RESEARCH ARTICLE

Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species

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Abstract

Butyrate is the preferred energy source for colonocytes and has an important role in gut health; in contrast, accumulation of high concentrations of lactate is detrimental to gut health. The major butyrate-producing bacterial species in the human colon belong to the *Firmicutes*. *Eubacterium hallii* and a new species, *Anaerostipes coli* SS2/1, members of clostridial cluster XIVa, are able to utilize lactate and acetate via the butyryl CoA : acetate CoA transferase route, the main metabolic pathway for butyrate synthesis in the human colon. Here we provide a mathematical model to analyse the production of butyrate by lactate-utilizing bacteria from the human colon. The model is an aggregated representation of the fermentation pathway. The parameters of the model were estimated using total least squares and maximum likelihood, based on *in vitro* experimental data with *E. hallii* L2-7 and *A. coli* SS2/1. The findings of the mathematical model adequately match those from the bacterial batch culture experiments. Such an *in silico* approach should provide insight into carbohydrate fermentation and short-chain fatty acid cross-feeding by dominant species of the human colonic microbiota.

Introduction

The human colonic microbiota has a number of important roles, including the anaerobic degradation of carbohydrates not absorbed in the upper digestive tract. This results in the production of short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate, which have been recognized for their health-promoting effects (Topping & Clifton, 2001). In the fermentation process, lactate is a key intermediate produced via the homofermentative or heterofermentative pathways. Lactate participates in a reversible reaction with another key component of the fermentation process, pyruvate. It is thus a precursor of the formation of acetate, propionate and butyrate in species such as *Megasphaera* and *Veillonella*. Lactate can also be a cosubstrate for the sulphate-reducing species *Desulfovibrio piger* (Marquet *et al.*, 2009).

Because lactate is involved in different metabolic reactions and is also absorbed by the host, it is detected at low levels in healthy subjects (Duncan *et al.*, 2007). Lactate-utilizing bacteria are important for the prevention of lactate accumulation in the large intestine. High concentrations of lactate have been associated with short bowel syndrome (Kaneko *et al.*, 1997), ulcerative colitis (Vernia *et al.*, 1988; Hove *et al.*, 1994), and can lead to neurotoxicity and cardiac arrhythmia (Chan *et al.*, 1994). Lactate accumulation also causes acidosis in ruminants (Ewaschuk *et al.*, 2005) and lameness in horses (Rowe *et al.*, 1994).

In vitro ^{13}C nuclear magnetic resonance studies have identified lactate and acetate as important precursors of butyrate production in human faecal samples (Bourriaud *et al.*, 2005; Morrison *et al.*, 2006; Belenguer *et al.*, 2008). Furthermore, *in vivo* rodent studies confirmed the importance of lactate utilization for the production of beneficial compounds such as butyrate (Sato *et al.*, 2008).

Butyrate plays an important role in gastrointestinal tract homoeostasis. It acts on gut receptors that modulate gut motility and inflammatory response (Brown *et al.*, 2003; Tazoe

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et al., 2008). Butyrate is the main energy source for colonic epithelial cells, and a preventive agent of intestinal pathologies such as inflammatory bowel disease and colorectal cancer (McIntyre *et al.*, 1993; Pryde *et al.*, 2002; Scheppach & Weiler, 2004; Hamer *et al.*, 2008). Moreover, butyrate concentrations vary dramatically in obese individuals following different carbohydrate diets (Duncan *et al.*, 2007).

Within the last decade, butyrate-producing bacteria, mostly belonging to the *Firmicutes* phylum, have been isolated from the human gastrointestinal tract (Barcenilla *et al.*, 2000). Among them, *Faecalibacterium prausnitzii* and *Roseburia* spp. are unable to use lactate (Duncan *et al.*, 2004a, b). Conversely, *Anaerostipes caccae, Eubacterium hallii* L2-7 and the proposed new species *Anaerostipes coli* SS2/1 (Walker *et al.*, 2011), are lactate-fermenting, butyrate-producing bacteria. *Eubacterium hallii* and *A. coli* belong to clostridial cluster XIVa (*Lachnospiraceae*). *Anaerostipes coli* is a dominant member of the colonic microbiota recognized for its importance in butyrate production (Walker *et al.*, 2011). The abundance of *E. hallii* in human faeces ranges from 1% to 3% of total bacteria (Harmsen *et al.*, 2002; Louis & Flint, 2009).

Butyrate can be synthesized from two metabolic pathways: butyrate kinase and butyryl CoA : acetate CoA transferase (Miller & Wolin, 1996; Diez-Gonzalez *et al.*, 1999; Duncan *et al.*, 2002). *In vitro* studies have identified the latter mechanism as the dominant one in the human colonic ecosystem (Louis *et al.*, 2004). Pyruvate is the central pivot of butyrate synthesis. It is oxidized to acetyl coenzyme A (acetyl-CoA), which is further routed to acetate or butyrate. Acetate is produced via acetate kinase. This pathway generates energy in the form of ATP. For butyrate formation, two molecules of acetyl-CoA are condensed to one molecule of acetoacetyl-CoA, and subsequently reduced to butyryl-CoA. Butyryl CoA : acetate CoA transferase transfers the CoA moiety to external acetate, leading to the formation of acetyl-CoA and butyrate (Duncan *et al.*, 2002). In addition to restoring NAD^+ in the cell, more energy may be obtained from butyrate formation in bacteria with a membrane-associated NADH : ferredoxin oxidoreductase-linked proton motive force (Seederof *et al.*, 2008; Louis & Flint, 2009).

In vivo and *in vitro* studies of the human gastrointestinal tract are hampered by both ethical and technical constraints, including the low percentage of cultured bacteria (Suau *et al.*, 1999; Eckburg *et al.*, 2005). Mathematical *in silico* models based on biological knowledge will help in understanding and predicting the patterns of carbohydrate fermentation and metabolite cross-feeding occurring in the human gastrointestinal tract. Mathematical models have been used previously to study anaerobic reactors (see, e.g. Batstone *et al.*, 2002). Given the processing similarities between anaerobic bioreactors and the human large bowel, the anaerobic digestion modeling represents a framework for modelling the human colonic ecosystem. Up to now, there have been very few dynamic models for the metabolic reactions driven by colonic bacterial species. Amaretti *et al.* (2007) developed a mathematical model to describe the kinetics in *Bifidobacterium adolescentis* MB 239 grown on different substrates. Other mathematical approaches have analysed the butyrate production from labelled substrates (Duncan *et al.*, 2004a, b; Morrison *et al.*, 2006). Recently, ^{13}C-labelled glucose was used to map the fermentation metabolic fluxes in human colonic microbiota (de Graaf *et al.*, 2010).

Complementary to animal studies (e.g. Le-Blay *et al.*, 1999), *in silico* models that predict butyrate formation should provide valuable insights into the structure and dynamics of human colonic microbiota, suggest interesting additional *in vitro* or *in vivo* experiments, and help in developing strategies to promote gut health.

The mathematical model construction process should obey the principle of parsimony (also known as Occam's razor), striving to build the least complex model that can still adequately represent the system under study. The aim of the work reported here was to develop a parsimonious mathematical model to describe the conversion of lactate and acetate into butyrate by key human colonic bacterial species. The model was constructed based on the butyryl CoA : acetate CoA transferase pathway for butyrate formation and was assessed by computing *in vitro* data obtained for *E. hallii* L2-7 and *A. coli* SS2/1 (Duncan *et al.*, 2004b).

Materials and methods

Mathematical model

Figure 1 illustrates a simplified scheme of the butyryl CoA : acetate CoA transferase pathway. The kinetic model developed is an aggregated representation of this pathway. A simplified schematic representation is displayed in Fig. 1 (inset box). The dynamics of all variables depends only on the concentrations of lactate and the number of bacteria (OD).

The mathematical model was derived by formulating mass-balance differential equations for a batch system. The model equations are then:

$$\frac{\mathrm{d}}{\mathrm{d}t}x_{\mathrm{la}} = Y\rho_{\mathrm{la}} \tag{1}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}s_{\mathrm{la}} = -\rho_{\mathrm{la}} \tag{2}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}s_{\mathrm{ac}} = Y_{\mathrm{ac}}\rho_{\mathrm{la}} \tag{3}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}s_{\mathrm{bu}} = Y_{\mathrm{bu}}\rho_{\mathrm{la}} \tag{4}$$

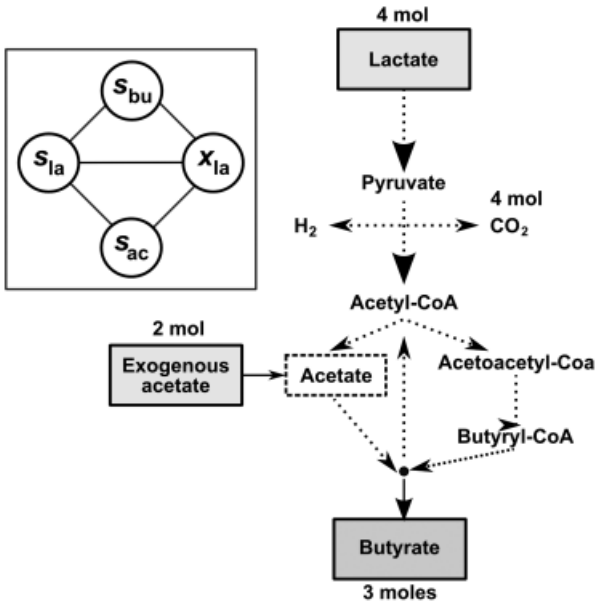

Fig. 1. Simplified scheme of the butyryl CoA : acetate CoA transferase pathway for butyrate production (adapted from Papoutsakis, 1984; Duncan *et al.*, 2004b). The inset is a graphical representation of the mathematical model. The model variables are the bacterial concentration (x_{la}), lactate concentration (s_{la}), acetate concentration (s_{ac}) and butyrate concentration (s_{bu}).

The model variables (also called state variables) are the bacterial concentration x_{la} in $OD_{650\,nm}$, assumed to be proportional to molar concentration, lactate concentration s_{la}, acetate concentration s_{ac} and butyrate concentration s_{bu} in mM.

The function ρ_{la} is the consumption rate of lactate. This is expressed by the standard Monod equation:

$$\rho_{la} = k_m \frac{s_{la} x_{la}}{K + s_{la}} \tag{5}$$

The parameter k_m is the lactate consumption rate constant. It is expressed in mM lactate per OD h^{-1}. The Monod constant K is in mM lactate. Y (OD mM^{-1} lactate) is the biomass yield according to the consumed lactate. The yield factors Y_{ac} (mM acetate mM^{-1} lactate) and Y_{bu} (mM butyrate mM^{-1} lactate) represent the molar change of acetate and butyrate to the consumed lactate. The yield factors are related to the stoichiometry of the metabolic conversions. Based on the reactions for butyrate production (Papoutsakis, 1984) and lactate consumption (Costello *et al.*, 1991), the following set of reactions is derived to group the intermediate steps of butyrate:

$$CH_3CHOHCOOH + H_2O \rightarrow CH_3COOH + 2H_2 + CO_2 \tag{a}$$

$$CH_3CHOHCOOH + CH_3COOH \rightarrow CH_3CH_2CH_2COOH + H_2O + CO_2 \tag{b}$$

It should be noticed that reactions(a) and (b) are used as a simplification of the complete mechanism in order to provide stoichiometric relationships between metabolites. In addition to the above reactions, carbon from lactate contributes to bacterial growth.

Let $(1 - f_{la})$ be the fraction of lactate used for bacterial growth. Denote by η_a the part of the remaining fraction f_{la} of lactate that is converted in reaction (a). Then, the part involved in reaction (b) is $\eta_b = 1 - \eta_a$.

Exploiting the stoichiometry, we get:

$$Y_{ac} = f_{la}(\eta_a - \eta_b) \tag{6}$$

$$Y_{bu} = f_{la}\eta_b \tag{7}$$

The biomass yield factor Y is decomposed as:

$$Y = \alpha\beta(1 - f_{la}), \tag{8}$$

where α is a conversion factor between OD and bacterial concentration and β is the number of bacteria produced per mole of lactate consumed for the bacterial growth.

Note that Y_{ac} is a net factor representing the difference between production and consumption of acetate. Thus, if Y_{ac} is positive, it means that lactate is routed to reaction (a) in a higher proportion than to reaction (b).

Stoichiometry imposes the following constraints on Y_{ac} and Y_{bu}:

$$0 \leq Y_{bu} \leq 1 \tag{9}$$

$$0 \leq Y_{bu} + Y_{ac} \leq 1 \tag{10}$$

Moreover, assuming that the fraction of lactate devoted to bacterial growth is low (f_a is close to 1) we get:

$$Y_{ac} + 2Y_{bu} - 1 \cong 0, \tag{11}$$

which gives an approximate relation between the number of butyrate moles produced per time unit (dn_{bu}) and the number of moles of lactate and acetate utilized per time unit ($-dn_{la}$ and $-dn_{ac}$):

$$dn_{bu} \approx \frac{-dn_{la} - dn_{ac}}{2} \tag{12}$$

A very important feature of the model is that, from Eqns (1) and (2) we get:

$$\frac{d}{dt}(x_{la} + Ys_{la}) = 0, \tag{13}$$

which means that bacterial concentration x_{la} is related to lactate concentration s_{la} by a linear relationship with slope $-Y$. In the same way, acetate concentration s_{ac} and butyrate concentration s_{bu} are related to s_{la} by linear relationships with slope $-Y_{ac}$ and $-Y_{bu}$, respectively.

The mathematical model does not take into account biomass decay, and no distinction between D-lactate and L-lactate is attempted. For *E. hallii* L2-7, the sum of the two

isomers is considered a sole substrate. In the case of *A. coli* SS2/1, D-lactate is the sole substrate, because this strain was not able to utilize L-lactate.

Use of experimental data to estimate the model parameters

The parameters to be estimated are k_m, Y, Y_{ac}, Y_{bu} and K. Experimental data formerly obtained (Duncan *et al.*, 2004b) were used to estimate these parameters. The published data are means of triplicates with pure cultures of either *E. hallii* L2-7 (DSM 17630; accession number AJ270490) or *A. coli* SS2/1 (DSM 23942) providing the inoculum. *Eubacterium hallii* L2-7 was isolated from a 2-year-old healthy infant (Barcenilla *et al.*, 2000) and *A. coli* SS2/1 from a healthy adult female (Louis *et al.*, 2004) consuming a westernized diet. Bacterial strains were grown in YCFA medium containing (per 100 mL) 0.1 g casitone, 0.25 g yeast extract, 0.4 g $NaHCO_3$, 0.1 g cysteine, 0.045 g K_2HPO_4, 0.045 g KH_2PO_4, 0.09 g NaCl, 0.009 g $MgSO_4 \cdot 7H_2O$, 0.009 g $CaCl_2$, 0.1 mg resazurin, 1 mg haemin, 1 μg biotin, 1 μg cobalamin, 3 μg *p*-aminobenzoic acid, 5 μg folic acid and 15 μg pyridoxamine. Final concentrations of the SCFA in the medium were 46 mM acetate, 9 mM propionate and 1 mM each of isobutyrate, iso-valerate and valerate. The medium was adjusted to pH 6.7 and placed in Hungate tubes that were flushed with CO_2 and heat-sterilized. Heat-labile vitamins were added after the medium was autoclaved to give a final concentration of 0.05 μg mL^{-1} thiamine and 0.05 μg mL^{-1} riboflavin. The glucose-supplemented medium as a carbon source contained a final concentration of 10 mM . Each test bacterial strain was inoculated into triplicate tubes and growth measured spectrophotometrically as $A_{650\,nm}$. Growth rates were calculated in exponential phase. The medium was supplemented with 35 mM DL-lactate (pH 6.7 ± 0.1), of which approximately 16 mM were in the L-lactate form. The *in vitro* experiments were carried out in Hungate tubes under anaerobic conditions. Fermentation was monitored by synchronous measurements of the $OD_{650\,nm}$ and concentrations of glucose, lactate (DL and L), acetate and butyrate. Concentrations of L-lactate and glucose were determined by enzymatic methods. Concentrations of SCFA and DL-lactate were determined by capillary GC. D-Lactate was estimated as the difference between total lactate and L-lactate concentrations.

Parameter estimation focused on *E. hallii* L2-7 and *A. coli* SS21/1 experiments in DL-lactate-containing medium (fig. 1b and e in Duncan *et al.*, 2004b). Data in the lag phase were not considered.

The linear relationships between concentrations were assessed using total least squares (Vanhuffel & Vandewalle, 1989; Björck, 1996). The usual least squares method was not used because in the linear relationships that we analyse, the independent variable (lactate concentration) is not error-free. The total least squares provided estimates of the initial concentrations and of the yields. The estimated mean square measurement errors were then calculated. A parameter estimation of the complete model was then carried out by the maximum likelihood approach. The previously estimated Y, Y_{ac} and Y_{bu} values were used to compute plausible starting values in the optimization step. Similarly, for the initial bacterial, lactate, acetate and for butyrate, the estimated values together with estimated SDs generated plausible values of the initial concentrations.

In many situations, the concentration of lactate will be very small compared with the Monod constant K. An estimation was therefore performed for the complete model and also for a simplified version where the following approximation:

$$\rho_{la} \cong \frac{k_m}{K} s_{la} x_{la}, \tag{14}$$

valid when $s_{la} < K$, was made. Note that in this case, only the ratio k_m/K can be estimated.

The cost function to be optimized was derived, assuming that the measurement errors followed a normal distribution with unknown and diagonal covariance matrix (e.g. Walter & Pronzato, 1997). The Matlab® toolbox IDEAS (Muñoz-Tamayo *et al.*, 2009) was used to estimate the model parameters. IDEAS uses a quasi-Newton unconstrained optimization method for its optimization step. Once the estimation is completed and the constraints imposed by stoichiometry are satisfied, the approximate 95% confidence intervals for the parameters are calculated based on the computation of the Fisher information matrix (e.g. Walter & Pronzato, 1997). IDEAS can be freely downloaded from the web page http://www.inra.fr/miaj/public/logiciels/ideas/index.html.

Results and discussion

The construction of the mathematical model describing the kinetics of butyrate production by *E. hallii* L2-7 and *A. coli* SS2/1 was based on standard models of anaerobic reactors. We adapted such models to the specificities of human colonic bacteria. For instance, lactate contribution to butyrate is not considered in mathematical models for anaerobic reactors (Costello *et al.*, 1991; Skiadas *et al.*, 2000), whereas lactate is an important precursor of butyrate production in the human colon.

Utilization of lactate and acetate to produce butyrate in the metabolic pathway catalysed by *E. hallii* L2-7 and *A. coli* SS2/1 is shown in Fig. 2. Both strains were provided with 35 mM of DL-lactate, from which approximately 16 mM was in the L-lactate form. *Eubacterium hallii* L2-7 utilized all the available lactate, whereas *A. coli* SS2/1 could only utilize

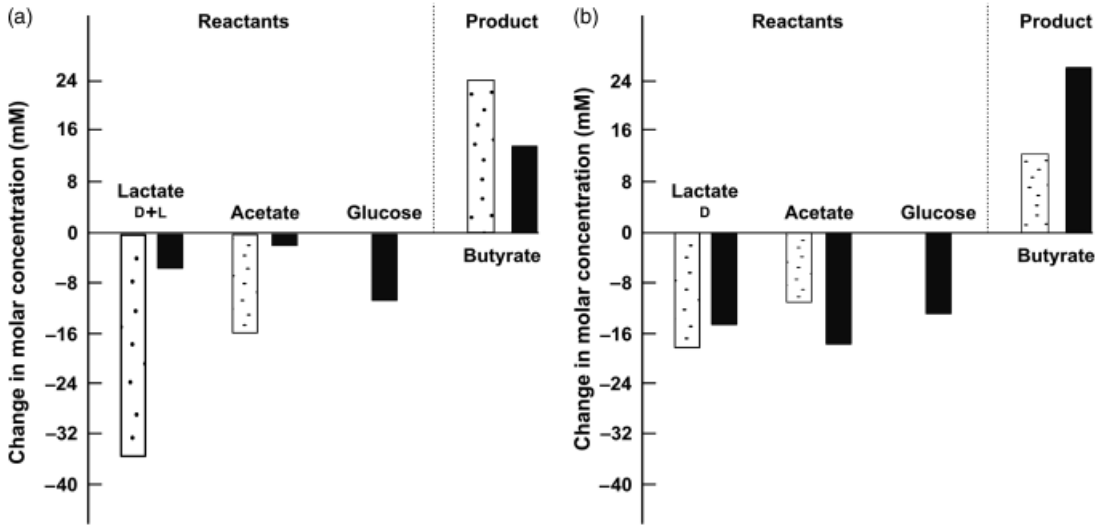

Fig. 2. Substrate utilization and product formation in *Eubacterium hallii* L2-7 (a) and *Anaerostipes coli* SS2/1 (b) cultures. Dotted bars, medium with lactate; solid bars, medium with lactate plus glucose.

D-lactate. The data of Duncan *et al.* (2004b) also showed that in spite of the difference in lactate utilization by the bacterial strains, the butyrate produced is about half the number of reacting moles of lactate plus acetate in both cases. The addition of glucose to lactate-containing medium repressed lactate utilization by *E. hallii* L2-7 but had little effect on lactate metabolism by *A. coli* SS2/1.

The linear relationships between concentrations are corroborated by the experimental data for both strains (Fig. 3). Estimated yield factors, initial concentrations and measurement error SDs are given in Table 1. These results agree with the structure of our model based on the butyryl CoA : acetate CoA transferase pathway.

Parameter estimation

Maximum likelihood estimation was performed for several starting values of the parameters, and for several values of the initial concentrations (Table 1). For each parameter, the mean and SD of the estimates were computed. For both strains, the k_m parameter was the most sensitive to the initial concentration, with an SD of 15%. For the other parameters, the SDs ranged from 3% to 14% of the mean. A multistart routine on the initial guess of the parameters was implemented to reduce the risk of finding local minima. The Monod model, in its simplified version, adequately represents the *in vitro* data from both strains (Fig. 4). Data corresponding to *A. coli* SS2/1 are more scattered, thus the fit is less satisfactory than for *E. hallii* L2-7. A discrepancy for acetate concentration was observed for both strains. In the case of *E. hallii* L2-7, this mismatch occurs for the first three sampling points. According to the model, acetate

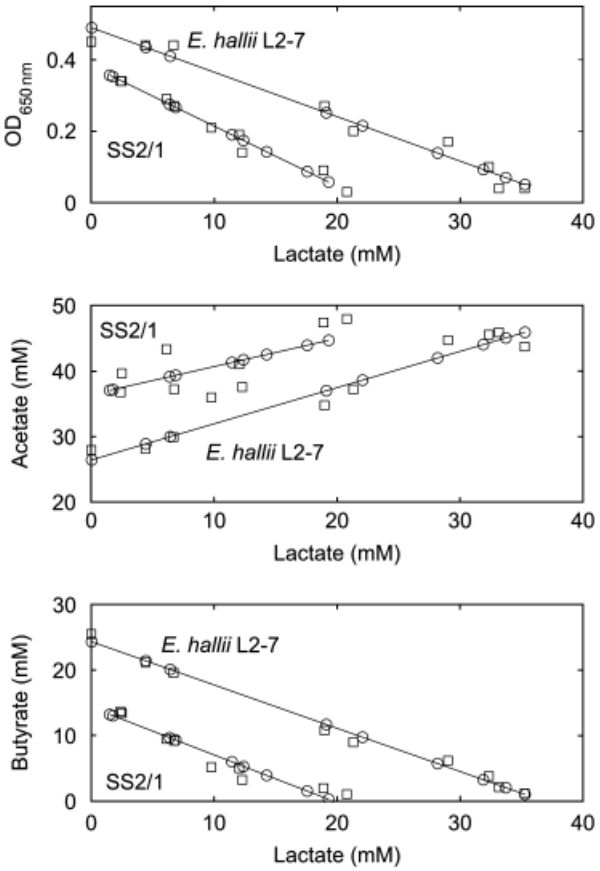

Fig. 3. Estimation by total least squares (TLS) of the linear relationships between lactate concentration and bacterial OD, acetate concentration and butyrate concentration. □, experimental concentrations; ○, estimated concentrations by the TLS algorithm.

Table 1. Total least squares estimates of the yield factors: measured initial concentrations, estimated initial concentrations and estimated mean quadratic errors for biomass and substrates

Yield factors		Y (OD mM^{-1} lactate)	Y_{ac} (mM acetate mM^{-1} lactate)	Y_{bu} (mM acetate mM^{-1} lactate)
Eubacterium hallii L2-7		0.012	− 0.55	0.66
Anaerostipes coli SS2/1		0.017	− 0.43	0.73
Initial concentrations	s_{la} (mM) Measured Estimated (error)	x_{la} (OD) Measured Estimated (error)	s_{ac} (mM) Measured Estimated (error)	s_{bu} (mM) Measured Estimated (error)
E. hallii L2-7	35.27 35.3 (0.49)	0.04 0.05 (0.025)	43.71 45.9 (1.76)	1.18 1.01 (0.68)
Anaerostipes coli SS2/1	20.8 19.3 (1.23)	0.03 0.06 (0.016)	47.94 44.7 (3.61)	1.05 0.26 (0.56)

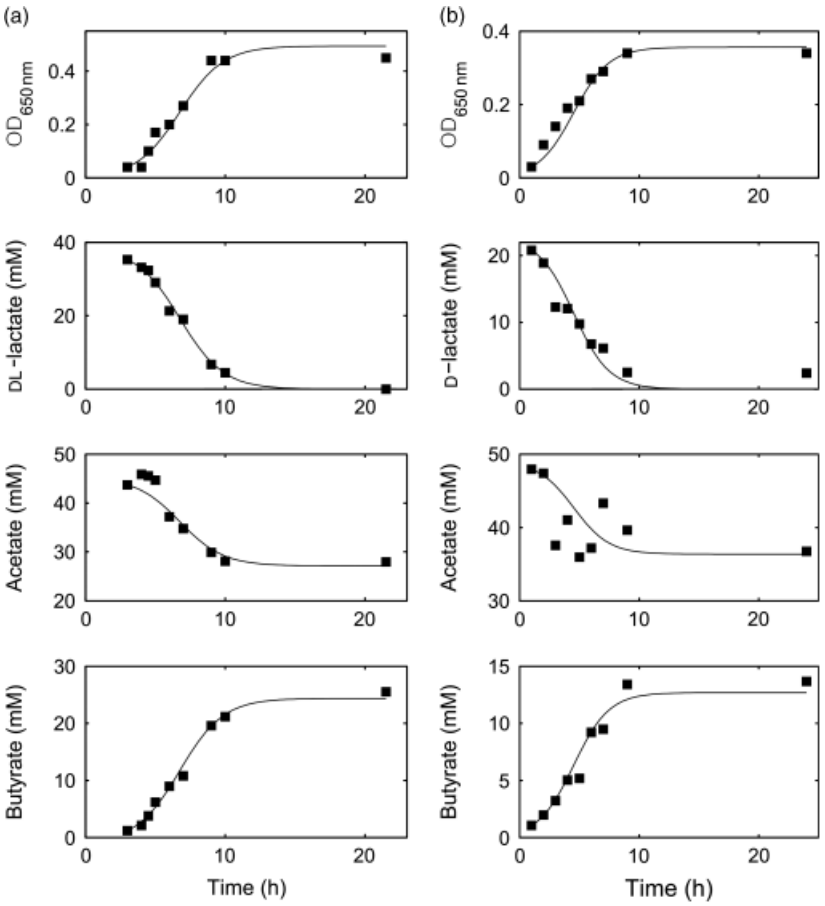

Fig. 4. Outputs of the kinetic model developed (solid lines) and experimental data (squares). (a) *E. hallii* L2-7 (b) *A. coli* SS2/1. The proposed model adequately represents the experimental data.

concentration will either always increase or always decrease. This transient mismatch may be due to measurement uncertainty, but most likely to the presence of carbon sources in YCFA medium from the yeast extract, not accounted for by the model. For *E. hallii* L2-7, the mathematical model slightly overestimated the biomass concentration at the final time point. Apart from measurement uncertainty, this could be explained by the fact that the kinetic model considers that biological activity is growth-associated. As bacteria can remain metabolically active without cell division, the OD in batch cultures is not always a very good indicator of bacterial activity.

Table 2. Estimates of the model parameters for the simplified kinetic model with their approximate 95% confidence intervals

	k_m/K (1 per OD h^{-1})	Y (OD mM^{-1} lactate)	Y_{ac} (mM acetate mM^{-1} lactate)	Y_{bu} (mM butyrate mM^{-1} lactate)
Eubacterium hallii L2-7	1.205 ± 0.082	0.013 ± 0.001	-0.556 ± 0.061	0.666 ± 0.032
Anaerostipes coli SS2/1	1.412 ± 0.148	0.015 ± 0.001	-0.404 ± 0.194	0.678 ± 0.058

Table 2 shows model parameters estimated with the quadratic kinetic function in Eqn(14) with their approximate 95% confidence intervals. As expected, the yield factor estimates are very close to the values obtained with total least squares computation only. The confidence intervals are narrow for both strains.

We also assessed the parameter accuracy for the complete Monod model (results not shown here). The SDs of the yield factors compare with those of the simplified model. However, k_m and the affinity constant K show high SDs, as was expected. Experimental data with higher lactate concentrations would be necessary to provide correct estimates of these parameters.

Reaction stoichiometry

The equations from the simplified set of reactions for butyrate production have enabled us to present stoichiometric relationships between metabolites. For instance, the number of moles of butyrate formed is approximately half the number of reacting moles of lactate plus acetate. This relationship was indeed verified by the experimental results. In addition, the stoichiometry is in agreement with observations on other butyrate-producing bacteria grown in glucose or fructose, where the conversion of acetate and bacterial growth ceased with the disappearance of glucose or fructose (Duncan *et al.*, 2004a; Falony *et al.*, 2006) or, in this case, lactate. It can be deduced that, in a given bacterial strain, lactate may be the sole carbon source to produce butyrate; only if the acetate kinase activity is high enough to produce acetate for the conversion of butyryl CoA to butyrate (by butyryl CoA : acetate CoA transferase).

Model limitations

The model adequately describes the kinetics of butyrate production by *E. hallii* L2-7 and *A. coli* SS2/1. However, more experimental data are necessary to perform a formal validation of the model.

The parameters were estimated from *in vitro* data obtained under a specific set of conditions and these could change for experiments carried out under other conditions. For instance, lactate consumption rate and SCFA production are known to depend on hydrogen partial pressure (Costello *et al.*, 1991; Macfarlane & Macfarlane, 2003). Unfortunately, for the experiments studied here, hydrogen was not measured and its effect not evaluated. It has been shown that lactate utilization and fermentation processes by human faecal microbiota depended on pH (Belenguer *et al.*, 2007), and it has been reported that the estimates of kinetic parameters can also change with pH conditions (Amaretti *et al.*, 2007). The model presented here does not take into account the influence of pH or of hydrogen partial pressure on the kinetics. Empirical mathematical expressions show that the effects of pH and H_2 on the kinetic rates have been developed for anaerobic reactors (Costello *et al.*, 1991; Batstone *et al.*, 2002) and can be used for our model. Additional experimental work (Fig. 5) has revealed that *E. hallii* L2-7 growth rate decreased from 0.18 h^{-1} at pH 6.5 to 0.13 h^{-1} at pH 5.5 at an initial concentration of 35 mM DL-lactate. Surprisingly, *A. coli* SS2/1 was stimulated at pH 5.5 (0.31 h^{-1}) compared with pH 6.5 (0.13 h^{-1}). Very interestingly, this suggests that the relative contribution of these two species to lactate utilization may vary with pH and, consequently, in proximal (pH 5.5) and distal colon (pH up to 6.8).

Environmental conditions impose thermodynamic limitations on the spectrum of fermentation products. The Gibbs free energy change ($\Delta G'$) of the reactions is the driving force that determines the metabolite concentration profile. Thus, the initial concentration and accumulation of metabolites will strongly affect the reaction progress. *In vitro* experiments with the ruminal butyrate-producing bacterium *Megasphaera elsdenii* showed the impact of the initial concentration of acetate on butyrate metabolism. Under low initial concentrations of extracellular acetate (< 4 mM), the production rate of acetate was higher than its consumption rate. When the initial concentration increased, the production decreased, whereas consumption increased concomitantly with an increased butyrate production (Hino *et al.*, 1991). For the experimental data analysed here, the acetate consumption rate was higher than its production rate, indicating that exogenous acetate is required to achieve maximal growth. In the kinetic model, this implies a negative value for Y_{ac} yield factor, so η_a is < 0.5. Under conditions where acetate production is higher than consumption, the model structure advocated here will remain identical, but η_a will be > 0.5 and Y_{ac} will become positive. This shift in the reaction process can be handled mathematically using thermodynamic-based models as proposed by Rodríguez *et al.* (2008).

Application of the model and perspectives

Due to a number of ethical and technical limitations, including the limited number of cultured species available

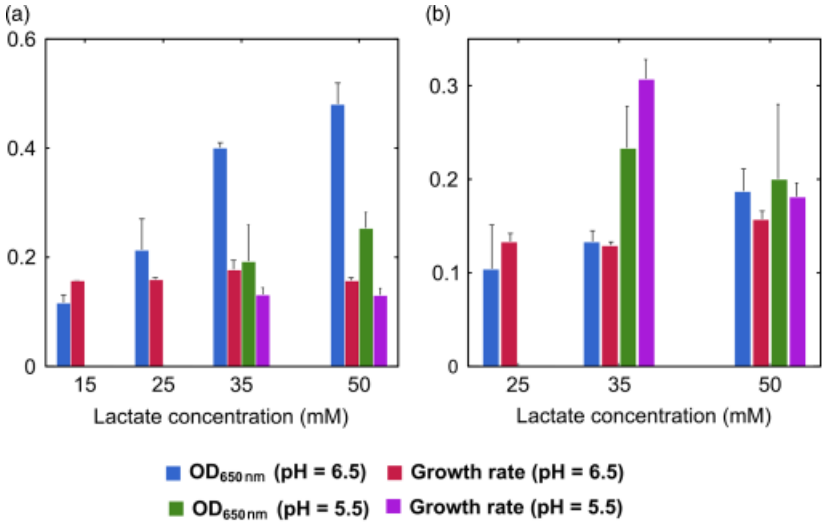

Fig. 5. Influence of YCFA medium initial pH and lactate concentrations on the growth of (a) *Eubacterium hallii* L2-7 and (b) *Anaerostipes coli* SS2/1. Growth rates are expressed per hour.

for metabolic studies, the human colon has remained largely unexplored for years. However, there is recent evidence that many representatives of the most abundant and metabolically active species have been isolated (Flint *et al.*, 2007; Tap *et al.*, 2009; Walker *et al.*, 2011) and their genomes sequenced within the Human Microbiome Project (Turnbaugh *et al.*, 2007). *In vitro* models based on experimental data from these isolates have proved very useful to study metabolism and interspecies interactions (Belenguer *et al.*, 2006; Falony *et al.*, 2006) and to provide better representations of the complexity of the gut ecosystem (Macfarlane & Macfarlane, 2007). Furthermore, simplified models in gnotobiotic rodents provide central information on *in vivo* bacterial metabolism and its consequences for host epithelial genetic responses (Mahowald *et al.*, 2009). However, extrapolation of these studies to the human colonic ecosystem is not trivial. *In silico* models, such as the one proposed here, offer complementary approaches that can be used to address the complexity of the large intestine.

Metagenomic studies of the microbiota from the human gastrointestinal tract confirmed the importance of the metabolic pathway studied here: the genes involved in butyrate production demonstrated high odds ratios compared with all available bacterial genomes or human genome (Gill *et al.*, 2006), and European MetaHit project (data not shown).

The mathematical model presented here focuses on *E. hallii* L2-7 and *A. coli* SS2/1; both are part of the dominant phylogenetic core of the human intestinal microbiota (Tap *et al.*, 2009; Walker *et al.*, 2011). These two strains differ in their affinity for lactate enantiomers. Whereas *E. hallii* was able to use both D- and L-lactate, *A. coli* SS2/1 only utilized D-lactate. Nevertheless, the model takes these differences into account. Even if the amount of data studied here is limited, the mathematical model structure should not be restricted to *E. hallii* L2-7 or *A. coli* SS2/1 as it could be applied to other phylogenetically distinct bacterial groups that share enzymatic reactions. Kinetic rates may differ between species, as demonstrated for the two strains analysed here; however, the model structures should be identical. Other observed differences between *E. hallii* and *A. coli* are their response to pH, in addition to *A. coli* showing greater resistance to glucose repression. Further refinement of the model will be required to take this behaviour into account.

Additional data are required to extrapolate our results to the human colonic ecosystem. Interesting data to be integrated in our approach include growth experiments with other key butyrate-producing bacteria that are unable to utilize lactate, such as *Roseburia intestinalis* and *F. prausnitzii*. *In vitro* experiments on microbial interactions between hydrogen utilizers and butyrate producers as presented by Chassard & Bernalier-Donadille (2006) provide useful data to build more representative models. Mathematical model approaches such as the one we developed to study homoacetogenesis reaction by *Blautia hydrogenotrophica* (Muñoz-Tamayo *et al.*, 2008) could also be coupled with the model presented here to allow valuable cross-feeding interactions to be studied.

An important application of the present work is the integration of butyrate metabolism into a model that includes the complete trophic chain of carbohydrate fermentation as well as transport phenomena such as SCFA absorption (Muñoz-Tamayo *et al.*, 2010). The estimation of the parameters of the resulting complex model is particularly challenging because of the scarcity of data. The analysis of *in vitro* bacterial growth experiments, such as the present study, is essential in the definition of prior knowledge to tackle the estimation problem for the complete model. This model could then address the impact of different carbon sources with butyrogenic effects. Such an approach would contribute to the development of strategies for enhancing butyrate production in relation to its health-promoting effects.

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