MINIREVIEW

Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine

Petra Louis & Harry J. Flint

Microbial Ecology Group, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK

Correspondence: Petra Louis, Microbial Ecology Group, Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK. Tel.: +44 1224 712751; fax: +44 1224 716687; e-mail: p.louis@abdn.ac.uk

Received 17 December 2008; accepted 15 January 2009.
First published online 13 February 2009.

DOI:10.1111/j.1574-6968.2009.01514.x

Editor: Rustam Aminov

Keywords
butyrate; large intestine; gut health; colonic microbiota; anaerobic metabolism.

Abstract

Butyrate-producing bacteria play a key role in colonic health in humans. This review provides an overview of the current knowledge of the diversity, metabolism and microbial ecology of this functionally important group of bacteria. Human colonic butyrate producers are Gram-positive firmicutes, but are phylogenetically diverse, with the two most abundant groups related to *Eubacterium rectale*/*Roseburia* spp. and to *Faecalibacterium prausnitzii*. Five different arrangements have been identified for the genes of the central pathway involved in butyrate synthesis, while in most cases butyryl-CoA : acetate CoA-transferase, rather than butyrate kinase, appears to perform the final step in butyrate synthesis. Mechanisms have been proposed recently in non-gut *Clostridium* spp. whereby butyrate synthesis can result in energy generation via both substrate-level phosphorylation and proton gradients. Here we suggest that these mechanisms also apply to the majority of butyrate producers from the human colon. The roles of these bacteria in the gut community and their influence on health are now being uncovered, taking advantage of the availability of cultured isolates and molecular methodologies. Populations of *F. prausnitzii* are reported to be decreased in Crohn's disease, for example, while populations of *Roseburia* relatives appear to be particularly sensitive to the diet composition in human volunteer studies.

Introduction

Butyrate-producing bacteria represent a functional group, rather than a coherent phylogenetic group, within the microbial community of the human large intestine. Despite their heterogeneity, however, there are good reasons why they should provide a compelling focus for a minireview. First, butyrate plays a key role in maintaining human gut health, as the major source of energy to the colonic mucosa, and as an important regulator of gene expression, inflammation, differentiation and apoptosis in host cells (Scheppach & Weiler, 2004; Pajak *et al.*, 2007; Hamer *et al.*, 2008). Second, there is recent evidence that butyrate formation can play a special role in bacterial energy metabolism (Herrmann *et al.*, 2008; Li *et al.*, 2008; Seedorf *et al.*, 2008) and this implies that certain features of energy metabolism and microbial ecology may be shared between phylogenetically distinct groups of butyrate-producing bacteria. Third, the butyrate producers that colonize the human gut are strict anaerobes that have generally been regarded as difficult to grow in culture. Recent efforts to isolate them have been successful, however, and are providing entirely new information on some of the most dominant members of the gut microbial community.

In an earlier review, we briefly considered the role of butyrate in protecting human colonic health, and what was then known about butyrate-producing bacteria from the human intestine (Pryde *et al.*, 2002). Subsequent years have witnessed a significant expansion in our knowledge and understanding of butyrate-producing bacteria and of the microbial production of butyrate in the large intestine. The aim of the present article is to provide a concise update on progress in these areas.

Diversity, phylogeny and abundance of butyrate-producing bacteria

The ability to produce butyrate is widely distributed among Gram-positive anaerobic bacteria that inhabit the human colon. Information on the phylogenetic diversity of butyrate

Table 1. Major butyrate-producing bacteria isolated from the human colon

		16S rRNA gene-based probes								Flagella	Metabolism			
Bacterial species/group	Cluster*	Rrec 584	Rint 623h†	Eram 997	Ehal 1469	Acac 194	Ceut 705	Ecyl 387	Fpra 655		Acetate utilization	Lactate utilization	Butyrate kinase	Typical abundance in faeces‡ (% total bacteria)
Eubacterium rectale	XIVa	+	+							+	+	–	–	0–6 } 2–15
Roseburia intestinalis	XIVa	+	+							+	+	–	–	
Roseburia faecis	XIVa	+	+							+	+	–	–	
Roseburia hominis	XIVa	+	+							+	+	–	–	
Roseburia inulinivorans	XIVa		+							+	+	–	–	0–1.5
Butyrivibrio fibrisolvens	XIVa									+	+	–	–	
Eubacterium ramulus	XIVa			+						+	+	–	–	0–0.1
Eubacterium hallii	XIVa				+						+	dl-L	–	0–3
Anaerostipes caccae	XIVa					+					+	dl-L	–	
SSC/2, SS2/1§	XIVa										+	d-L	–	0–2
SS3/4, GM2/1§	XIVa										+	–	–	
Coprococcus catus GD/7	XIVa										+	dl-L	–	
Coprococcus eutactus L2-50	XIVa						+				–	–	+	
Coprococcus comes A2-232	XIVa										–	–	+	
Eubacterium cylindroides	XVI							+			–	–	–	0–3
Faecalibacterium prausnitzii	IV								+		+	–	–	2–15
Subdoligranulum variabile	IV										ND	ND	ND	
Anaerotruncus colihominis	IV										ND	ND	ND	

*16S rRNA gene clusters as defined by Collins *et al*. (1994); also referred to as the *Clostridium coccoides* (XIVa) and *Clostridium leptum* (IV) clusters.
†Group-specific probe used with a helper oligonucleotide (Aminov *et al*., 2006).
‡Approximate estimates are based on data from FISH microscopy, real-time PCR and sequencing of amplified 16S rRNA genes for a limited number (c. 30) of volunteers (e.g. Schwiertz *et al*., 2000; Suau *et al*., 2001; Hold *et al*., 2003; Eckburg *et al*., 2005; Aminov *et al*., 2006; Walker *et al*., 2008).
§Cultured isolates belonging to two abundant species/genera that have yet to be named (Duncan *et al*., unpublished data).
ND, not determined.

producers has come largely from cultural studies, allied to DNA sequence analysis and sequence-based detection methods (Barcenilla *et al*., 2000; Schwierz *et al*., 2000; Hold *et al*., 2003; Aminov *et al*., 2006). Numerically, two of the most important groups appear to be *Faecalibacterium prausnitzii*, which belongs to the *Clostridium leptum* (or clostridial cluster IV) cluster, and *Eubacterium rectale/Roseburia* spp., which belong to the *Clostridium coccoides* (or clostridial cluster XIVa) cluster of firmicute bacteria (Table 1). Estimates based on FISH and real-time PCR detection indicate that each of these groups typically accounts for around 5–10% of the total bacteria detectable in faecal samples from healthy adult human subjects. Although the number of cultured isolates remains limited, in the case of the *Roseburia/E. rectale* group, the available isolates appear to cover much of the 16S rRNA gene sequence diversity that is revealed by direct PCR amplification from faecal samples (Aminov *et al*., 2006). Because all the cultured representatives of this group produce butyrate, this suggests that this group consists mainly, if not exclusively, of butyrate producers. On the other hand, recent analysis of the *C. leptum* group of human gut bacteria, which includes *F. prausnitzii*, by fluorescence-activated cell sorting suggests that many phylotypes remain uncultured and functionally uncharacterized (Lay *et al*., 2007).

The distribution of butyrate production among firmicute bacteria is uneven, and the abundant *C. coccoides* and *C. leptum* clusters include both producers and nonproducers of butyrate (Pryde *et al*., 2002). Therefore, a functionally based approach appears to be desirable for the enumeration of butyrate-producing bacteria. Amplification of the recently described butyryl-CoA : acetate CoA transferase gene (discussed further below) using degenerate primers that recognize multiple phylogenetic groups represents a promising route (Louis & Flint, 2007).

Butyrate synthesis

Butyrate-producing bacteria carry out fermentative metabolism, i.e. they gain energy in the form of ATP by substrate-level phosphorylation during oxidative substrate breakdown. The resulting reducing equivalents (in the form of NADH) are transferred onto metabolic intermediates, leading to the formation of large amounts of reduced end products such as butyrate. Fermentative bacteria generally possess alternative pathways leading to the formation of

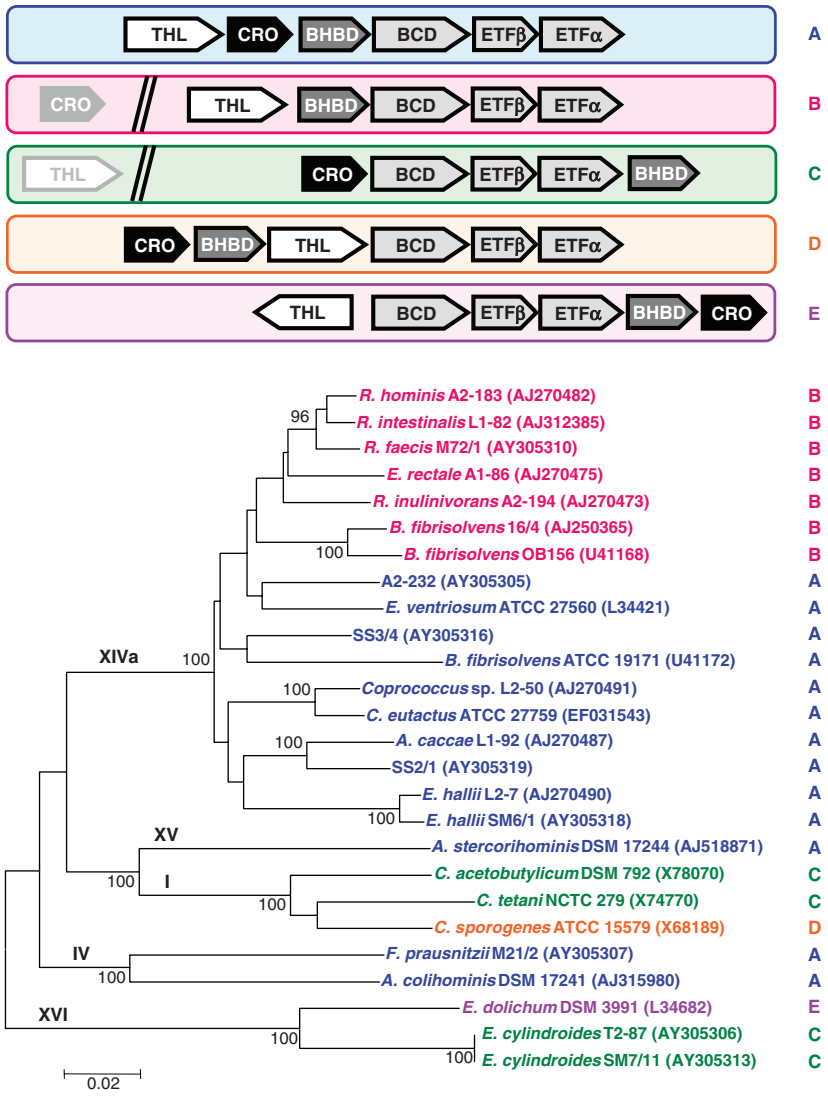

Fig. 1. Genomic arrangement of genes for the formation of butyryl-CoA from acetyl-CoA. Data are from Louis *et al*. (2007a) and from an analysis (P. Louis, unpublished data, November 2008) of complete draft genome sequences from HGMI (Genome Sequencing Center at Washington University School of Medicine in St. Louis, http://genome.wustl.edu/pub/organism/Microbes/Human_Gut_Microbiome). Colour coding and alphabetical key indicate the distribution of each gene arrangement within the phylogenetic tree based on 16S rRNA gene sequences (positions 44–1441 of the *Escherichia coli* numbering system of Brosius *et al*., 1978). Bootstrap values >90 (per 100 replications) are shown at branch points. Clostridial clusters (Collins *et al*., 1994) are indicated by roman numbers. BCD, butyryl-CoA dehydrogenase; BHBD, β-hydroxybutyryl-CoA dehydrogenase; CRO, crotonase; ETFα, electron transfer protein α; ETFβ, electron transfer protein β; and THL, thiolase.

different end products. Many butyrate producers from the gut environment can also produce lactate, formate, hydrogen and carbon dioxide, and the relative proportions of the different products formed depend on the environmental conditions. Thus, the level of ATP production and maintenance of redox balance can be modulated through shifts between alternative pathways in response to carbon source availability and hydrogen partial pressure (Macfarlane & Macfarlane, 2003).

Radioisotope analysis of faecal suspensions has revealed that the Embden–Meyerhof–Parnas pathway is the main route for the catabolism of glucose and that butyrate is formed by condensation of two molecules of acetyl-CoA (Miller & Wolin, 1996). Three different gene arrangements were recently reported among human gut bacteria for the pathway leading from acetyl-CoA to butyryl-CoA, and these arrangements seemed to be well conserved phylogenetically (Louis *et al*., 2007a). An analysis of complete draft genome sequences of butyrate-producing human gut isolates from the Human Gut Microbiome Initiative (http://www.genome.wustl.edu/hgm/HGM_frontpage.cgi) revealed that this conservation in gene arrangement was found for all further bacteria from clostridial cluster XIVa, but two additional gene arrangements were found in cluster I and cluster XVI (Fig. 1). It remains to be established whether these differences in gene organization reflect differences in pathway regulation.

It has recently been proposed that butyrate production might be used not only to regenerate NAD^+ from NADH produced in the energy-generating steps of substrate breakdown, but that it might also lead to further energy gains (Herrmann *et al*., 2008). During the conversion of crotonyl-CoA to butyryl-CoA by the butyryl-CoA dehydrogenase (Bcd) electron-transferring flavoprotein (Etf) complex, a proton motive force may be generated with a membrane-

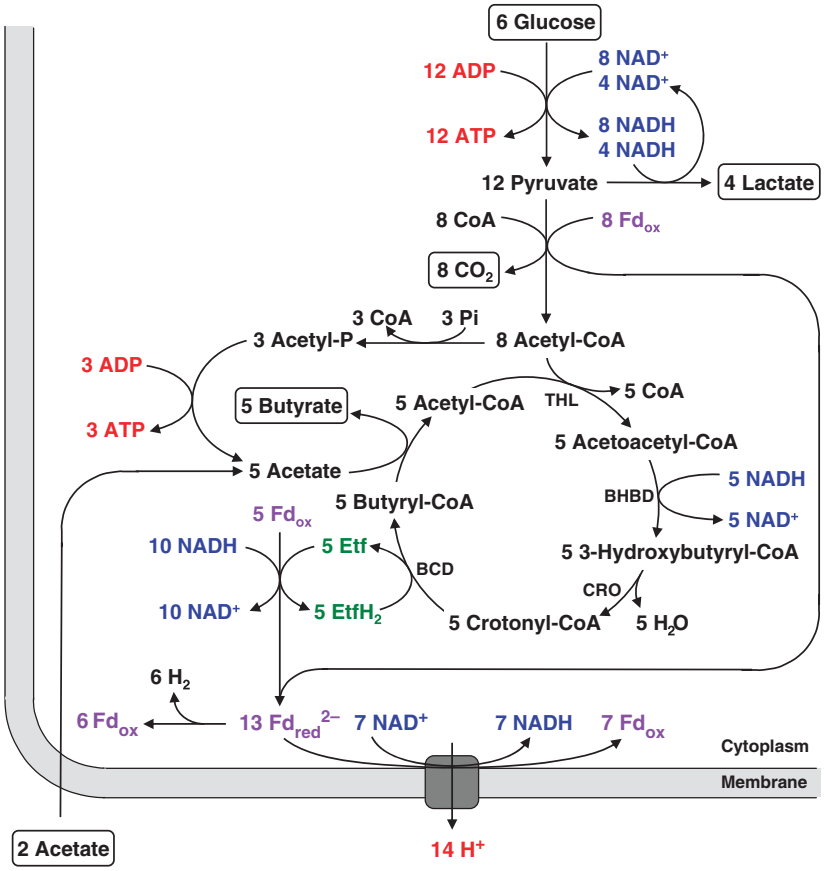

Fig. 2. Hypothetical scheme of butyrate generation from glucose in human colonic butyrate producers; adapted from Herrmann *et al*. (2008) and Seedorf *et al*. (2008). Energy gain results from ATP synthesis by substrate-level phosphorylation and generation of a proton motive force as detailed in the main text. Steps corresponding to the genes shown in Fig. 1 are indicated (abbreviations see Fig. 1). For mechanistic details, refer to Herrmann *et al*. (2008). Note that the stoichiometry can differ depending on the environmental conditions [e.g. hydrogen partial pressure, which influences the level of hydrogen generation from reduced ferredoxin (Fd^{2-}_{red})] and the activity of biosynthetic pathways. For simplicity, only the generation of CO_2 and reduced ferredoxin from pyruvate is shown; formate may be generated, however, if pyruvate-formate lyase rather than pyruvate-ferredoxin oxidoreductase is utilized for acetyl-CoA formation.

associated NADH : ferredoxin oxidoreductase (Fig. 2). A detailed genomic and biochemical analysis of *Clostridium kluyveri* is fully consistent with this hypothesis (Seedorf *et al*., 2008; Li *et al*., 2008). While the original proposals on energy conservation were developed for the metabolism of ethanol and acetate, and of amino acids, by *Clostridium* spp., they appear equally applicable to the metabolism of carbohydrates by butyrate producers from the human colon, as suggested in Fig. 2. Because the genes for Bcd–Etf are found in all human colonic butyrate producers (Fig. 1, Louis *et al*., 2007a), these important new findings on the energetics of butyrate production therefore point the way towards future studies into the regulation of fermentative metabolism in the gut.

After reduction to butyryl-CoA, butyrate can be formed either with the enzymes phosphotransbutyrylase and butyrate-kinase via butyryl-phosphate or with the enzyme butyryl-CoA : acetate CoA-transferase, which utilizes acetate as a co-substrate and generates acetyl-CoA. A screen of 38 butyrate-producing human gut isolates, covering a phylogenetically wide range of representatives of clostridial cluster XIVa and some cluster IV and XVI strains, indicated that the butyryl-CoA : acetate CoA-transferase route is far more prevalent in this ecosystem than the butyrate kinase route (Louis *et al*., 2004). This is in agreement with stable isotope studies determining the incorporation of acetate into butyrate (Duncan *et al*., 2004a). For *Coprococcus eutactus* L2-50, which uses the butyrate kinase route, only 28% of butyrate was derived from acetate, whereas for several strains using the butyryl-CoA : acetate CoA-transferase route it was found to be >85%. A high level of acetate incorporation was also found in faecal incubations on several substrates, which appears to be consistent with a dominant role for the butyryl-CoA : acetate CoA-transferase route, in the mixed community (Duncan *et al*., 2004a).

The gene encoding butyryl-CoA : acetate CoA-transferase has recently been identified in several species of human gut bacteria. Based on their sequences, these genes form a lineage separate from 4-hydroxybutyrate CoA-transferases; the overexpressed recombinant enzyme was able to utilize either butyryl-CoA or propionyl-CoA as a substrate (Charrier *et al*., 2006). The butyryl-CoA : acetate CoA-transferase gene is not closely linked to the other butyrate pathway genes, but lies within a different genomic region in four human gut bacteria for which sequence information is currently available (Louis *et al*., 2007a). It seems likely that possession of butyryl-CoA : acetate CoA-transferase must provide some selective advantage in the environment of the colon where acetate concentrations are high (typically

>30 mM). A real-time PCR assay using degenerate primers has now been developed for this gene, which allows semi-quantitative monitoring of changes in the major butyrate producers present in the human colonic microbiota (Louis & Flint, 2007).

Substrate utilization

A wide range of polysaccharides can be utilized by different butyrate-producing firmicutes, suggesting that these bacteria make an important contribution to colonic fermentation of dietary components. *Roseburia intestinalis* was identified as a major xylan-degrading bacterium in the human colon, although this activity is not shared by many other related species (Duncan *et al.*, 2006; Chassard *et al.*, 2007). Starch utilization appears to be widespread, and starch-utilizing *Roseburia* relatives produce a major extracellular amylase (or neopullulanase) that appears to be anchored to the cell surface via a sortase-mediated mechanism (Ramsay *et al.*, 2006). The ability to utilize long-chain inulin is found in *Roseburia inulinivorans* (Duncan *et al.*, 2003, 2006) and *F. prausnitzii* (Duncan *et al.*, 2002). Certain species show considerable metabolic versatility. Notably, *R. inulinivorans* is able to grow on fucose as a result of the induction of pathways, leading to the formation of propionate and propanol rather than butyrate (Scott *et al.*, 2006).

Bacteria related to *Eubacterium hallii* and *Anaerostipes caccae* (Schwiertz *et al.*, 2002), within the *C. coccoides* cluster, were shown to be able to convert acetate and lactate into butyrate, in addition to producing butyrate from carbohydrates (Duncan *et al.*, 2004b; Sato *et al.*, 2008). An unnamed, but abundant, group of bacteria represented by the strains SSC/2 and SS2/1 showed similar activity, but were only able to use D-lactate, and not L-lactate. Bacterial conversion of lactate to butyrate by mixed human faecal bacteria has been demonstrated by several groups using stable isotopes (Bourriaud *et al.*, 2005; Belenguer *et al.*, 2006, 2007; Morrison *et al.*, 2006). Lactate utilization helps to explain why lactate concentrations typically remain low in healthy subjects, whereas lactate accumulates at a reduced pH when its utilization is curtailed (Belenguer *et al.*, 2007). Metabolic crossfeeding from lactate-producing to lactate-utilizing bacteria may also be a factor in the butyrogenic effect of certain dietary substrates (Belenguer *et al.*, 2006).

Several human colonic *Roseburia* species were recently shown to actively metabolize linoleic acid, forming either vaccenic acid or a hydroxyl-18:1 fatty acid that can act as precursors for the health-promoting conjugated linoleic acid (CLA) *cis*-9, *trans*-11-18:2 (Devillard *et al.*, 2007). This activity is also seen among related butyrate-producing bacteria from the rumen, which is an important site for CLA formation. It is not clear, however, whether CLAs are likely to be formed to any significant extent in the human large intestine under normal dietary conditions (Kamlage *et al.*, 1999).

Impact of prebiotics and other nondigestible dietary carbohydrates on butyrate formation

Diet provides the main energy source for the gut microbiota and is thus expected to play a major role in dictating which bacteria are able to thrive in the large intestine. Certain dietary constituents, for example resistant starch, are known to increase the bacterial production of butyrate in the large intestine (reviewed in Louis *et al.*, 2007b). This effect may result from the direct stimulation of polysaccharide-utilizing, butyrate-producing bacteria. An additional factor, however, may be the contribution of metabolic crossfeeding from other active polysaccharide-metabolizing bacteria, leading to stimulation of butyrate producers. This effect has been demonstrated to occur in *in vitro* cocultures between bifidobacteria and butyrate-producing species. Coculture with lactate-utilizing strains can result in crossfeeding of the fermentation products acetate and lactate, while a second mechanism is via crossfeeding of oligosaccharide breakdown products released from the polysaccharide substrate (Duncan *et al.*, 2004b; Belenguer *et al.*, 2006; Falony *et al.*, 2006).

Dietary intake can also affect the pH in the proximal colon, and this appears likely to be a key factor determining butyrate production. In an *in vitro* fermentor study with a faecal inoculum, it was found that the two major butyrate-producing bacterial groups, *Roseburia/E. rectale* species and *F. prausnitzii*, thrived at pH 5.5, whereas their population declined dramatically at pH 6.5, with *Bacteroides* spp. becoming dominant. In accordance with the population changes, butyrate was the main fermentation product at pH 5.5, while acetate and propionate became the main products at pH 6.5. The ability of several cultured strains to grow at a low pH was in agreement with these findings (Walker *et al.*, 2005). Thus, changes in pH are likely to affect the community structure and microbial activity in the colon. Diets containing high levels of carbohydrate that is nondigestible in the upper gut lead to higher levels of bacterial fermentation in the colon, which results in mildly acidic pH values in the proximal colon (Cummings & Macfarlane, 1991) that seem likely to promote butyrate formation.

Recent studies have examined the impact of low-carbohydrate weight-loss diets on the faecal microbiota of obese male human volunteers (Duncan *et al.*, 2007, 2008). The greatest response was a fourfold reduction in the *Roseburia/E. rectale* group of butyrate-producing bacteria after 4 weeks of very low total carbohydrate intake (24 g day^{-1}, 6 g NSP fibre day^{-1}) and this was accompanied by a fourfold reduction in faecal butyrate concentration. This suggests

that this group of gut bacteria is particularly dependent on carbohydrate (or fibre) intake to maintain its population in the colon. As discussed above, it is possible that the decrease in pH that results from active fermentation of carbohydrates in the proximal colon may be important in allowing these bacteria to compete against Gram-negative carbohydrate-utilizing bacteria such as *Bacteroides* spp.

Prebiotics, nondigestible food ingredients that beneficially affect the host by modulating the intestinal microbiota, have traditionally targeted non-butyrate-producing lactic acid bacteria, which are generally regarded as having beneficial effects (Gibson *et al.*, 2004). However, the complexity of the gut microbiota makes it likely that the response to prebiotics is more complex. A recent human volunteer study on the effect of the prebiotic inulin found a significant increase in the relative levels not only of *Bifidobacterium* species but also of *F. prausnitzii* (Ramirez-Farias *et al.*, 2009). As discussed above, the stimulation of this butyrate-producing group may contribute to the butyrogenic effect that is often found after inulin consumption (Gibson *et al.*, 2004), although other mechanisms such as metabolic crossfeeding and environmental effects (especially pH) are also likely to play a role (Flint *et al.*, 2007).

Alternative approaches to promoting butyrate production in the colon

As the potential benefit of increasing butyrate levels in the colon, especially for the treatment of gut diseases, is increasingly being recognized, alternative approaches are being developed to deliver butyrate to the colon (Hamer *et al.*, 2008). Bajka *et al.* (2008) recently demonstrated in rats fed a high-protein diet that consumption of butyrylated starch in comparison with nonbutyrylated starch led to a significant increase of caecal butyrate levels with a concomitant decrease of DNA damage in colonocytes. Furthermore, encapsulation of butyrate itself (Roda *et al.*, 2007) or starch (Rose *et al.*, 2008) may lead to improved delivery of butyrate to the distal colon. Butyryl-L-carnitine has been proposed as a potential prodrug for the treatment of inflammatory bowel disease, providing colonocytes with L-carnitine and butyrate, both of which exert anti-inflammatory effects (Srinivas *et al.*, 2007).

Populations of colonic butyrate-producing bacteria in health and disease

A number of investigations have shown that certain butyrate-producing firmicute bacteria are reduced in inflammatory bowel disease (Sokol *et al.*, 2007). In particular, populations of *F. prausnitzii* in faecal samples and on the gut mucosa appear to be reduced in Crohn's disease (Manichanh *et al.*, 2006). This may have a negative impact on the supply of butyrate to gut epithelial cells, but in addition recent evidence strongly suggests that *F. prausnitzii* produces a separate anti-inflammatory factor (Sokol *et al.*, 2008). Indeed, the risk of recurrence of Crohn's disease following surgical resection was reported to be increased in patients whose mucosal *F. prausnitzii* populations were low (Sokol *et al.*, 2008).

Another feature of Crohn's disease is the detection of antibodies against commensal bacteria. Antibodies against flagellar antigens include ones that target flagella of bacteria that are related to *E. rectale* and *Roseburia* spp. (Duck *et al.*, 2007). The explanation for decreased populations of firmicute bacteria in inflammatory bowel disease is not yet clear. The immune response might play a role in shaping the composition of the community, but changes in the gut environment and in gut transit are also likely to be of major importance.

Conclusions

The application of molecular tools to the study of the human gut microbiota has led to a dramatic change in our view of the abundance of certain bacterial groups, especially strict anaerobes belonging to the firmicutes, in recent years. Butyrate producers are an abundant and phylogenetically diverse group of bacteria that are likely to play an important role in maintaining gut health, primarily through the production of butyrate. Evidence is mounting that some butyrate producers possess additional traits that may benefit health, for example helping to stabilize lumenal pH by the consumption of lactate, or by exerting anti-inflammatory effects through currently unknown mechanisms. On the other hand, potentially deleterious effects, for example via toxin production, or adverse immune responses to flagella, also require further exploration, and new genomic information on available cultured strains will be invaluable here. Cultured strains will also facilitate the future development of novel probiotics and prebiotics. Thus, a combination of studies using cultured isolates and culture-independent approaches will be necessary to further elucidate the function of butyrate producers and their interplay with other gut bacteria, diet and the host. Importantly, new insights have been obtained recently into the importance of the butyrate pathway in Gram-positive anaerobic bacteria for energy supply. Further investigations on this topic appear to be particularly relevant to understanding the ecology and energetics of butyrate producers as a functional group within gut communities.

Acknowledgements

The Rowett Institute of Nutrition and Health receives support from the Scottish Government Rural and Environment Research and Analysis Directorate. We are grateful to Sylvia Duncan for critically reading this manuscript.

References

Aminov RI, Walker AW, Duncan SH, Harmsen HJM, Welling GW & Flint HJ (2006) Molecular diversity, cultivation, and improved detection by fluorescent *in situ* hybridization of a dominant group of human gut bacteria related to *Roseburia* spp. or *Eubacterium rectale*. *Appl Environ Microb* **72**: 6371–6376.

Bajka BH, Clarke JM, Cobiac L & Topping DL (2008) Butyrylated starch protects colonocyte DNA against dietary protein-induced damage in rats. *Carcinogenesis* **29**: 2169–2174.

Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C & Flint HJ (2000) Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microb* **66**: 1654–1661.

Belenguer A, Duncan SH, Calder G, Holtrop G, Louis P, Lobley GE & Flint HJ (2006) Two routes of metabolic cross-feeding between bifidobacteria and butyrate-producing anaerobes from the human gut. *Appl Environ Microb* **72**: 3593–3599.

Belenguer A, Duncan SH, Holtrop G, Anderson E, Lobley G & Flint HJ (2007) Impact of pH on lactate formation and utilization by human fecal microbial communities. *Appl Environ Microb* **73**: 6526–6533.

Bourriaud C, Robins RJ, Martin L, Kozlowski F, Tenaileau E, Cherbut C & Michel C (2005) Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J Appl Microbiol* **99**: 201–212.

Brosius J, Palmer ML, Kennedy PJ & Noller HF (1978) Complete nucleotide sequence of a ribosomal RNA gene from *Escherichia coli*. *P Natl Acad Sci USA* **75**: 4801–4805.

Charrier C, Duncan GJ, Reid MD, Rucklidge GJ, Henderson D, Young P, Russell VJ, Aminov RI, Flint HJ & Louis P (2006) A novel class of CoA-transferase involved in short-chain fatty acid metabolism in butyrate-producing human colonic bacteria. *Microbiology* **152**: 179–185.

Chassard C, Goumy V, Leclerc M, Del'homme C & Bernalier-Donadille A (2007) Characterization of the xylan-degrading microbial community from human faeces. *FEMS Microbiol Ecol* **61**: 121–131.

Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal J, Garcia P, Cai J, Hippe H & Farrow JA (1994) The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**: 812–826.

Cummings JH & Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* **70**: 443–459.

Devillard E, McIntosh F, Duncan SH & Wallace RJ (2007) Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. *J Bacteriol* **189**: 2566–2570.

Duck LW, Walter MR, Novak J, Kelly D, Tomasi M, Cong Y & Elson CO (2007) Isolation of flagellated bacteria implicated in Crohn's disease. *Inflamm Bowel Dis* **13**: 1191–1201.

Duncan SH, Hold GL, Harmsen HJM, Stewart CS & Flint HJ (2002) Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen nov., comb. nov. *Int J Syst Evol Micr* **52**: 2141–2146.

Duncan SH, Scott KP, Ramsay AG, Harmsen HJM, Welling GW, Stewart CS & Flint HJ (2003) Effects of dietary substrates on competition between human colonic bacteria in an anaerobic fermentor system. *Appl Environ Microb* **69**: 1136–1142.

Duncan SH, Holtrop G, Lobley GE, Calder AG, Stewart CS & Flint HJ (2004a) Contribution of acetate to butyrate formation by human faecal bacteria. *Brit J Nutr* **91**: 915–923.

Duncan SH, Louis P & Flint HJ (2004b) Lactate-utilising bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microb* **70**: 5810–5817.

Duncan SH, Aminov RI, Scott KP, Louis P, Stanton TB & Flint HJ (2006) Proposal of *Roseburia faecis* sp. nov., *Roseburia hominis* sp. nov., and *Roseburia inulinivorans* sp. nov., based on isolates from human faeces. *Int J Syst Evol Micr* **56**: 2437–2441.

Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ & Lobley GE (2007) Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microb* **73**: 1073–1078.

Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P & Flint HJ (2008) Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obesity* **32**: 1720–1724.

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE & Relman DA (2005) Diversity of the human intestinal microbial flora. *Science* **308**: 1635–1638.

Falony G, Vlachou A, Verbrugge K & de Vuyst L (2006) Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting butyrate-producing colon bacteria during growth on oligofructose. *Appl Environ Microb* **72**: 7835–7841.

Flint HJ, Duncan SH, Scott KP & Louis P (2007) Interactions and competition within the microbial community of the human colon: links between diet and health. *Environ Microbiol* **9**: 1101–1111.

Gibson GR, Probert HM, Van Loo J, Rastall RA & Roberfroid MB (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* **17**: 259–275.

Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ & Brummer RJ (2008) Review article: the role of butyrate on colonic function. *Aliment Pharm Therap* **27**: 104–119.

Herrmann G, Jayamani E, Mai G & Buckel W (2008) Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. *J Bacteriol* **190**: 784–791.

Hold GL, Schwiertz A, Aminov RI, Blaut M & Flint HJ (2003) Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in the human feces. *Appl Environ Microb* **69**: 4320–4324.

Kamlage B, Hartmann B, Gruhl B & Blaut M (1999) Intestinal micro-organisms do not supply associated gnotobiotic rats with conjugated linoleic acids. *J Nutr* **129**: 2212–2217.

Lay C, Dore J & Rigottier-Gois L (2007) Separation of bacteria from the *Clostridium leptum* subgroup from the colonic

microbiota by fluorescence-activated cell sorting or group-specific PCR using 16S rRNA gene oligonucleotides. *FEMS Microbiol Ecol* **60**: 513–520.

Li F, Hinderberger J, Seedorf H, Zhang J, Buckel W & Thauer RK (2008) Coupled ferredoxin and crotonyl coenzyme A (CoA) reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Eft complex from *Clostridium kluyeri*. *J Bacteriol* **190**: 843–850.

Louis P & Flint HJ (2007) Development of a semi quantitative degenerate real-time PCR-based assay for estimation of numbers of butyryl-coenzyme A (CoA) CoA transferase genes in complex bacterial samples. *Appl Environ Microb* **73**: 2009–2012.

Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS & Flint HJ (2004) Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol* **186**: 2099–2106.

Louis P, McCrae SI, Charrier C & Flint HJ (2007a) Organization of butyrate synthetic genes in human colonic bacteria: phylogenetic conservation and horizontal gene transfer. *FEMS Microbiol Lett* **269**: 240–247.

Louis P, Scott KP, Duncan SH & Flint HJ (2007b) Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* **102**: 1197–1208.

Macfarlane S & Macfarlane GT (2003) Regulation of short-chain fatty acid production. *P Nutr Soc* **62**: 67–72.

Manichanh C, Rigottier-Gois L, Bonnaud E *et al.* (2006) Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* **55**: 205–211.

Miller TL & Wolin MJ (1996) Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Appl Environ Microb* **62**: 1589–1592.

Morrison DJ, Mackay WG, Edwards CA, Preston T & Weaver LT (2006) Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? *Brit J Nutr* **96**: 570–577.

Pajak B, Orzechowski A & Gajkowska B (2007) Molecular basis for sodium butyrate-dependent proapoptotic activity in cancer cells. *Adv Med Sci* **52**: 83–88.

Pryde SE, Duncan SH, Hold GL, Stewart CS & Flint HJ (2002) The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett* **217**: 133–139.

Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G & Louis P (2009) Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Brit J Nutr* **101**: 533–542.

Ramsay AG, Scott KP, Martin JC, Rincon MT & Flint HJ (2006) Cell associated α-amylases of butyrate-producing firmicute bacteria from the human colon. *Microbiology* **152**: 3281–3290.

Roda A, Simoni P, Magliulo M, Nanni P, Baraldini M, Roda G & Roda E (2007) A new oral formulation for the release of sodium butyrate in the ileo-cecal region and colon. *World J Gastroentero* **13**: 1079–1084.

Rose DJ, Keshavarzian A, Patterson JA, Venkatachalam M, Gillevet P & Hamaker BR (2008) Starch-entrapped microspheres extend *in vitro* fecal fermentation, increase butyrate production, and influence microbiota pattern. *Mol Nutr Food Res* doi: 10.1002/mnfr.200800033/.

Sato T, Matsumoto K, Okumura T, Yokoi W, Naito E, Yoshida Y, Nomoto K, Ito M & Sawada H (2008) Isolation of lactate-utilising butyrate-producing bacteria from human feces and *in vivo* administration of *Anaerostipes caccae* strain L2 and galacto-oligosaccharides in a rat model. *FEMS Microbiol Ecol* **66**: 528–536.

Scheppach W & Weiler F (2004) The butyrate story: old wine in new bottles? *Curr Opin Clin Nutr* **7**: 563–567.

Schwiertz A, Hold GI, Duncan SH, Gruhl B, Collins MD, Lawson PA, Flint HJ & Blaut M (2002) *Anaerostipes caccae* gen. nov., sp. nov., a new saccharaolytic acetate-utilising butyrate-producing bacterium from human faeces. *Syst Appl Microbiol* **25**: 46–51.

Schwiertz A, Le Blay G & Blaut M (2000) Quantification of different *Eubacterium* spp. in human faecal samples with species-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microb* **66**: 375–382.

Scott KP, Martin JC, Campbell G, Mayer C-D & Flint HJ (2006) Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium *Roseburia inulinivorans*. *J Bacteriol* **188**: 4340–4349.

Seedorf H, Fricke WF, Veith B *et al.* (2008) The genome of *Clostridium kluyveri*, a strict anaerobe with unique metabolic features. *P Natl Acad Sci USA* **105**: 2128–2133.

Sokol H, Lepage P, Seksik P, Dore J & Marteau P (2007) Molecular comparison of dominant microbiota associated with injured versus healthy mucosa in ulcerative colitis. *Gut* **56**: 152–154.

Sokol H, Pigneur B, Watterlot L *et al.* (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn's disease patients. *P Natl Acad Sci USA* **105**: 16731–16736.

Srinivas SR, Prasad PD, Umapathy NS, Ganapathy V & Shekkawat PS (2007) Transport of butyryl-L-carnitine, a potential prodrug, via the carnitine transporter OCTN2 and the amino acid transporter $ATB^{o,+}$. *Am J Physiol Gastr L* **293**: G1046–G1053.

Suau A, Rochet V, Sghir A, Gramet G, Brewaeys S, Sutren M, Rigottier-Gois L & Dore J (2001) *Fusobacterium prausnitzii* and related species represent a dominant group within the human fecal flora. *Syst Appl Microbiol* **24**: 139–145.

Walker AW, Duncan SH, McWilliam Leitch EC, Child MW & Flint HJ (2005) pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microb* **71**: 3692–3700.

Walker AW, Duncan SH, Harmsen HJM, Holtrop G, Welling GW & Flint HJ (2008) The species composition of the human intestinal microbiota differs between particle-associated and liquid phase communities. *Environ Microbiol* **10**: 3275–3283.