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Factors affecting microbial metabolism in a human fecal fermentation model to evaluate prebiotics

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HIGHLIGHTS

- Multiple factors affect microbial fermentability in a human fecal batch culture.
- Inulin had a dose-dependent effect on microbial lactate and acetate generation.
- Rutin plus inulin increased propionate/butyrate ratio.
- Feces frozen at −80 °C provide a stable source of inoculum for batch cultures.

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ABSTRACT

In vitro fermentations using human fecal bacteria are an often used high through put strategy to evaluate the prebiotic potential of food ingredients. We investigated the influence of the substrate presented to fecal microbiota by monitoring the microbial short chain fatty acid production. We also examined the impact of cryopreserved glycerol stocks of feces on the performance of multiple batches of fermentations to examine the feasibility of using standardized inocula for multiple batches. Both composition and concentrations of bioactives (dietary fibre, polyphenols) were found to influence the metabolic capacity of human fecal microbiota. We also found that feces preserved at −80 °C provided a consistent and stable inoculum for performing batch fermentations for a duration of up to 3 years.

1. Introduction

Dietary fibre and polyphenols are key plant-based ingredients that enhance the growth of gut microbiota associated with improved human health [1]. Human intervention studies, while ideal for evaluating the prebiotic potential of functional food components [2], are expensive and time-consuming, considering the vast variety and different concentrations of foods that could potentially be tested. *In vitro* simulations of the human colonic milieu using human fecal microbiota provide a rapid first-pass screening of prebiotic foods and ingredients through analysis of the fermentation products [1,3,4]. However, gut microbiota even from the same individual, show temporal variation in composition in response to factors such as diet, antibiotic intake, body weight and health status [5]. Given the need for a fecal inoculum that retains microbial activity for multiple batches of experiments, we evaluated the impact of the frozen fecal aliquots on the consistency of microbial metabolism in different batches of fermentation. Further, given that plant foods have diverse quantitative and qualitative profiles of fibre and phytochemicals, we examined the effect of substrates that vary in fibre and phytochemical concentration and composition on the fecal microbial fermentative capacity.

2. Materials and methods

Feces were obtained from 5 consenting healthy volunteers (approved by Northern X Regional Ethics Committee, New Zealand, #NTX/08/11/112). The feces were processed to slurries within 30 min of voiding and stored as 20% (w/v) feces (in anaerobic glycerol buffered saline, pH 7.2) at −80 °C.

Fermentations were set up to include digesta obtained by the *in vitro*

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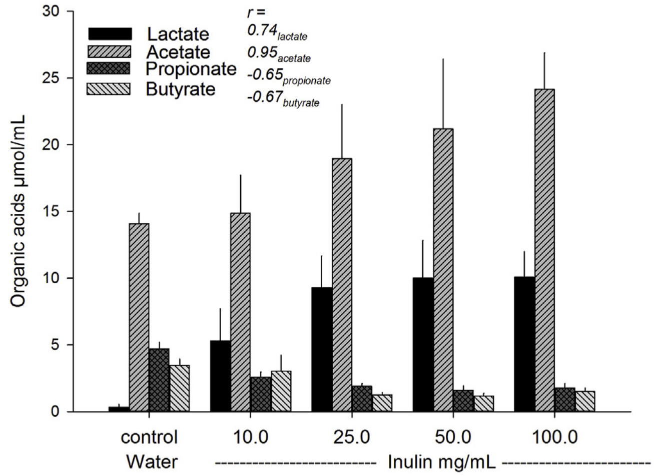

Fig. 1. Effect of inulin concentration on microbial acid production. Values are means ± SE, n = 5 fecal donors.

gastro-ileal digestion of test substrates, such as inulin or inulin with polyphenols at various concentrations, or water (as a control). A carbohydrate-free basal medium (CFBM) was used to study the effect of the substrates on microbial activity. Anaerobic fermentation was performed with 1% feces at 37 °C with shaking at 70 rpm. Aliquots of the fermenta were collected at 16 h and analysed for short chain fatty acids (SCFA) using high performance liquid chromatography as detailed previously [6].

The dose response effect of a pure prebiotic substrate was tested using inulin at concentrations of 10, 25, 50 and 100 mg/mL in CFBM. When testing the effect of polyphenols, quercetin (at 31.6 µg/mL) or its glycone, rutin (at 31.6, 100 or 316.0 µg/mL final concentrations) were added to the gastro-ileal digested inulin (10 mg/mL). The impact of the inoculum preservation at −80 °C was examined after performing fermentations with inulin (10 mg/mL) and water with different aliquots of the frozen slurries at multiple time points over 3 years. The protocols for fecal slurry preparation, gastrointestinal digestion and fermentation, SCFA analysis and statistics are detailed in supplementary file S1.

3. Results and discussion

Pearson's correlation co-efficient (*r*) indicated dose response relationships for inulin with lactate (0.74) and acetate (0.95), and inverse relationships with propionate (−0.65) and butyrate (−0.67) (Fig. 1). ANOVA testing of the difference between control and inulin fermenta and of the dose effect (within the four inulin concentrations) found higher lactate and acetate with the inulin than with the control ($P < 0.001$ and $P = 0.028$ respectively), and lower propionate and butyrate with inulin than with control ($P < 0.001$ and $P = 0.015$ respectively). As the concentration of inulin increased, an increase in lactate and acetate was observed, which is consistent with the effect of inulin in enhancing the growth of lactate-producing bifidobacteria and lactobacilli [7]. Acetate is produced in excess when the substrate is abundant, and propionate (and butyrate) are produced under conditions of carbon limitation, such as in the water control [8]. The propionogenic effect of pure rutin observed at concentrations as low as 10 µg/mL [6] has been shown to be reduced in the presence of background fibre in *ex vivo* fecal fermentations [9]. We observed a similar trend with no significant changes in SCFA concentrations in the presence of polyphenols, although the presence of rutin ≥100 µg/mL along with inulin (10 mg/mL) increased the propionate/butyrate ratio ($P < 0.05$, Fig. 2). Thus the concentration of the dietary fibre *and* the presence of polyphenols must be considered while evaluating the prebiotic potential of dietary components. Rutin is rapidly deglycosylated to quercetin by fecal microbiota including multiple species of the propionogenic *Bacteroides* [10]. Dietary supplementation with rutin-rich pectin and quercetin *in vivo* decreased the Firmicutes:Bacteroidetes ratio resulting in a gut microbial profile associated with metabolic control and body weight management [11,12].

Frozen aliquots of stabilised fecal inocula were used to carry out the fermentation of inulin at 10 mg/mL and control (water) in different experiments over 3 years. We analysed the SCFA profiles (Fig. 3) using

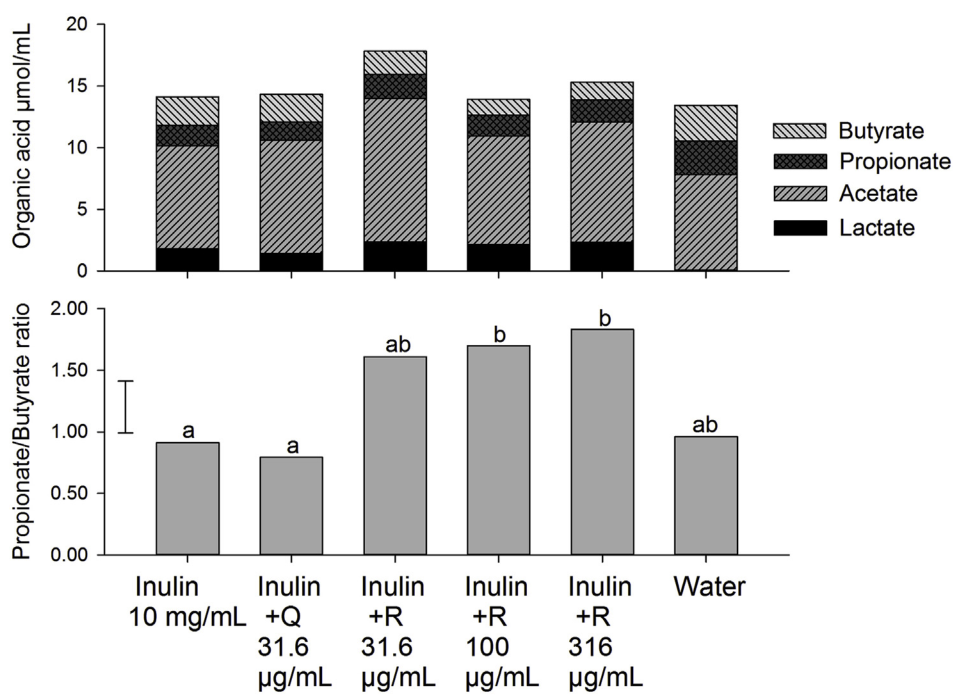

Fig. 2. Effect of co-incubation of inulin (10 mg/mL) with quercetin (Q) or rutin (R) on microbial acid production in fermenta inoculated with five fecal samples. *$P > 0.001$ compared to control. Letters after means indicate result of *post hoc* Tukey's tests; significantly different means ($P < 0.05$) are denoted by different letters.

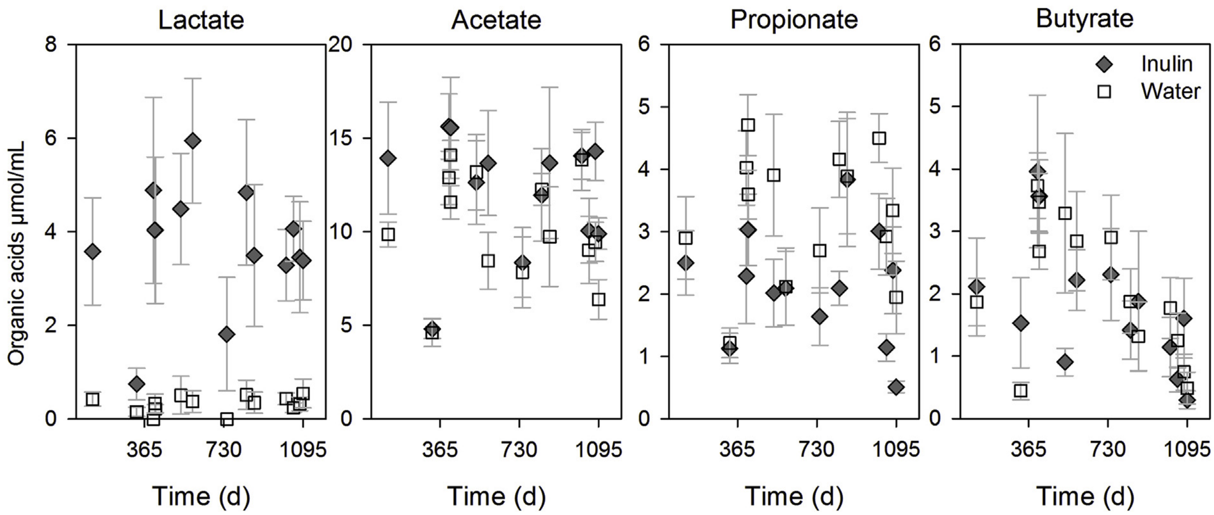

Fig. 3. Variation in microbial acid production over time. Substrates - inulin (10 mg/mL) and water control - were incubated with inocula obtained from frozen fecal slurries. Values are means ± SE, n = 5 fecal donors.

split plot ANOVA with experiments and substrates as factors, and donors-within-experiments as plots. Lactate, acetate and propionate showed significantly different responses to control and inulin across the experiments ($P < 0.001$). There was a significant experiment effect, with acetate, propionate and butyrate concentrations varying over time ($P = 0.005$, < 0.001 and < 0.001 respectively). However, the relative SCFA response to inulin and water remained consistent across experiments, with no significant experiment x substrate interactions. We propose that freezing provides a stable and consistent inoculum that, when used with appropriate controls, helps to normalise inter-experiment variations in terms of the microbial acid production for multiple batch fermentations. The use of frozen feces, when properly collected and preserved, eliminates the difficulties associated with fresh feces, including temporal and inter-individual variations in the microbiome [13].

The SCFA profile has been suggested to be a fairly rapid, robust and stable proxy for characterising the microbiota-modulating effects of dietary fibre, especially the metabolic activity of dominant bacterial taxa such as *Bacteroidaceae, Ruminococcaceae* and *Prevotellaceae* [14]. Given that different bacterial clusters generate these SCFAs, and the relative proportions of these acids were maintained over the 3 years, at least a partial, if not complete, preservation of the major bacterial consortia that generate these acids may be speculated. However, to confirm microbiome stability, 16S rRNA gene sequencing analysis would be required to interrogate the abundance of all the key bacteria within each sample.

In conclusion, appropriately processed and preserved fecal samples serve as a source of consistent and stable inocula for comparing batches of fermentations. This is the first longitudinal study, over a duration of 3 years, examining the metabolic activity of cryopreserved fecal microbiota. Further, the concentration of the dietary fibre and the presence of polyphenols in the substrate influence microbial metabolism. This information is particularly useful when evaluating the prebiotic potential of foods that vary in the composition and concentration of fibre and polyphenols - two major components that modulate gut microbiota.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnim.2018.12.003.

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