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Determination of potential metabolic pathways of human intestinal bacteria by modeling growth kinetics from cross-feeding dynamics

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ABSTRACT

Microbial cross-feeding is essential for a healthy commensal bacteria community in the human gut. Here we present mathematical models that account for the various types of cross-feeding by human commensal intestinal bacteria. The model bacteria include a mixed but unknown microbial community (fecal slurry), *Eubacterium hallii*, *Roseburia intestinalis*, *Roseburia inulinivorans*, and *Anaserostipes caccae*. These mathematical models demonstrate that a carbon balance approach together with chemical kinetic analysis and parameters estimated from model fitting can be used to determine which of several potential metabolic pathways are employed by cross-feeding bacterial communities to produce their metabolites. The approach can be used to estimate growth kinetics either if the population of bacteria is known, or if the population is mixed and unknown. Based on chemical kinetic analysis, an alternative view of the metabolic pathway of *E. hallii* is proposed. The modeling suggested that the production of butyrate by *E. hallii* from lactate and acetate was a second rather than a third-order reaction. Furthermore, the process by which both *R. inulinivorans* and *R. intestinalis* degraded carbohydrates and acetate was a second order reaction, and the consumption ratio was found to be approximately 1 mM FE oligofructose to 1 mM acetate for both *Roseburia* strains. As well as estimating metabolic parameters, the approach has also suggested candidate metabolic pathways for those systems that could be tested experimentally.

1. Introduction

In nature, bacteria are primarily found in mixed microbial populations. This close association leads to diverse social dynamics, in particular the evolution of microbial cross-feeding. Several types of microbial cross-feeding have been reported. For instance, when *Bacteroides thetaiotaomicron* degrades oligofructose and inulin, monomers and other oligosaccharides are released into the environment, which can then be used by other bacteria, such as bifidobacteria (Falony, 2009; Falony, Calmeyn, Leroy, & De Vuyst, 2009). Other bacteria, including *Eubacterium hallii*, can utilize acetate and lactate, two metabolites that are often produced from bifidobacteria fermentation of carbohydrates (Duncan, Louis, & Flint, 2004; Muñoz-Tamayo et al., 2011). Bacteria such as *Roseburia intestinalis* have been shown to degrade monosaccharides and oligosaccharides simultaneously with acetate (Falony, Verschaeren et al., 2009).

While cross-feeding is a key component of microbial communities, mechanistic mathematical models of this phenomenon are still in their infancy. The merits of previously published mathematical models have been discussed elsewhere (Van Wey, Cookson, Roy, McNabb, Soboleva et al., 2014). The types of model used have ranged from empirical to mechanistic models (Amaretti et al., 2007; Baranyi & Roberts, 1994; Bull & Harcombe, 2009; Fgaier, Kalmokoff, Ells, & Eberl, 2014; Janssen et al., 2006; Muñoz-Tamayo et al., 2011; Poschet, Vereecken, Geeraerd, Nicolaï, & Van Impe, 2005; Van Impe, Poschet, Geeraerd, & Vereecken, 2005; Wintermute & Silver, 2010).

Recent works have considered bacterial cross-feeding and metabolic pathways (Harcombe et al., 2014; Kettle, Louis, Holtrop, Duncan, & Flint, 2015). For instance Harcombe et al. (2014) have proposed a system of equations that predicts population proportions and spatial dynamics (COMETS) which was validated against 1) a synthetic two species system in which the populations are symbiotic, both requiring metabolites produced by the other bacteria in order to grow, and 2) a synthetic three species system which includes a level of competitiveness and mutualistic behavior. While the particular bacteria used to validate the model were engineered to have the specified behavior, this work demonstrates that bacteria growth can

Abbreviations: MCMC, Markov Chain Monte Carlo; EPS, extracellular polymeric substance; OD, optical density; CFU, colony-forming unit; FE, fructose equivalents; RMSE, root mean square error.

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be predicted based on an individual optimality postulate, which is consistent with the premise underlying the co-culture predictions by (Van Wey et al., 2014; Van Wey Lovatt, 2013). In the work of Harcombe et al. (2014), the metabolic pathways were "manually curated reconstructions, or from automated pipelines that construct models from annotated genomes and high-throughput data".

Kettle et al. (2015) developed a system of 10 functional bacteria groups to describe the dynamics in the human colon. They also assumed that the metabolic pathways proposed in the literature were accurate rather than testing their validity before implementation but, as acknowledged in their manuscript, the metabolic pathways are easy to change, and could be updated with new knowledge of the pathways.

Van Wey, Lovatt, Roy, and Shorten (2016) modified a previously published model (Van Wey et al., 2014) to reduce the number of fitting parameters required to describe monoculture growth kinetics and to predict co-culture growth kinetics. Both articles demonstrated that kinetic parameters obtained by fitting a model to monoculture data could be used to predict co-culture behavior. These predictions were then validated against experimental data. Furthermore, it was shown that since the rate of metabolite production is proportional to the rate of substrate degradation, the proportionality constant (metabolic conversion constant) can be determined directly from the experimental data rather than be used as a fitting parameter (Van Wey et al., 2015). In these studies, cross-feeding by bifidobacteria resulted from the accumulation of monosaccharides and oligosaccharides during extracellular degradation of inulin by *Bacteroides thetaiotaomicron*.

In this article, we propose additional mathematical models which describe the aforementioned types of cross-feeding. The first is a modification of our previously published model (Van Wey et al., 2014; Van Wey et al., 2016) based on the work of Amaretti et al. (2007). Published time-series, co-culture data from experiments using fecal slurries often have a heterogeneous and unknown microbial population. The lack of *a priori* information about such slurries makes it difficult to predict the bacterial characteristics. Thus, an appropriate mathematical model should not be restricted to dealing with individual microbial species, but should be adaptable to represent a mixed population. Here we demonstrate that our previously published model can be applied to a mixed microbial population and account for a primary substrate, the accumulation of molecules from extracellular degradation of the primary substrate, utilization of the accumulated molecules by a mixed microbial population, and the subsequent production of metabolites from both substrates. Specifically, we use this model to describe the accumulation of peptides from casein degradation, as well as the production of metabolites and bacterial growth of a mixed bacterial population from the utilization of both the casein and accumulated peptides.

The second model was motivated by the work of Muñoz-Tamayo et al. (2011). Using Michaelis–Menten kinetics, we describe *Eubacterium hallii*'s utilization of both acetate and lactate, two metabolites produced by bifidobacteria in the largest quantities. Based on chemical kinetic analysis and a carbon balance approach we propose an alternative view of the metabolic pathway.

The third model describes the simultaneous use of saccharides and a single bacterial metabolite by *Roseburia intestinalis* and *Roseburia inulinivorans*. In this instance the use of either substrate depends on the presence of the other.

The fourth model describes the simultaneous degradation of either a metabolite and carbohydrate source or two metabolites by *Anaserostipes caccae*. This case differs from that of the *Roseburia* strains and *E. hallii*, because *A. caccae* can only utilize acetate in the presence of another substrate (lactate or carbohydrate); however, the presence of acetate is not required for the ability to degrade either lactate or the carbohydrate.

2. Materials & methods

2.1. Monoculture and co-culture data

Monoculture and co-culture experimental data were obtained from the literature using image capturing software. The specific experimental designs for the generation of data (bacteria growth, substrate degradation, and metabolite production) used in this work are detailed in the original literature (Duncan et al., 2004; Falony, 2009; Falony, Vlachou, Verbrugghe, & De Vuyst, 2006; Falony, Verschaeren et al., 2009; Macfarlane & Allison, 1986).

For consistency between data sets and to represent the yield of bacteria in meaningful units, the bacteria concentrations that were reported in optical density (OD) were converted to colony-forming units (CFU)/ml and then $\log_{10}$ transformed. The $\log_{10}$ transformation, rather than the bacterial population density, was used for convenience since much of the experimental data reported by Falony and colleagues was reported in $\log_{10}$(CFU/ml) rather than CFU/ml. We assumed that $\mathrm{CFU/ml} = 2\times10^8(\mathrm{OD}) + 4\times10^6$ or $\mathrm{CFU/ml} = 5\times10^8(\mathrm{OD})$ for OD_{600} and OD_{650}, respectively (Ericksen, Wu, Lu, & Lehrer, 2005; Kim, Chung, Lee, & Choi, 2012). There is no standard conversion from OD to CFU/ml because the conversion depends on the particular bacteria and conditions. OD can be affected by the accumulation of EPS and cannot distinguish between live or dead cells. For instance Myers, Curtis, and Curtis (2013) report values as high as 3.78×10^{10} ($\mathrm{CFU/OD}_{660}$) for *Rhodobacter sphaeroides* and as low as 1.61×10^7 ($\mathrm{CFU/OD}_{550}$) for *Chlorella vulgarus*. Thus, the equations used here are approximations. However, the estimated specific growth rate parameter value in the following equations is invariant to the conversion equation between OD and CFU because it is based on a species-specific fixed doubling time under ideal growth conditions. The yield is insensitive to the constant term in the linear conversion equation but is sensitive to a change in the leading coefficient. This is not necessarily problematic, because the "real" yield value, corresponding to a strain-specific OD to CFU/ml conversion equation, will be proportional to the estimated value in this work, with the proportionality constant being the ratio of the strain-specific leading coefficient to our assumed value of either 2×10^8 or 5×10^8 for OD_{600} and OD_{650}, respectively.

2.2. General mechanistic model for bacterial growth in monoculture

In general, bacteria growth from the fermentation of a single substrate with quantity S (measured in mM fructose equivalents (FE) or mM carbon, for example), in monoculture can be described using Monod kinetics,

$$\frac{dS}{dt} = -\frac{\mu}{Y_{X/S}}\left(\frac{S}{K_S+S}\right)X \tag{1a}$$

$$\frac{dB}{dt} = \frac{1}{\ln(10)}\left(\frac{\mu S}{K_S+S} - k_d\right) \tag{1b}$$

$$\frac{dP_i}{dt} = -k_i\frac{dS}{dt}, \tag{1c}$$

where t is time, μ (h^{-1}) is the specific growth rate of the bacteria, X (CFU/ml), $Y_{X/S}$ (CFU/ml (mM FE)$^{-1}$) is the yield, $B = \log_{10}X$ ($\log_{10}$(CFU/ml)), K_S (mM FE) is the Monod constant, k_d ($\log_{10}$(CFU/ml) h^{-1}) is the death rate of the bacterium, and the metabolic conversion constant, k_i (mM P_i (mM FE)$^{-1}$), varies depending on the metabolite, P_i, being produced. Given that many bacteria are unable to degrade long chain polysaccharides (De Vuyst & Leroy, 2011; Falony, Lazidou et al., 2009), we define the total substrate, $S_T(t) = S_U + S(t)$, to be the sum of undegradable, $S_U = (1-a)S_T(0)$, and degradable, $S(0) = aS_T(0)$, fractions, where $S_T(0)$ is the total initial quantity of substrate and a is the portion of the substrate that the bacterium is capable of degrading