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A case study: Using microbial abundance data to mathematically calculate organic acid production by human faecal microbiota within an in vitro batch fermentation

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ABSTRACT

The amount and ratio of organic acids from carbohydrate fermentation by the gut microbiota is dependent upon both the ability of members of the microbiota to exploit undigested dietary carbohydrate and the type of organic acids they have the capability to generate. Additionally, some bacteria feed upon carbohydrate degradation products, as well as organic acids produced by other bacteria, further affecting the overall organic acid profile. Experimentally unentangling these complex trophic food webs is challenging.

Here we demonstrate a mathematical model that calculates the organic acid profile resulting from an *in vitro* human faecal microbial fermentation of kiwifruit cell wall polysaccharides, parameterised with organic acid and microbial abundance data. The model was based on our hypothesis that in conditions of carbohydrate excess, changes in the abundance of members of the microbiota will be reflected in an equivalent change in those organic acids they have the capability to produce. We used microbial abundance data to calculate changes in organic acids over time, and the resulting data was shown to recapitulate the measured organic acid profiles. We also demonstrate here the successful mathematical simulation of the metabolic cross-feeding which occurs upon simulated digestion and fermentation of a fresh whole fruit carbohydrate. We assign the relative propensity of various members of a complex microbial community to generate selected acids within the organic acid profiles. We anticipate this case study to be a starting point for more sophisticated research and clinical tools to investigate and predict the interactions with food, the microbiota and the host.

1. Introduction

The human large bowel harbours a numerous, diverse and complex microbiota, which collectively outnumber the cells making up the rest of our bodies, and are regarded as an integral metabolic organ. Microbial metabolism contributes by a range of metabolic transformations and signalling mechanisms to the host epithelial cells, the local gut immune cells, and impacts upon systemic immunity and energy deposition and utilisation. Collectively, the microbiota and the human host comprise a superorganism (Lederberg, 2000). Common human conditions with substantial impact on morbidity or mortality such as obesity/metabolic syndrome (Ley, Turnbaugh, Klein & Gordon, 2006; Turnbaugh et al., 2006; Turnbaugh et al., 2009), atopic disease (Storro, Avershina, & Knut, 2013), inflammatory bowel disease and rheumatoid arthritis (Bornigen et al., 2013) have been associated with alterations, deficiencies or differences in the composition and activity of the microbiota through mechanisms that are only now beginning to be untangled.

Aside from endogenous (host) secretions, the predominant carbohydrate source for the gut microbiota is plant carbohydrates which escape digestion and survive largely intact to reach the large bowel. There are a range of carbohydrates which may be fermented by the microbiota, from plant storage oligosaccharides and polysaccharides such as (resistant) starch and inulin to plant cell wall pectins,

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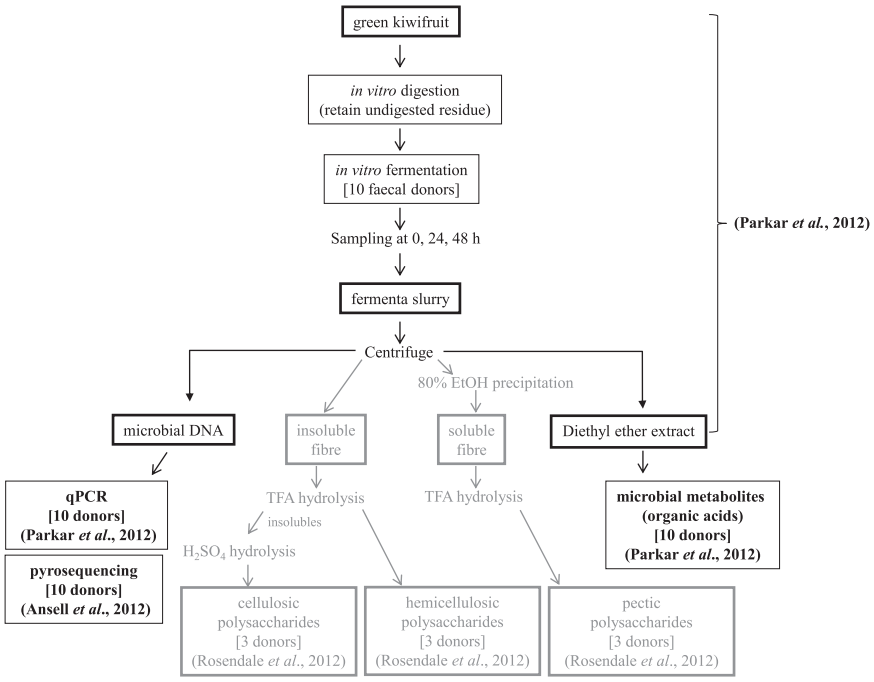

Fig. 1. Summary flow chart of experimental procedures (in black) used to generate data for this model. Bold font denotes data used for this model. Other elements (grey) included for completeness, but not used to generate data for this model.
Adapted from Rosendale et al. (2012).

hemicelluloses and cellulose. Here these carbohydrates are subject to a range of degradation and utilisation strategies by a complex food web of primary, secondary and subsequent feeders, employing a range of external, cell-associated and internal degradative enzymes, binding/recognition systems and transporters (Flint, Bayer, Rincon, Lamed, & White, 2008). Upon fermentation, microbial waste products are excreted, commonly as organic acids, methane, carbon dioxide and accompanying sulphurous and nitrogenous by-products (Louis, Scott, Duncan, & Flint, 2007; Macfarlane & Macfarlane, 2003). The major organic acid fermentation products are acetate, propionate and butyrate, although formate, lactate and succinate are also found. Some of these acids are in turn used by secondary feeders to result in formation of other acids, as summarised in Fig. 1.

Microbial organic acid by-products, particularly acetate, propionate and butyrate, are associated with a number of health benefits to the host. These have been summarised elsewhere (for example, Fava, Lovegrove, Tuohy, & Gibson, 2008; Rosendale, Cookson, Roy, & Vetharaniam, 2011) but include use as an energy source (brain, heart and muscle), increased defensin secretion; altered lipid metabolism (acetate can be used as a de novo lipogenesis substrate, whilst propionate inhibits HMG-CoA synthase and reductase and reduces acetate absorption and fatty acid synthesis resulting in decreased hepatic lipogenesis); and butyrate has immunomodulatory function and potential anticancer roles in inhibition of proliferation and induction of apoptosis. In addition, these acids plus lactate and succinate decrease gut pH, resulting in increased bile salt solubility, increased mineral absorption, decreased ammonia absorption and decreased pathogen growth.

Previously, we have examined these organic acids produced by the human microbiota. We used a simulated batch *in vitro* digestion and fermentation model, with pectin-, hemicellulose- and cellulose-containing green and gold kiwifruit as a carbohydrate source, incubated with faecal material from ten different donors, and we compared bacterial numbers by real-time quantitative PCR and microbial glycosidase activities against model substrates at three time points (0 h, 24 h and 48 h) (Parkar et al., 2012); with pyrosequence data (Ansell, Parkar, Paturi, Rosendale, & Blatchford, 2012); and performed a detailed pilot study with three of these donors to determine their precise green kiwifruit carbohydrate utilisation (Rosendale et al., 2012). There we found that kiwifruit cell wall carbohydrates were used to a different extent by the various donor microbiota, yet resulted in quite similar response in terms of microbial by-product formation and changes in abundance of certain members of the faecal microbiota, despite different starting ecologies. We concluded that consistent functional changes (organic acid production) were the most relevant assessment of gut health benefits of food. We have previously described the need for mathematical modelling of microbial organic acid production to guide and realise improvements in personalised nutrition and wellness (Rosendale et al., 2011).

The generation of microbial community data from 16S next-generation sequencing or chip arrays, often accompanied by some or all of these fermentation by-product data, are becoming commonplace. Now we are finding that we need to turn to sophisticated data collection technologies and data analyses in attempts to extract meaning or understanding from these vast bacterial catalogues. Increasingly, attempts are made to prove or correlate these data with such things as neuroendocrine function, immunomodulation, local and systemic energy deposition and utilisation, xenobiotic metabolism, regulation of toxicity response elements in the gut, liver and immune systems, gut morphology and barrier function, mood, and sleep (Hanage, 2014). Yet it could be argued that from the perspective of attaining and maintaining gut health a key factor is the production of fermentation by-products from the microbial fermentation of indigestible dietary carbohydrate (e.g. in terms of protecting from colon cancer see O'Keefe, 2008; O'Keefe at al., 2009; Ou, DeLany, Zhang, Sharma, & O'Keefe, 2012, 2013). It is the distillation of this complex system into simple outputs that we attempt here: "if these bacterial numbers change in response to fermentable carbohydrate, what will the acid profile look like? " We are trying to infer metabolic information from community profiling data. This knowledge would then complement the further studies looking at the host response to those acids. This model would also allow us to posit some hypotheses on the basis of the predicted role of certain members of the microbial community:

Here we describe a mathematical model that predicts the organic