

MiniReview

The microbiology of butyrate formation in the human colon

Susan E. Pryde¹, Sylvia H. Duncan, Georgina L. Hold², Colin S. Stewart,
Harry J. Flint^{*}

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

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Abstract

Butyrate arising from microbial fermentation is important for the energy metabolism and normal development of colonic epithelial cells and has a mainly protective role in relation to colonic disease. While certain dietary substrates such as resistant starch appear to be butyrogenic in the colon, it is not known to what extent these stimulate butyrate production directly, e.g. by promoting amyolytic species, or indirectly, e.g. through cross-feeding of fermentation products. Cultural and molecular studies indicate that the most numerous butyrate-producing bacteria found in human faeces are highly oxygen-sensitive anaerobes belonging to the Clostridial clusters IV and XIVa. These include many previously undescribed species related to *Eubacterium*, *Roseburia*, *Faecalibacterium* and *Coprococcus* whose distribution and metabolic characteristics are under investigation. A better understanding of the microbial ecology of colonic butyrate-producing bacteria will help to explain the influence of diet upon butyrate supply, and to suggest new approaches for optimising microbial activity in the large intestine.

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1. Introduction

The anaerobic microbial communities of the mammalian large intestine and rumen produce the short chain fatty acids (SCFA) acetate, propionate and butyrate as their main non-gaseous fermentation end products. SCFA are assimilated by the mammalian host, and provide a high proportion of the total energy gained from the diet in herbivores, especially ruminants. In humans the overall contribution of SCFA towards the host's energy requirement is far lower, but they have an important influence on colonic health (reviewed [1,2]). In particular butyrate is the preferred energy source for the colonic mucosa and has been implicated in protection against colitis and colorectal cancer [3–5]. Production of butyrate by mixed human fae-

cal microflora in vitro is influenced by the growth substrate with resistant starch, in particular, being regarded as butyrogenic (reviewed [6,7]). The microbiological basis for the effects of diet and individual variation upon gut metabolism is, however, poorly understood, and this brief review considers our current knowledge of the physiology, identity and ecology of butyrate-producing bacteria from the human large bowel.

2. Microbial fermentation, butyrate and gut health

Acetate, propionate and butyrate are all taken up by the colonic mucosa, but butyrate is transported preferentially and appears to be the preferred energy source for colonocytes [8,9]. Approximately 95% of the butyrate produced by colonic bacteria is transported across the epithelium, but concentrations in portal blood are usually undetectable as a result of rapid utilisation. In addition to its role as a fuel, butyrate influences gene expression, primarily through its action as a non-competitive inhibitor of histone deacetylases, leading to hyperacetylation of chromatin [10]. These effects are also elicited, but to a lesser extent, by other SCFA [10]. In addition, however, specific

^{*} Corresponding author. Tel.: +44 (1224) 712751;
Fax: +44 (1224) 716687.
E-mail address: h.flint@rri.sari.ac.uk (H.J. Flint).

¹ Present address: Food Standards Agency, St Magnus House, Guild Street, Aberdeen AB11 6NJ, UK.

² Present address: Department of Medicine and Therapeutics, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK.

butyrate response elements have been identified [11]. Furthermore, butyrate has anti-inflammatory effects that result from inhibition of activation of the transcription factor NF- κ B, and consequent reduced formation of proinflammatory cytokines [12,13]. Recent microarray analyses have revealed more precisely the extent of butyrate effects upon gene expression [14]. Depending on its concentration, butyrate can inhibit growth or promote differentiation of human cells in tissue culture, and can induce apoptosis in tumour cells, while also acting as a trophic factor for cells in intact tissues (reviewed [10]).

These interactions have important consequences for the health of the colonic epithelium. The observation that butyrate promotes apoptosis and inhibits growth of cancer cells in vitro [15] is consistent with a protective role against colorectal cancer in vivo [16,17]. Cells in colon carcinomas that overexpress cyclooxygenase 2 become resistant to butyrate-induced apoptosis, but butyrate is proposed to be a factor in suppressing pre-cancerous cells at an earlier stage in their progression [10]. Other studies support a role in preventing and/or ameliorating conditions such as ulcerative colitis [1,2,5,6,8]. It is proposed that an inadequate supply of energy to colonocytes, 70% of which is normally obtained from butyrate, can be a causative factor in colitis. Thus dextran sulfate, which inhibits butyrate oxidation without affecting glucose metabolism, induces colitis when given orally to mice [18] and it has been proposed that sulfide toxicity results largely from inhibition of the energy supply to colonocytes from butyrate [19]. Excellent reviews [5,6,10] critically discuss the evidence for and against the view that low concentrations of SCFA, and of butyrate in particular, increase the risks of both colorectal cancer and inflammatory bowel diseases.

The question of what is an 'optimal' concentration of butyrate is complex. Cells cultured in vitro show growth arrest at concentrations (1–10 mM) lower than those seen in faeces, but the relevant concentration in vivo is likely to be that experienced by growing cells within the intact crypt epithelium [10]. The supply of butyrate to the colonic epithelium depends largely on the fermentation of dietary components that are incompletely digested in the small intestine. Faecal concentrations are not a good guide to production rates because a very high proportion of the SCFA is taken up by the colonic mucosa. There is, however, substantial evidence to indicate that different dietary polysaccharides affect the amounts and relative molar proportions of SCFA in the gut of pigs, rodents and humans. For example (see Fig. 1) in vitro studies with mixed human faecal bacteria suggest that starch fermentation yields a higher molar proportion of butyric acid among the SCFA products than pectin fermentation (reviewed [7]). Resistant starch from the diet that escapes small intestinal digestion is therefore likely to be butyrogenic [6,7] and there is also some evidence (e.g. [17,20]) that fructo-oligosaccharides can be butyrogenic. Numerous studies have been undertaken with animal models to explore the link

between the provision of 'low digestible' dietary carbohydrates, the supply of SCFA and protection against experimentally induced tumours (e.g. [3,16,17]; reviews [1,2,5,6,10]). Resistant starch appears to be more effective than non-starch polysaccharides in dietary fibre in protecting against colon cancer [6].

Direct supply of butyrate, or of butyrate 'carriers' such as tributyrin, has been considered via oral [5,21] or rectal enema [22] routes as a treatment for ulcerative colitis. Less consideration seems to have been given to the possible use of butyrate-producing bacteria as probiotics, but the targeted stimulation of native butyrate-producing bacteria by dietary prebiotics provides an obvious approach for delivering butyrate to its site of utilisation at the colonic mucosa. Delivery to the distal large bowel is of particular interest in view of the lower SCFA concentrations and higher incidence of polyps in this region [3,5,23]. Inhibition of starch digestion in the small intestine enhances butyrate production by delivering more starch to the large intestine [24] while the presence of dietary components such as wheat bran that affect transit [23] can shift the main site of starch fermentation distally down the intestine.

3. Butyrate-producing bacteria – diversity, phylogeny and culturability

Extensive past surveys of the cultivable human intestinal microflora [25,26] showed that the colon harbours significant populations of genera, such as *Clostridium*, *Eubacterium* and *Fusobacterium* that include butyrate-producing species. The relationships of these bacteria are, however, currently undergoing rapid revision in the light of information from 16S rRNA sequencing (e.g. [27]). Furthermore, the ability to amplify and sequence ribosomal genes directly from gut samples [28,29] has uncovered remarkable diversity in the human colonic flora, much of which appears to be distantly related to known species for which ribosomal sequences are available. Thus it is by no means certain that standard culturing techniques recover all butyrate producers from the human gut.

Recent work has combined molecular and cultural approaches to investigate the dominant butyrate-producing bacteria of the human colon. Specific oligonucleotide probes that recognise 11 known *Eubacterium* spp. detected only low numbers in human faeces [30]. On the other hand, 74 butyrate-producing isolates identified by sequencing and restriction fragment length polymorphism (RFLP) analysis of 16S rRNA genes [31] were recently found to include many new species, some of which, e.g. *Roseburia intestinalis* [32], produce particularly high levels of butyrate in vitro (Fig. 2). Also prominent among strains that produce > 10 mM butyrate in vitro were those related to *Fusobacterium prausnitzii*, which has now been reclassified as *Faecalibacterium prausnitzii* [33] in view of its lack of

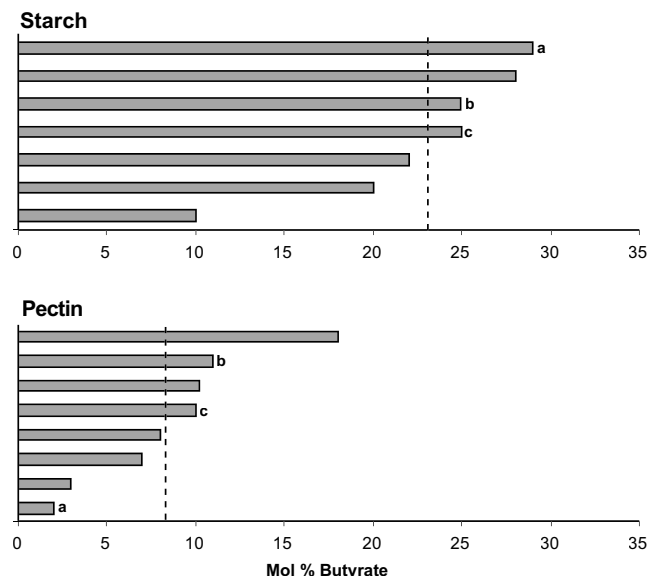


Fig. 1. Molar proportion of butyrate present among the SCFA products of fermentation of starch and pectin by mixed human faecal bacteria in vitro. Data are from a review by Cummings [7]: each point represents a different published study (three of these studies, marked a, b and c, included both substrates). The broken line indicates the mean butyrate proportion for each substrate.

relatedness to true *Fusobacterium* species. Oligonucleotide probing suggests that *F. prausnitzii*-related strains are among the most abundant bacteria in human faeces [34]. As further probes are designed for different groups of butyrate-producing bacteria, these will yield definitive information on their distribution and abundance in the human gut. Additional work on isolation and molecular phylogeny appears necessary in particular to recover non-saccharolytic as well as saccharolytic species. Several butyrate-producing species have proved to require SCFA for growth, emphasising the importance of the isolation medium.

A phylogenetic tree based on 16S rRNA gene sequences from *Clostridium*-related bacteria of gut origin is shown in Fig. 3. It can be noted that butyrate-producing species of *Eubacterium*, *Roseburia* and *Clostridium* are interspersed with other genera such as *Ruminococcus* that are not known to produce butyrate. In conclusion, although most of the butyrate-producing bacteria so far cultured from the human gut are related to the Clostridia, they are widely distributed across several clusters defined by 16S rRNA analyses, including clusters I, IV, XIVa, XV and XVI.

4. Anaerobic metabolism and butyrate synthesis

Butyrate is formed from two molecules of acetyl CoA yielding acetoacetyl CoA, which is then converted, via the intermediates L(+)- β -hydroxybutyryl CoA and crotonyl CoA, to butyryl CoA [35]. Thereafter, butyryl CoA may

yield butyrate via butyrate kinase or via butyryl CoA: acetate CoA transferase (Fig. 4). In the latter reaction, first detected in a soil bacterium, *Clostridium kluveri*, butyryl CoA is exchanged with exogenously derived acetate to yield acetyl CoA and butyrate.

Butyryl CoA:acetate CoA transferase but not butyrate kinase activity was detected in six *Roseburia* and *F. prausnitzii* strains studied from human faeces [36]. These strains also showed net utilisation of acetate in the growth medium, and acetate is a growth requirement for *F. prausnitzii* [33]. In *C. kluveri*, which also has an absolute growth requirement for acetate, it is thought that exogenously supplied acetate may help in the disposal of reducing equivalents [37]. A seventh, *Coprococcus*-related strain, L2-50, that was a net acetate producer was found to possess both butyrate kinase and acetyl CoA transferase [36]. The significance of these metabolic differences has yet to be established, but labelling studies (e.g. [38]) suggest that significant amounts of free acetate contribute to carbon in butyrate in mixed human faecal incubations. Other fermentation products detected for butyrate-producing strains include H_2 , CO_2 , formate and lactate, with D-lactate being produced by *F. prausnitzii* strains and L-lactate by other species examined [32,33,36].

Diez-Gonzalez et al. [39] distinguished two metabolic types of *Butyrivibrio fibrisolvens* which is the major producer of butyrate in the rumen. One group, represented by the type strain D1 (ATCC 19171), expressed the enzyme butyrate kinase, but apparently not butyryl CoA:acetate CoA transferase. D1 produced little lactate and its growth was not stimulated by acetate. Strains of the second group (represented by NCDO 2223 in Fig. 3) possessed butyryl CoA:acetate CoA transferase, but not butyrate kinase. These strains converted 75% of the glucose supplied into lactate in the absence of acetate, but when acetate was added produced mainly butyrate. The two metabolic

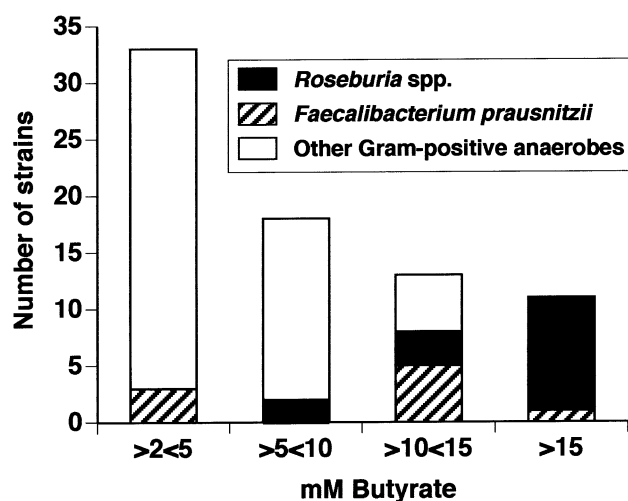


Fig. 2. In vitro butyrate production by 74 human faecal bacterial isolates obtained from three individuals [31]. Identification of isolates was by 16S rRNA gene sequencing and RFLP analysis.

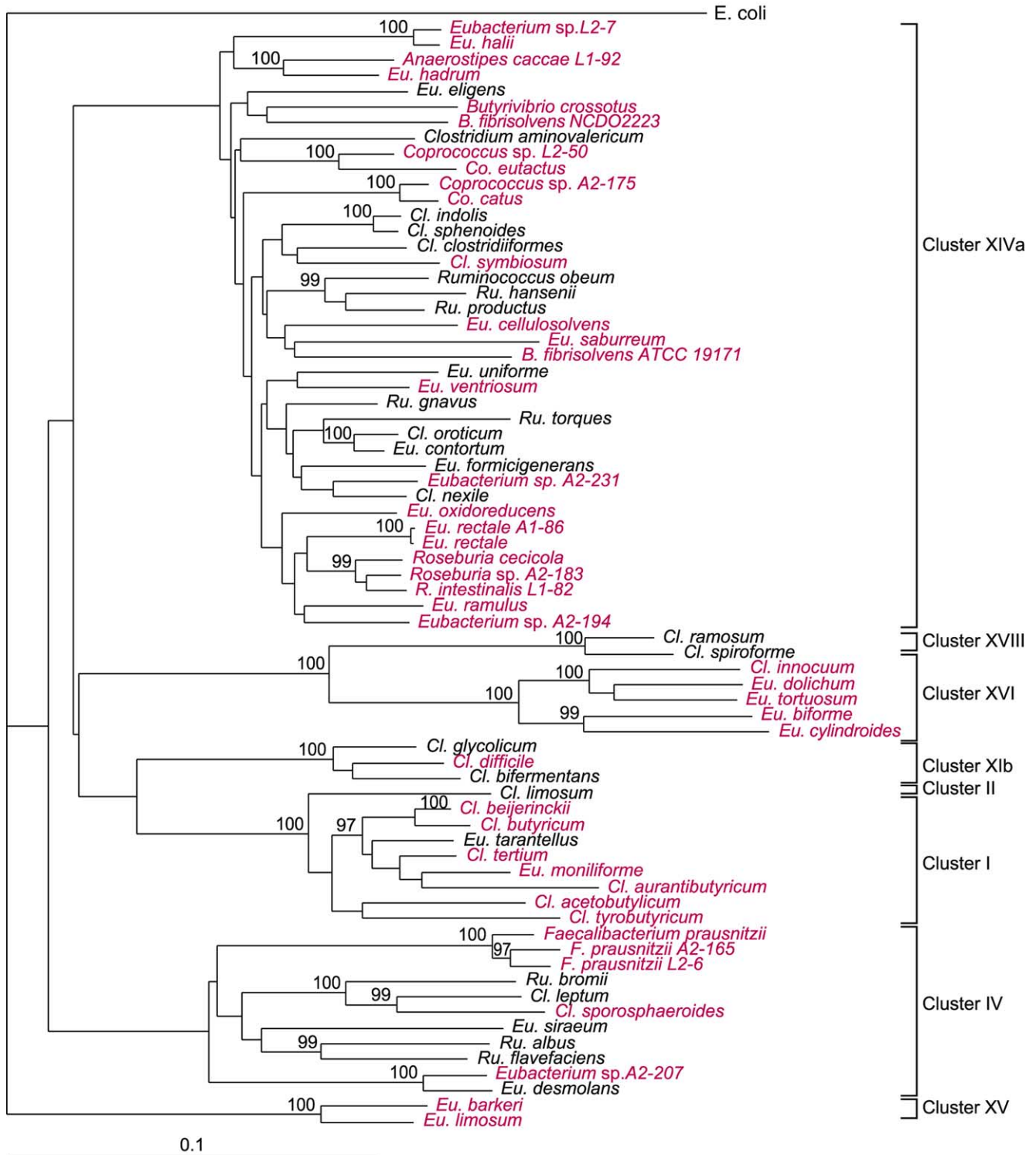


Fig. 3. Phylogenetic tree showing the inter-relationship of 16S rRNA sequences from butyrate- (red) and non-butyrate-producing bacteria (black). Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points: values of 96% or more were considered significant. The scale bar represents genetic distance (10 substitutions per 100 nucleotides).

groups apparently correspond to two distinct phylogenetic groups defined by 16S rRNA analyses [27] (Fig. 3). A single human faecal strain of *B. fibrisolvens* 16/4 [31] appears to be related to the second group of rumen *B. fibrisolvens* strains. Further studies should reveal how close the

parallels are between the metabolic behaviour of the human and ruminal butyrate producers.

There is very little information on the genetic determination and regulation of butyrate pathway enzymes in gut bacteria. Most of the available information derives from

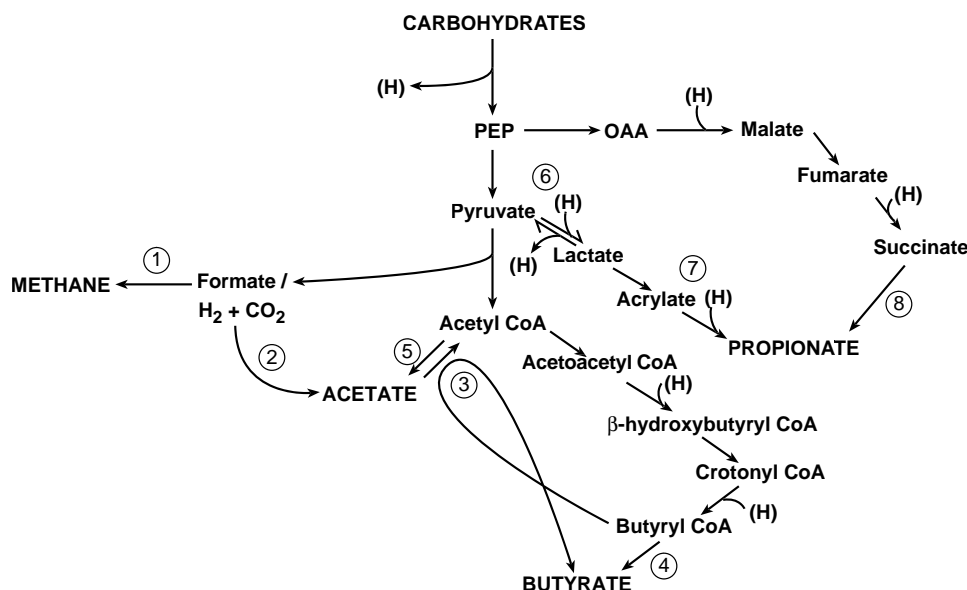


Fig. 4. Schematic representation of pathways for carbohydrate fermentation in the large intestine. 1 = Methanogenesis, 2 = reductive acetogenesis, 3 = butyryl CoA:acetate CoA transferase, 4 = phosphotransbutyrylase/butyrate kinase, 5 = phosphotransacetylase/acetate kinase, 6 = lactate dehydrogenase, 7 = acrylate pathway, 8 = succinate decarboxylation.

industrial interest in solventogenic clostridia, which have the capacity to switch from butyrate production to the formation of acetone and butanol during late exponential growth [35]. In *Clostridium acetobutylicum* enzymes involved in converting acetyl CoA to butyrate (Fig. 4) are encoded by a gene cluster that also encodes flavoproteins [40], and homologous genes have been identified in the non-solventogenic gut pathogen *Clostridium difficile* [41]. Solvent formation is conferred by another gene cluster (sol) that is plasmid encoded in *C. acetobutylicum* but chromosomally encoded in *Clostridium beijerinckii*. A separate operon encodes phosphotransbutyrylase and butyrate kinase [42].

5. Factors affecting butyrate production in vivo

'Butyrogenic' substrates such as resistant starch may affect the colonic fermentation in a number of ways. Some may alter fermentative metabolism in individual bacteria – e.g. more reduced substrates might tend to promote butyrate formation because of its role as a hydrogen sink [35] (Fig. 4). On the other hand, most effects are presumed to be the result of microbial population changes. Thus there may be direct selection for increased populations of butyrate-producing species that are efficient primary degraders of the substrate. In the case of resistant starch, however, it is not yet clear whether the dominant butyrate producers include active primary degraders. Alternatively, certain butyrate producers may be able to compete particularly well as scavengers of partially degraded substrate (e.g. oligosaccharides) released by primary degraders. Indirect stimulation of butyrate production might also occur through increases in other fer-

mentation products such as acetate or (as suggested in [43]) lactate that can act as precursors of butyrate. Finally we must anticipate many more complex 'system' effects. The fermentation balance of the colonic ecosystem will be affected, for example, by redox potential, mucosal transport rates, gut turnover and motility and certain substrates may affect these variables, directly or indirectly. We should also note that both endogenous and dietary sources contribute to microbial fermentation in the large intestine, and the fraction due to endogenous sources can be significant on some diets [44]. The influence of host-derived substrates on butyrate formation is largely unknown.

The species/strain composition of the faecal bacterial flora varies between individuals, with some suggestion from twin studies that it is influenced by host genetic/maternal factors [45]. Individual variation is also observed in the relative proportions of SCFA formed on incubation of various substrates with faecal inocula [46]. In general we do not know to what extent inter-individual variation in the colonic flora, as distinct from diet-induced changes, influences the colonic fermentation, although variation in the potential for methanogenesis is well established [44,46]. The possible impact of inter-individual variation in microflora composition upon fermentation patterns is clearly a key question with regard to possible pro-/prebiotic strategies for optimising the fermentation balance.

6. Conclusions

It is sometimes tempting for the physiologist to treat the colonic ecosystem as a 'black box' whose inputs and outputs alone are of interest. However, the composition and behaviour of the colonic microbial community is of key

importance. The colonic ecosystem is subject to shifts in metabolism and species composition that can only be understood by dissecting out the dominant microbial groups and their interactions. Butyrate production is widely distributed among anaerobic bacteria belonging to the Clostridial subphylum. The Clostridial clusters XIVa and IV in particular include some potentially important butyrate producers related to *Roseburia* and *F. prausnitzii*, respectively that are abundant colonisers of the human gut. It is far from clear whether the full range of diversity has yet been cultured, or to what extent different species and functional groups vary in their distribution between individuals, and in their responses to alternative dietary substrates. A wider range of species-specific probes, allied to metabolic studies, will help to answer such questions. Further studies on newly described butyrate-producing bacteria from the human colon will help to unravel the effects of diet upon health, including microbial interactions with the immune system, and will help in the design of prebiotic or probiotic strategies for stimulating sub-optimal butyrate synthesis in the large intestine.

Note added in proof

Recent evidence indicates activation of the cytokine IL-18 in response to butyrate in human carcinoma-derived cell lines [Kalina, U. et al. (2002) *Eur. J. Immunol.* 32, 2635–2643]. It should be stressed that the effects of butyrate upon inflammation are complex, and the full picture is still emerging.

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