SESSION 2: FOOD APPLICATIONS

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# DEVELOPMENT AND VALIDATION OF AN IN VITRO NEW MODEL FOR INTESTINAL FERMENTATION WITH IMMOBILIZED CELLS

A delicate balance exists between the human intestinal microflora and its host. The gut microbiota is part of a complex ecosystem having significant impact on human health, particularly for infants in whom perturbation of gut microbiota can lead to major intestinal disorders and infections (Gibson, 2004). It is therefore important that gut microflora interactions be controlled and sustained in an optimal manner. The large intestine is by far the most intensively populated microbial ecosystem with several hundred species accounting for a total of $10^{11}$–$10^{12}$ bacteria per gram of contents and a total of $10^{14}$ viable, but 30 to 40 species comprise 99% of the intestinal flora (Fooks et al., 1998). Each human has a unique bacterial community in their faeces. The colonic microflora can be divided into species that are either potentially harmful or beneficial towards host welfare. Lactobacilli and bifidobacteria have been for long time considered as beneficial to health (Gibson and Roberfroid, 1995) and different strategies have been investigated in order to increase their number and/or activity in the colon: ingestion of bacterial strains (probiotics); dietary adjuncts (prebiotics) which promote the growth of beneficial bacteria in the colon; synbiotics, a mixture of probiotics and prebiotics. Despite increasing research in this field, our understanding of the bacterial ecosystem in the human colon remains very limited. This knowledge is necessary for demonstrating the effect of probiotics, prebiotics and other biological compounds.

Ethical and accessibility problems limit in vivo studies of gut microbiota, especially in healthy infants. Various *in vitro* approaches such as batch culture, single or multi–stage chemostats have been used to study adult, and infant colonic microbiota to a lesser extent (De Boever et al., 2001; Gibson and Fuller, 2000; Gibson 2004). Compared with batch cultures, continuous culture models are particularly well suited for ecological studies. However, all models are based on free–cell suspension cultures. These models may present stability problems such as loss of less competitive bacteria during experimental trials, long stabilization periods over 3 weeks, wash–out due to short retention times. As a result, the testing of different parameters successively, i.e. with the same microbiota, is prevented. Moreover, these models poorly reproduce the colonic ecosystem, characterized by bacteria in the immobilized state, growing in intimate associations on the surface of food particles, or forming biofilms on the intestinal epithelium. When steady state is reached, the total bacterial concentration (<$10^{10}$ CFU/ml) in free–cell cultures is lower than that ($10^{10}$–$10^{11}$ CFU/g) observed in intestinal contents.

To solve these problems observed with current *in vitro* models, and to more closely mimic the intestinal conditions in which bacteria are in close association with particulate material, we recently proposed the use of cell immobilization and continuous fermentation (Cinquin et al., 2004). We hypothesized that cell immobilization could improve *in vitro* colonic fermentation models by better mimicking the colonic environment and increasing cell density and stability over long periods. We developed a 3–stage chemostat with immobilized cells to simulate simultaneously proximal, transverse and distal infant colon.

Immobilization of baby feces in 1–2 mm diameter gel beads was based on a dispersion process in a two–phase system using a mixed gel of gellan gum (2.5%) and xanthan gum (0.25%) and sodium citrate (0.2%). The entrapment process and gel composition were selected since conditions are mild and preserve the integrity of the sensitive intestinal bacteria, and the gel matrix has suitable mechanical properties for long–term stability during continuous cultures with immobilized intestinal microbiota (tested for up to 54 days). Moreover, gel beads were easily dissolved in EDTA for microbiological analyses, with no apparent effects on the viability of the different populations.

A schematic illustration of the continuous in vitro intestinal model with immobilized cells is shown in Fig. 1. After bead pre–colonization using three successive pH–controlled batch the beads were inoculated (30% v/v) in a reactor continuously fed with a medium used to simulate chyme produced by an infant diet (Cinquin et al., 2004). Two free–cell reactors were placed in series with the immobilized cell reactor and all three reactors were operated with retention time and pH chosen to

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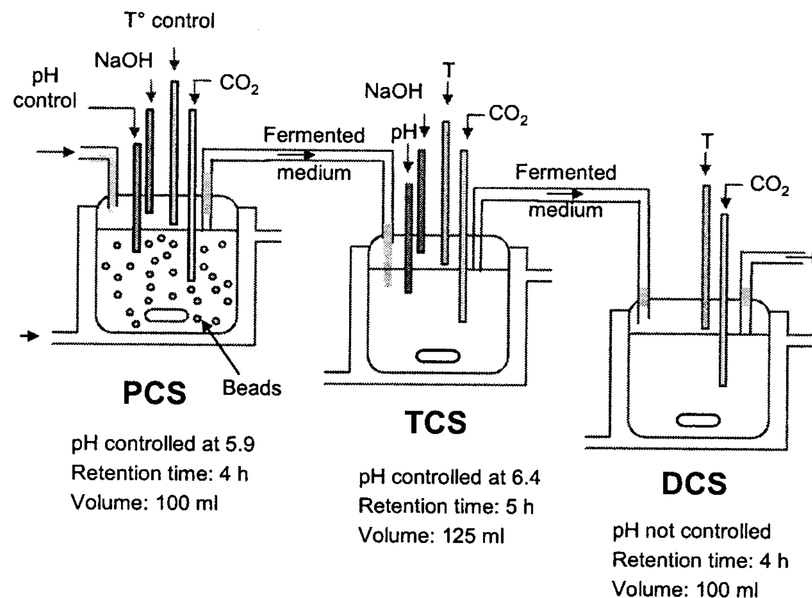


*Figure 1. Schematic representation of the continuous in vitro intestinal model with immobilized cells*

simulate proximal, transverse and distal colon conditions in infant. Bacterial composition and metabolic activity were monitored daily during two continuous cultures fed with a medium formulated to approximate the composition of infant chyme for two weeks and compared with in vivo data.

After an initial colonization–stabilization period of approximately 10 days, cell counts in beads were high and very close to that in the feces inoculum. The proportions of six bacterial marker groups measured by plating on selective media and fluorescence *in situ* hybridization (FISH) in beads and effluent medium, and the metabolic activities measured in effluent samples were similar to that in fecal inoculum used for immobilization and infant *in vivo* data. Our results showed that bacterial immobilization and continuous in vitro colonic fermentation can be used to accurately simulate intestinal fermentation over long time period, with preservation of main gut populations and activities and high stability.

We also used this *in vitro* model to test the effects of to compare the effects of purified exopolysaccharides (EPS) from *Lactobacillus rhamnosus* RW–9595M to a well–known prebiotic (fructo–oligosaccharide; FOS) on bacterial populations and activities. In agreement with literature data, FOS increased both bifidobacteria and lactobacilli, and decreased clostridia concentrations. On the other hand, EPS supplementation was deprived of any prebiotic effect in the infant colonic model.

To our knowledge, this work is the first reported study on the immobilization of complex fecal microbiota. The main advantage of bacterial immobilization is stability over long experimental trials, due to the continuous inoculation of the medium by shedding of free–cells from highly colonized beads retained in the reactor. This ability allowed the system to rapidly restore previous equilibrium. Compared with fermentation models using free–cells, the use of immobilized cells for modeling colonic fermentation presented other distinct advantages, including high cell density and the possibility of using precolonized beads with the same microbiota for testing different parameters in the same or in several experiments. Moreover, since it is known that bacterial metabolism is modified in biofilms, immobilization of the microbiota might then improve the colon modelization, by modifying bacterial physiology.

The *in vitro* intestinal model will be a useful tool for first step demonstration of the effects and mechanisms of action of a variety of food and pharmaceutical compounds on the intestinal microbiota, before carrying out more complex and very expensive experimentation with animal models and human. It may have important applications for testing probiotic bacteria with specific activities, prebiotics, antibiotic drugs or treatments for restoration of unbalanced intestinal flora.

**BIBLIOGRAPHY**

[1] Cinquin, C. et al. (2004) Sucessful immobilization of infant fecal flora and utilization in an *in vitro* colonic fermentation system. Microbial Ecology (in press, DOI: 10.1007/s00248–003–2022–7).

[2] De Boever et al. (2001) Development of a six–stage culture system for simulating the gastrointestinal microbiota of weaned infants. Microbial Ecology and Health Diseases 13:111–123

[3] Fooks et al. (1998) Prebiotics, probiotics and human gut microbiology. International Dairy Journal 9: 53–61.

[4] Gibson (2004). Gut reaction. Nature 424: 284–287.

[5] Gibson and Fuller (2000). Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. Journal of Nutrition 130: 391S–5S.

[6] Gibson and Roberfroid (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125: 1401–12.