rhamnosivorans
Escherichia coli

Roseburia inulinivorans
Blautia obeum
Salmonella enterica
Listeria spp.

Clostridium sphenoides
Escherichia coli
Saccharomyces cerevisiae

Lactobacillus buchneri

Hydroxyacetone

Propionaldehyde

Propionyl-CoA

Eubacterium hallii
Lactobacillus reuteri

2H2O

2H

Fig. 3
A. General equation:

\[
\text{glucose} + n \text{ acetate} \rightarrow (1 + n/2) \text{ butyrate} + 2 \text{ CO}_2 + (2 - n) \text{ H}_2
\]

(In the diagram \( y = (1 + n/2) \))

ATP/ glucose: \( 2 + (2 - y) + (2(y + n))/4 \) ATP

[assuming generation of 1 ATP per 4 H⁺]

---

B. Figure 4

- butyrate
- \( R. \text{ intestinalis} \) butyrate
- \( R. \text{ hominis} \) butyrate
- \( F. \text{ prausnitzii} \) butyrate
- \( \text{H}_2 \)
- ATP