Contribution of acetate to butyrate formation by human faecal bacteria

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Acetate is normally regarded as an endproduct of anaerobic fermentation, but butyrate-producing bacteria found in the human colon can be net utilisers of acetate. The butyrate formed provides a fuel for epithelial cells of the large intestine and influences colonic health. [1-13C]Acetate was used to investigate the contribution of exogenous acetate to butyrate formation. Faecalibacterium prausnitzii and Roseburia spp. grown in the presence of 60 mM-acetate and 10 mM-glucose derived 85–90 % butyrate-C from external acetate. This was due to rapid interchange between extracellular acetate and intracellular acetyl-CoA, plus net acetate uptake. In contrast, a Coprococcus-related strain that is a net acetate producer derived only 28 % butyrate-C from external acetate. Different carbohydrate-derived energy sources affected butyrate formation by mixed human faecal bacteria growing in continuous or batch cultures. The ranking order of butyrate production rates was amylopectin > oat xylan > shredded wheat > inulin > pectin (continuous cultures), and inulin > amylopectin > oat xylan > shredded wheat > pectin (batch cultures). The contribution of external acetate to butyrate formation in these experiments ranged from 56 (pectin) to 90 % (xylan) in continuous cultures, and from 72 to 91 % in the batch cultures. This is consistent with a major role for bacteria related to F. prausnitzii and Roseburia spp. in butyrate formation from a range of substrates that are fermented in the large intestine. Variations in the dominant metabolic type of butyrate producer between individuals or with variations in diet are not ruled out, however, and could influence butyrate supply in the large intestine.

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Abbreviation: MPE, molar % excess.

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SCFA are the main products of anaerobic microbial fermentation in the large intestine and affect colonic health by providing energy to the epithelial cells (Csordas, 1996). Butyrate, in particular, has important effects on development and gene expression in intestinal cells (Csordas, 1996; Avivi-Green et al. 2000; Mariadason et al. 2000; Cavaglieri et al. 2003) and is generally thought to play a protective role against colorectal cancer and colitis (McIntyre et al. 1993; Hague et al. 1995; Wachterhuser & Stein, 2000; Scheppach et al. 2001). The concentrations and, thus, relative proportions of different SCFA in the colon are affected by the composition of the diet, in particular the type and quantity of substrates that survive to the large intestine (Cummings, 1995; Macfarlane & Gibson, 1997; Wolin et al. 1999). The relationship between fermentable carbohydrates and metabolic output, however, depends critically on the composition of the microbial community and the metabolic characteristics of individual anaerobic bacteria. Understanding the interactions within the gut microbial community, therefore, is essential in devising strategies to enhance gut health by nutritional means (Pryde et al. 2002).

In pure culture, different anaerobic gut bacteria can produce a wide variety of products, including acetate, propionate, butyrate, succinate, lactate and ethanol, but only the first three are normally found as major products in the mixed gut ecosystem (Macfarlane & Gibson, 1997). This is largely explained by the fact that products such as lactate and succinate are efficiently utilised by certain groups of anaerobic bacteria. Although acetate reaches the highest concentration of any of the SCFA in faeces, it is known that many human faecal bacteria are net consumers of acetate in pure culture (Barcenilla et al. 2000). Acetate-consumers include two important groups of butyrate producers, Faecalibacterium prausnitzii and the Roseburia intestinalis/Eubacterium rectale, and many strains require acetate for optimal growth (Duncan et al. 2002a,c; Hold et al. 2003). Rather than just representing a stable endproduct, turnover of acetate in the colonic lumen would be expected, with a significant contribution by acetate to
butyrate synthesis. Interestingly, there are already indications from earlier work that labelled CO₂ can be incorporated first into acetate by reductive acetogenesis, and then into butyrate, when faecal material is incubated in the presence of glucose and endogenous substrates (Miller & Wolin, 1996; Wolin et al. 1999).

In the present study, the contribution of acetate to butyrate formation was investigated directly using [1³C]acetate. The present results show very substantial incorporation of acetate-C into butyrate, both in pure cultures of major butyrate-producing bacteria from the human gut (Barcenilla et al. 2000; Duncan et al. 2002a,c; Hold et al. 2003) and in batch and continuous-flow fermentor incubations of mixed bacterial populations from human faeces. We also demonstrated that the rate of butyrate formation and the contribution of acetate to butyrate formation can vary with the species of butyrate-producing bacterium and with the type of fermentable carbohydrate.

Materials and methods

Bacterial strains and maintenance

Bacterial strains were from the Rowett Research Institute culture collection; certain strains are also available from the DSM (Deutsches Sammlung von Mikroorganismen und Zellkulturen GMBH), as indicated later. Isolation of the butyrate-producing isolates Roseburia sp. (A2-181 and A2-183), Roseburia intestinalis (L1-82 (DSM14610T) and L1-8151), F. prausnitzii (A2-165) and Coprococcus-related sp. (L2-50) from human faeces was reported previously (Barcenilla et al. 2000). R. intestinalis strains L1-82 and L1-8151 were isolated from a healthy male infant (11 months old) and the Coprococcus related strain (L2-50) was isolated from a faecal sample from the same infant (2 years old); the two Roseburia sp. strains (A2-181 and A2-183) and the F. prausnitzii strain (A2-165) were all isolated from a female adult on a normal diet. The anaerobic medium M2 incorporating 2 g of the three C sources (glucose, soluble starch and cellubiose/l M2GSC medium) (Miyazaki et al. 1997) was used for the routine maintenance of the bacterial isolates. The medium was dispensed into Hungate tubes sealed with butyl septum stoppers (Bellco Glass Inc., Vineland, NJ, USA) following the anaerobic method, in which the medium is prepared and maintained under O₂-free CO₂ (Bryant, 1972).

For kinetic studies, the isolates (approximately 1 × 10⁷ cells per ml medium) were incubated at 37°C in the presence of 10 mM-glucose and 3 mM-acetate containing [1³C]acetate to give 6 molar % excess (MPE), similar to the conditions described by Duncan et al. (2002b). Tubes were prepared in triplicate and analysed after incubation for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 24 and 32 h.

Collection and preparation of faecal samples

Fresh faecal samples were provided by a healthy male volunteer (50 years old). The volunteer did not take any antibiotics or drugs known to influence faecal microbiota during the 6 months before the start of the studies. Each fermentor vessel was inoculated with 5 g wet weight of faeces. For the batch culture incubations, a faecal slurry (200 g/l) was prepared in anaerobic PBS and 1 ml slurry was added to 9 ml medium under anaerobic conditions.

Simulated human colonic fermentor studies

The studies were conducted in duplicate single-stage fermentor systems based on the model described by Macfarlane et al. (1989). The fermentor medium (Hillman et al. 1994) consisted of a mixture of the following C sources: potato starch (5·0 g/l) and xylan, pectin, amylopectin and arabinoxylan (0·6 g/l each). The initial mixture of carbohydrates in the feed flasks (described earlier) was subsequently replaced on a 7 d cycle with each of the specific compounds: amylopectin, pectin, inulin (dahlia; Dahlia, sp.), xylan, inulin (chicory; Cichorium intybus) and pancreatin-treated shredded wheat, in that order, to give a final concentration of 5·0 g/l, as described by Duncan et al. (2003). The shredded wheat was prepared by incubation with 10 mg pepsin/g at pH 2·3 for 30 min at 37°C. The pH was adjusted to 7·5 and material incubated with 40 mg pancreatin/g. The solids from the pre-treated material were pelleted by centrifugation at 6000 g for 15 min and washed twice in sterile water. The solid material was freeze-dried before use.

The growth medium was maintained under a stream of CO₂. The volume of the medium in the fermentor vessel was kept constant at 250 ml, with a flow rate of fresh medium (containing 2·5 mM-acetate) equal to one liquid pool turnover per d. Both the sterile medium feed flask and fermentor flasks were mixed by internal stirrer bars powered by external stirring units. A pH controller delivered sterile solutions of 0·5 M-HCl or 0·5 M-NaOH to maintain pH 6·5–6·8; the temperature was maintained at 37°C using a thermal jacket. The fermentor vessel was inoculated through a port in the top with a faecal suspension from the same volunteer. Freshly voided faeces (5 g), suspended under O₂-free CO₂ in 20 ml 50 mM-phosphate buffer with the type of fermentable carbohydrate.

Anaerobic fermentor medium (described earlier; an SCFA mixture containing acetate (28 mM plus 2 mM-[1³C]acetate, i.e. a mixture at 6·7 MPE), propionate (9 mM), valerate

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(1·2 mm), isovalerate (1·2 mm) and isobutyrate (1·2 mm) (with the exception of one batch of medium that contained amylopectin (5 g/l) plus acetate at a final concentration of 60 mm and 7 MPE with regard to [1-13C]acetate)) was dispensed in 9 ml portions into Hungate tubes under a stream of CO2. The model dietary substrates were the same as those used in the fermentor studies, but with chicory inulin excluded and glucose included, and the medium was heat sterilised. After cooling, the medium was inoculated with 1 ml faecal slurry (200 g/l) under CO2 and incubated at 37°C. Sample tubes were processed in triplicate to measure SCFA concentrations and 13C enrichments at 0, 2, 4, 6, 8, 10, 12, 24, 30 and 48 h.

**Analysis of 13C Acetate and 13C Butyrate Enrichments**

Concentrations of SCFA were measured either by the GC method based on conversion to the tert-butyldimethylsilyl derivative as described by Richardson et al. (1989), or by isotope dilution. For the latter technique, to portions of the incubation media removed at each time point were added known quantities of [1-13C]acetate and [1-13C]butyrate to raise the enrichments to approximately 50 MPE (i.e. by addition of an equal mass). These enrichments were then determined (see later) and the concentrations determined from the isotopic dilution as described in principle previously (Calder et al. 1999).

Other portions (1 ml) of the media were acidified with 0·5 ml concentrated HCl for measurement of isotopic enrichments. The MS in these samples (and those for isotope dilution measurements) were converted to the tert-butyldimethylsilyl derivatives (Richardson et al. 1989) and analysed by GC–MS on a Hewlett Packard 5899A MS coupled to a HP 5890 GC (Hewlett Packard, Stockport, Cheshire). The temperatures of the GC injector and of the interface line were 280 and 250°C respectively. The column was connected directly to the ion source of the MS. The MS was operated under electron impact conditions (m/z) 117 and 118, but for butyrate M+1 was also quantified to determine any butyrate formed from two [1-13C]acetate molecules (i.e. m/z 145, 146 and 147 were monitored). In practice, the amounts of double-labelled butyrate formed (i.e. from condensation of two labelled acetate molecules) were close to that predicted by the laws of probability from the square of the acetate enrichment. Enrichments in 13C were expressed as MPE according to the equation:

\[
\text{enrichment (MPE)} = \frac{Ri - Ro}{1 + (Ri - Ro)} \times 100,
\]

where \( Ri \) and \( Ro \) are the ratios of the peak areas for enriched and natural abundance (background) samples respectively. For the concentration determinations, appropriate corrections were applied for the enrichments in the sample.

**Kinetic model of acetate metabolism**

**Batch cultures.** For convenience the model units are expressed as C2 units, i.e. 2 mol for butyrate formation, while for glucose it is assumed that metabolism of 1 mol glucose provides 2 mol acetate (or acetyl–CoA) with the remaining C lost as CO2. Let \( i \) denote a time interval starting at time \( t_0 \) and ending at \( t_1 \). The production of acetate during interval \( i \), denoted by \( F_a(i) \), is obtained from the dilution of [1-13C]acetate:

\[
F_a(i) = \left( \frac{E_a(t_2)}{E_a(t_1)} - 1 \right) \times C_a(t_0),
\]

where \( E_a \) is the acetate enrichment (MPE) and \( C_a(t_0) \) is the acetate concentration at \( t_0 \). Net acetate production during interval \( i \) (\( F_a(net)(i) \)) is given by:

\[
F_{ace}(i) = C_a(t_1) - C_a(t_0).
\]

A negative value indicates a net loss. Butyrate production during interval \( i \) (\( F_b(i) \)) is given by:

\[
F_b(i) = C_b(t_1) - C_b(t_0),
\]

where \( C_b \) is the butyrate concentration. It was assumed that the butyrate is not further metabolised (SH Duncan and GE Lobley, unpublished results) so that net production also reflects total production of butyrate. To determine the contribution of production of butyrate from acetate, the enrichment of the newly produced butyrate was compared with the acetate enrichment. Let \( E_b \) (produced during \( i \)) be the enrichment of the newly produced butyrate (in C2 units) during time interval \( i \):

\[
E_b(produced~during~i) = \frac{E_b(t_1)C_b(t_1) - E_b(t_0)C_b(t_0)}{C_b(t_1) - C_b(t_0)}.
\]

Since in molar terms 1 mol butyrate is derived from 2 mol acetate, then the enrichment of total acetate in single–labelled (M+1) butyrate is half that of the butyrate, i.e. \( E_b = 0.5 \) MPE. This simplification is possible because of the negligible double-labelled butyrate formed (i.e. where both C2 units derive from [1,13C]acetate). Then butyrate production from acetate during time interval \( i \) (\( F_{ba}(i) \)) is given by:

\[
F_{ba}(i) = \frac{E_b(produced~during~i)}{E_a(i)} \times F_b(i),
\]

where \( E_a(i) \) is the average of \( E_a(t_0) \) and \( E_a(t_1) \). Let the final time point be at \( T \). For the single-strain batch cultures \( T \) was taken when glucose had disappeared i.e. \( T = 7·0, 6·0, 5·0, 14·5, 9·0 \) and 14·5 h for strains A2-181, A2-183, L2-50, A2-165, L1-82 and L1-8151 respectively. For the mixed culture batch cultures \( T \) was taken as 24·0 h. To obtain flows
per d, the flows for each time interval were aggregated and then multiplied by 24/\(T\):

\[
F_a = \frac{24}{T} \sum F_a(t); \quad F_b = \frac{24}{T} \sum F_b(t);
\]

\[
F_{ba} = \frac{24}{T} \sum F_{ba}(t); \quad F_{anet} = \frac{24}{T} \sum F_{anet}(t).
\]

The proportion of butyrate derived from acetate (\(p\)) is then given by:

\[
p = \frac{F_{ba}}{F_b}.
\]

For the single-strain batch cultures, the model was extended by including an intracellular C2 pool of acetate and acetyl-CoA, which exchanges with the exogenous acetate pool. It was assumed that butyrate production is via this pool (see later, Fig. 2). Assuming that the molar glucose conversion to 2 mol acetyl-CoA also flows via the intracellular C2 pool, then:

\[
\text{influx} = \text{glucose loss} \times \frac{p}{1 - p}
\]

\[
\text{efflux} = \text{influx} - F_{anet}
\]

**Fermentor studies.** A single dose (2 mmol) of enriched acetate was added to the fermentor at \(t = 0\). The enrichment of butyrate as C2 units at time \(t\) is given by \(E_b(t) = \frac{MPE_b(t)}{2}\). The acetate and butyrate enrichments at time \(t\) are then modelled by:

\[
E_a(t) = A \times \exp(-k_a \times t)
\]

and

\[
E_b(t) = L \times \left(\exp(-k_b \times t) - \exp(-k_b \times t)\right)
\]

where \(L = A \times k_b \times C_b / (k_b - k_b \times C_a)\), and \(k_a, k_b, L, k_{ba}\) (d\(^{-1}\)) are fractional rate constants (Shipley & Clark, 1972). Models 1 and 2 were fitted to the acetate and butyrate enrichments giving estimates of \(A, k_a, L\) and \(k_{ba}\). Then the acetate flux, butyrate production, and production of butyrate from acetate (denoted by \(F_a, F_b\) and \(F_{ba}\) respectively, all in mmol C2/l per d) are given by:

\[
F_a = k_a \times C_a,
\]

\[
F_b = k_b \times C_b,
\]

and

\[
F_{ba} = k_{ba} \times C_a,
\]

where

\[
k_{ba} = L \times (k_b - k_b) \times C_b / (A \times C_a).
\]

The proportion of butyrate derived from acetate is then:

\[
p = \frac{F_{ba}}{F_b}.
\]

**Statistical analyses**

Models 1 and 2 were fitted to the acetate and butyrate enrichments from the fermentor study using non-linear regression in Genstat edition 6 (release 6.1; Lawes Agricultural Trust, Rothamsted, Herts., UK). To test for differences between substrates, ANOVA was applied to the quantities of interest with fermentor as a blocking factor and with substrate as treatment effect.

**Results**

Concentrations are expressed conventionally as mm. In contrast, and for ease of comparison, calculations and values in tables describing metabolite flows and mass transfers are expressed in C2 units, i.e. 1 per mol acetate and 2 per mol butyrate. The flow from glucose is taken as two C2 (acetyl-CoA) units, since the CO2 or HCOOH (formic acid) released in forming acetyl-CoA has been considered irrelevant.

**Studies with isolated bacteria**

Incorporation of [\(^{13}\)C]acetate into butyrate was studied in six strains of anaerobic bacteria (listed in Table 1) representing abundant groups of bacteria in the human gut (Roseburia, F. prausnitzii and Coprococcus relatives) from human faeces (Barcenilla et al. 2000). For all the pure strains studied, production of butyrate and consumption (or production) of acetate ceased between 5 and 14 h, concomitant with the disappearance of the trace of [\(^{13}\)C]acetate from the medium (Fig. 1). Similarly, changes in butyrate enrichments and total label incorporation also ceased when all the glucose was consumed (Fig. 2). As observed previously (Barcenilla et al. 2000), four of the anaerobic bacterial strains (Roseburia spp. A2-181, A2-183, L1-82, L1-8151) isolated from human faeces showed some net utilisation of the acetate initially present in the growth medium (Table 1). These strains also gave the highest net production of butyrate (42–72 nmol C2/d per l; Table 1). Two strains, Roseburia sp. A2-183 and F. prausnitzii A2-165, consumed little or no acetate and their butyrate production matched utilisation of C2 units from glucose (Table 1). Despite this, in all of these strains the proportion of butyrate-C derived from acetate, based on [\(^{13}\)C] transfers, was > 85% (Table 1). This was best explained by fitting a model that included considerable interchange of extracellular and intracellular acetate across the cell membrane. This model allowed for estimation of the absolute inward and outward transport rates (Fig. 3; Table 1).

In contrast, the Coprococcus-related strain L2-50 was a net acetate producer with only small amounts of butyrate synthesised. Of this, only 28% was derived from acetate and little exchange occurred between extracellular and intracellular acetate (Table 1).

**Effect of substrate on acetate metabolism and butyrate production by mixed faecal bacteria**

Duplicate continuous culture vessels that received identical faecal inocula were used to investigate the influence of
alternative polysaccharide energy sources upon fermentation. Acetate concentrations remained stable over the 7 d of incubation and ranged from 20.2 mM (shredded wheat) to 28.8 mM (pectin; Table 2). These concentrations were considerably in excess of that in the inflow medium (2.5 mM) and indicated that substantial net acetate production occurred within the fermentors for all substrates. Butyrate concentrations were greatest with amylopectin (5.3 mM) and lowest with pectin (3.1 mM) as substrate (Table 2), while propionate concentrations ranged from 9.1 mM (pectin) to 26.8 mM (mixed substrate; Table 2).

For all fermentors, a single exponential model fitted well the kinetics of $[^{13}C]$acetate-enrichments (illustrated for xylan, Fig. 4), with $93\%$ of the observed variance accounted (results not shown). For all substrates, $42\%$ of the acetate flux through the fermentor was due to the infusion, the remainder being a consequence of carbohydrate fermentation. Butyrate productions were in proportion to butyrate concentrations and encompassed a twofold range for single carbohydrate sources in the order amylopectin > xylan > shredded wheat > dahlia inulin > chicory inulin > pectin. In all cases, the majority

Table 1. Medium concentrations, net mass changes (mmol C2/d per l) and flows between acetate, butyrate and glucose in bacterial strains isolated from human faeces*

<table>
<thead>
<tr>
<th></th>
<th>Roseburia sp. A2-181</th>
<th>Roseburia sp. A2-183</th>
<th>Roseburia intestinalis L1-82</th>
<th>Roseburia intestinalis L1-8151</th>
<th>Coprococcus-like L2-50</th>
<th>Faecalibacterium prausnitzii A2-165</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentrations (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate at t0</td>
<td>56.0</td>
<td>53.8</td>
<td>61.4</td>
<td>60.6</td>
<td>54.5</td>
<td>57.1</td>
</tr>
<tr>
<td>Acetate at Td</td>
<td>50.1</td>
<td>53.2</td>
<td>53.8</td>
<td>49.6</td>
<td>55.6</td>
<td>57.5</td>
</tr>
<tr>
<td>Butyrate at Td</td>
<td>10.9</td>
<td>9.0</td>
<td>12.4</td>
<td>13.3</td>
<td>1.7</td>
<td>9.4</td>
</tr>
<tr>
<td><strong>Flows (mmol C2/d per l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate (F_{acet})</td>
<td>-20.3</td>
<td>-2.3</td>
<td>-20.2</td>
<td>-18.2</td>
<td>5.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Glucose (F_{glu})</td>
<td>-65.8</td>
<td>-65.8</td>
<td>-44.2</td>
<td>-34.2</td>
<td>-8.1</td>
<td>-29.8</td>
</tr>
<tr>
<td>Butyrate (F_{b})</td>
<td>71.7</td>
<td>67.7</td>
<td>63.9</td>
<td>42.3</td>
<td>12.4</td>
<td>29.5</td>
</tr>
<tr>
<td>Acetate production (F_{a})</td>
<td>51.3</td>
<td>47.5</td>
<td>48.6</td>
<td>32.8</td>
<td>6.0</td>
<td>20.5</td>
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<tr>
<td><strong>Butyrate formed:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>From acetate (F_{ba})</td>
<td>62.6</td>
<td>59.8</td>
<td>57.2</td>
<td>37.9</td>
<td>3.4</td>
<td>26.5</td>
</tr>
<tr>
<td>From other</td>
<td>9.1</td>
<td>7.9</td>
<td>6.7</td>
<td>4.4</td>
<td>9.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Proportion from acetate (p)</td>
<td>0.87</td>
<td>0.88</td>
<td>0.89</td>
<td>0.90</td>
<td>0.28</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Transmembrane transport</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influx</td>
<td>453</td>
<td>501</td>
<td>376</td>
<td>293</td>
<td>3.1</td>
<td>263</td>
</tr>
<tr>
<td>Efflux</td>
<td>432</td>
<td>499</td>
<td>356</td>
<td>275</td>
<td>8.5</td>
<td>263</td>
</tr>
</tbody>
</table>

Td, time point at which glucose had disappeared (see p. 917).

* For details of the kinetic model, see p. 917.

Fig. 1. Concentrations of acetate (●) and butyrate (▲) detected in the media during incubation of Roseburia-like isolate sp. A2-183 (A), Faecalibacterium prausnitzii A2-165 (B), Coprococcus-like sp. L2-250 (C) and R. intestinalis L1-8151 (D). ⅼ, Time at which glucose was depleted. For details of procedures, see p. 917.
of butyrate-C arose from external acetate, with contributions ranging from 56 (pectin) to 90 (xylan)%.

The simplified batch culture technique for the faecal samples yielded similar qualitative results to the fermentors with regard to butyrate production (Tables 3 and 4). When corrected for basal conditions (utilisation of peptones and other constituents of the medium) net use of 10-mM glucose was matched by quantitative production of acetate and butyrate in 2:3 proportion (expressed as C₂ units). Most substrates showed net production of acetate, except for amylopectin (zero) and dahlia inulin (net consumption). Butyrate production rates differed fivefold between substrates in the order dahlia inulin > amylopectin > xylan > shredded wheat > pectin (Table 3). The most notable difference with the fermentor data was the improved butyrogenesis with dahlia inulin. For most substrates the proportion of butyrate carbon derived from exogenous acetate was similar to that observed with the fermentor (c.f. Tables 2 and 3), the exceptions were dahlia inulin and pectin, where both showed greater contributions during the batch culture incubations. Interestingly, when the initial acetate concentration was doubled (from 30 to 60 mM) in the presence of amylopectin the contribution to butyrate-C was also substantially increased, although there was no obvious effect on butyrate production.

Fig. 2. Molar % excess (MPE) for acetate (●) and butyrate (▲) detected in the media during incubation of Roseburia-like isolate sp. A2-183 (A), Faecalibacterium prausnitzii A2-165 (B), Coprococcus-like sp. L2-250 (C) and R. intestinalis L1-8151 (D) in the presence of [13C]acetate. For details of procedures, see p. 917.

Fig. 3. Transfers (mmol C₂/d per l) between glucose, acetate and butyrate in cultures of Roseburia sp. A2-183, calculated from the kinetic model described on p. 917. Values in the boxes represent the net change in substrate pools. Fₐ, butyrate production; ➔, ➔, Flow between pools. For details of procedures, see p. 917. The value of +0-4 for intracellular C₂ refers to the difference between butyrate formation and glucose plus acetate input. This may reflect the increase in total intracellular C₂ units due to growth in cell numbers. Although the strain shown here gave little net acetate uptake, acetate uptake was significant in other related strains.
Discussion

There is much interest in using nutritional manipulation to optimise SCFA (and in particular butyrate) production in the large intestine so as to provide appropriate sources of energy to tissue epithelial cells to help in the prevention of cancer and colitis. This requires an understanding of the responses of key groups of gut bacteria to dietary energy sources that are available in the large bowel.

Metabolism of butyrate-producing bacteria

The present study and previous studies (Barcenilla et al. 2000) have clearly demonstrated that the *Roseburia* spp. and *F. prausnitzii* strains present in human faeces produce butyrate and in many strains this is associated with net consumption of both acetate and carbohydrate. In net terms, glucose appeared to dominate the C mass balance in pure batch cultures provided with 10 mM-glucose and 60 mM-acetate, but the isotope studies indicated that most of the butyrate-C (approximately 85%) was derived from external acetate, with only 15% arising directly from glucose. Since butyrate synthesis ceased when the glucose supply was exhausted, carbohydrate is apparently required, probably to provide a source of reducing power for butyrate synthesis from acetyl-CoA as well as energy for growth.

At the same time, these strains show active interchange between internal and external C2 pools (Fig. 3), with inflow from external acetate nearly sixfold the production of C2-units from glucose. The role of this rapid exchange remains unclear, but may be linked to the observation that both *F. prausnitzii* and *Roseburia* spp. apparently lack butyrate kinase activity and, instead, rely on butyryl CoA: acetate CoA transferase for butyrate synthesis (Duncan et al. 2002a). Butyryl CoA: acetate CoA transferase appears to be a soluble enzyme (Duncan et al. 2002a) that depends on cytosolic acetate as a substrate.

Other groups of human faecal bacteria that produce butyrate exhibit different behaviour, however. In the *Coprococcus*-related strain L2-50, for example, the majority of butyrate-C probably arose from glucose, with...
Table 4. Change in concentrations of the three major SCFA (acetate, propionate and butyrate) during batch culture incubations (48 h)*
(Mean values and standard deviations for three replicates)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Acetate Mean ± SD</th>
<th>Propionate Mean ± SD</th>
<th>Butyrate Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>8.79 ± 1.20</td>
<td>1.29 ± 0.20</td>
<td>3.65 ± 0.13</td>
</tr>
<tr>
<td>Glucose (10 mM)</td>
<td>10.67 ± 1.91</td>
<td>2.03 ± 0.17</td>
<td>9.06 ± 0.19</td>
</tr>
<tr>
<td>Amylopectin†</td>
<td>11.81 ± 3.44</td>
<td>3.80 ± 0.82</td>
<td>18.20 ± 2.20</td>
</tr>
<tr>
<td>Amylopectin‡</td>
<td>6.22 ± 3.49</td>
<td>4.62 ± 0.85</td>
<td>20.03 ± 0.69</td>
</tr>
<tr>
<td>Pectin</td>
<td>13.97 ± 3.28</td>
<td>2.61 ± 0.31</td>
<td>5.80 ± 0.14</td>
</tr>
<tr>
<td>Inulin</td>
<td>8.81 ± 1.63</td>
<td>2.32 ± 1.02</td>
<td>33.29 ± 0.39</td>
</tr>
<tr>
<td>Xylan</td>
<td>3.44 ± 4.86</td>
<td>2.26 ± 0.48</td>
<td>13.76 ± 0.62</td>
</tr>
<tr>
<td>Shredded wheat</td>
<td>6.79 ± 3.58</td>
<td>3.91 ± 1.24</td>
<td>12.48 ± 4.42</td>
</tr>
</tbody>
</table>

* For details of kinetic models and procedures, see p. 917.
† i.e. After subtracting any SCFA present in the medium initially (normally 30 mM in the case of acetate).
‡ Indicates that the start concentration of acetate was 60 mM (instead of about 30 mM as in the other incubations).

Impact of carbohydrate source

Continuous flow fermentors allow the long-term impact of carbohydrate sources and their metabolism to be monitored under steady-state conditions. The conditions studied here are probably closest to those in the lumen of the transverse colon, since pH may be somewhat <6.5 proximally and slightly higher distally (Macfarlane & Gibson, 1997). Use was also made here of the quicker batch culture approach. Both fluxes and transfers needed to be determined in order to compare butyrate production between these two systems: simple comparison of concentrations would be misleading because there is continuous liquid outflow (and inflow) in the open system, while SCFA accumulate in the closed-batch system. It should also be noted that since the continuous flow fermentor experiment involved presenting different carbohydrate sources in succession, it cannot be ruled out that the order of substrate addition could have been a factor affecting the fermentation of a given substrate (Duncan et al. 2003).

In fact most of the carbohydrate sources gave similar relative butyrate outputs, with pectin being the least butyrogenic substrate in both batch and continuous flow systems, but dahlia inulin produced the most butyrate in the batch cultures, and amylopectin the most in the continuous cultures. These findings are in agreement with previous in vitro studies (e.g. Englyst et al. 1987; Titgemeyer et al. 1991; Weaver et al. 1992; Duncan et al. 2003). Inulin and starch have been reported to be butyrogenic in vivo studies in human subjects and in gnotobiotic rats with a human-associated microflora (Weaver et al. 1992; Wolin et al. 1999; Kleessen et al. 2001; Topping & Clifton, 2001).

A marked difference between batch and continuous culture systems was seen in the relative production of butyrate and propionate, with higher proportions of the latter being produced in the open system than in batch culture, although the same medium and faecal donor were used in both cases. This could be related to the lack of pH control and endproduct removal in the batch incubations, to differences in O2 levels, or to shifts in the fermentor community during the initial 7 d equilibration period.

In conclusion, the net and dynamic contributions of acetate to butyrate formation by mixed human faecal bacteria appear consistent with the behaviour of isolated bacteria related to Roseburia spp. and F. prausnitzii in pure culture; this implies a major role for these bacteria in butyrate synthesis in vivo. Most of the carbohydrate sources tested, with the possible exception of pectin, appear suitable as butyrogenic substrates, despite the fact that they do not necessarily support growth of the individual butyrate-producing bacterial species in pure culture. It is clear from this work, and from other studies (Duncan et al. 2002b), that the major butyrogenic species depend for their survival and activities on other bacteria, including net producers of acetate and species capable of degrading a variety of complex carbohydrates. A possible factor in the reported butyrogenic effect of resistant starch, for example, might be that acetate produced by amylolytic bifidobacteria became re-routed into butyrate by bacteria such as those considered here. Nutritional strategies aimed at delivering butyrate to the human colon need to take these interactions into account.
Bacterial conversion of acetate to butyrate

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References


