

Modulation of the human gut microbiota by prebiotics is pH dependent. By W.S.F. Chung¹, A.W. Walker¹, F. Farquharson¹, P. Louis¹, J. Vermeiren², D. Bosscher², S.H. Duncan¹ and H.J. Flint¹, ¹Rowett Institute of Nutrition & Health, University of Aberdeen, Aberdeen, UK and ²Cargill R&D Centre Europe, Vilvoorde, Belgium

Prebiotics are non-digestible food ingredients usually in the form of complex carbohydrates that can benefit the health of the host by stimulating or altering the growth of certain bacterial species in the gut¹. Prebiotic substrates of interest include apple pectin (Sigma) and inulin (Oliggo-Fiber DS2, Cargill inc., avDP<10). In addition to providing suitable growth substrates, prebiotics are likely to influence intestinal pH through the production of short chain fatty acids (SCFAs). We decided therefore to investigate how different physiological pHs may influence the effects of prebiotics upon the gut microbiota.

Continuous flow anaerobic fermentors were used to investigate which species within the faecal microbiota of healthy human volunteers were promoted by single polysaccharide substrates, apple pectin or inulin. As pH is a potentially important factor governing the competition between bacterial species², it was decided to shift the controlled fermentor pH to investigate microbial competition across a physiologically relevant pH range. Two fermentors were run in parallel and received an identical inoculum, but one was shifted in the sequence pH 5.5, 6.0, 6.4, 6.9 (Fermentor 1) and the other in the opposite sequence pH 6.9, 6.4, 6.0, 5.5 (Fermentor 2). For each substrate, separate experiments were conducted with faecal inocula from three different donors.

Changes in major bacterial groups were assessed by analysis of amplified 16S rRNA genes using qPCR. The results suggested selective stimulation of particular species by apple pectin across the pH range. *Lachnospiraceae* abundance decreased significantly as pH increased from 5.5 to 6.9 only with inulin (Figure 1B) whereas *Bacteroides* spp. proportion increased significantly as pH increased from 5.5 to 6.9 in both inulin (Figure 1A) and apple pectin fermentors (Figure 2A). This effect was observed in all three donors regardless of whether the fermentors were run with either the high or low pH at the start.

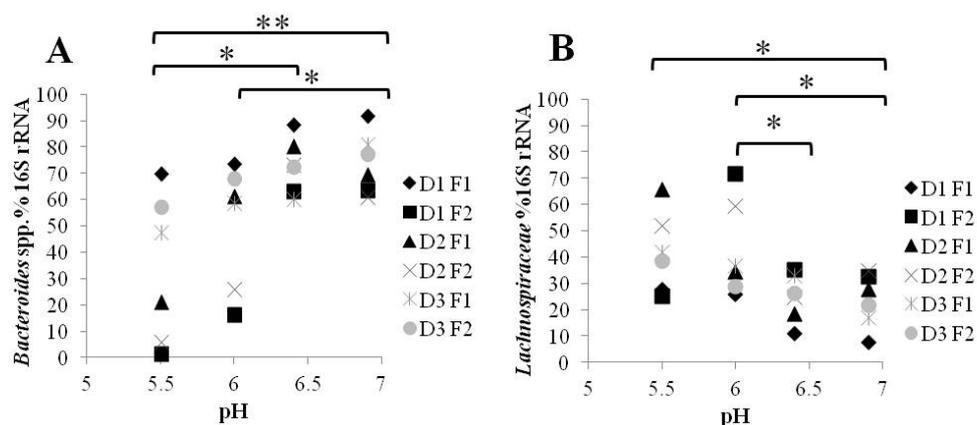


Fig 1. Effect of pH on colonic microbial community *Bacteroides* spp. (A) and *Lachnospiraceae* (B) with inulin (Oliggo-Fiber DS2) as energy source. D1, D2, D3 = Donor 1, 2, 3. F1= Fermentor 1 with pH shift up 5.5, 6.0, 6.4, 6.9. F2= Fermentor 2 with pH shift down 6.9, 6.4, 6.0, 5.5. *p<0.05, **p<0.005.

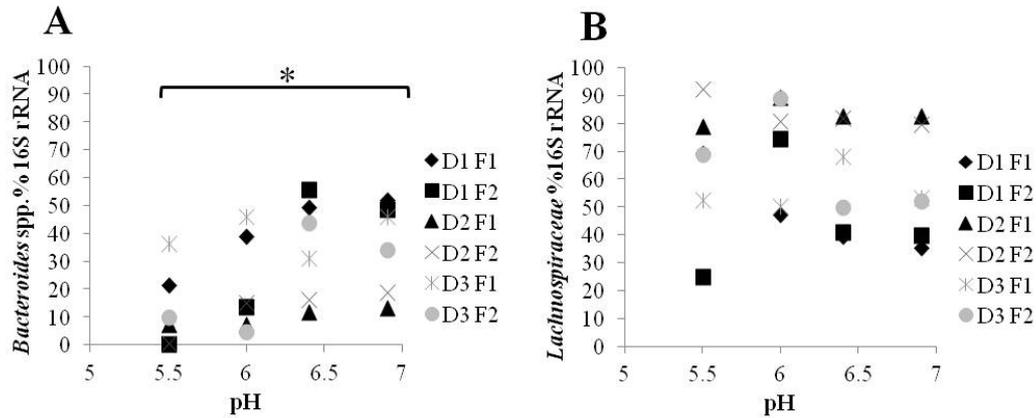


Fig 2. Effect of pH on colonic microbial community *Bacteroides* spp. (A) and *Lachnospiraceae* (B) with apple pectin (Sigma) as energy source. D1, D2, D3 = Donor 1, 2, 3. F1= Fermentor 1 with pH shift up 5.5, 6.0, 6.4, 6.9. F2= Fermentor 2 with pH shift down 6.9, 6.4, 6.0, 5.5. * $p < 0.05$.

SCFA concentrations were measured throughout the inulin and apple pectin fermentor experiments. The modulation of the microbial community correlated with the changes in the short chain fatty acid metabolite profiles, suggested by the proportion of *Bacteroides* spp. and *Prevotella* spp. present in the fermentor community (as determined by qPCR) and the percentage of propionate present at the same time point (Figure 3).

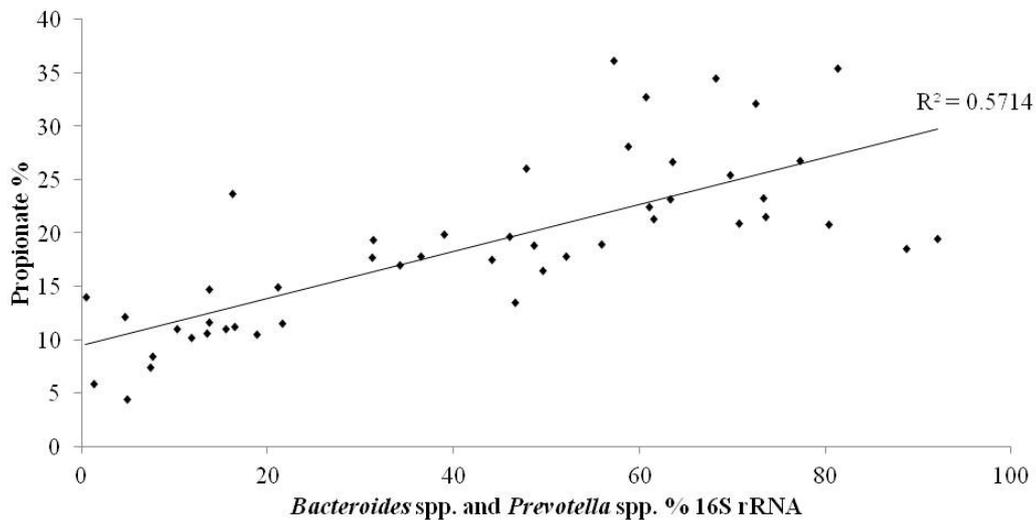


Fig 3. Propionate producers *Bacteroides* spp. and *Prevotella* spp. show a correlation between microbial metabolite production and microbial community population in apple pectin (Sigma) and inulin (Oligo-Fiber DS2) fermentors.

In conclusion, the effect of prebiotics on major groups within the human gut microbiota is pH dependent and microbial metabolite production correlates with the microbial community population present. In the future, the in vitro fermentor system will allow modelling of the microbial community with controlled pH and substrate input.

1. Macfarlane GT and Macfarlane S. Fermentation in the Human Large Intestine Its Physiologic Consequences and the Potential Contribution of Prebiotics. *J Clin Gastroenterol* 2011; 45: S120-S127.

2. Walker A, Duncan S, Leitch E, et al. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 2005; 71: 3692-3700.