IMPROVED IN VITRO GAS FERMENTATION METHOD TO ASSESS PREBIOTIC POTENTIAL OF INDIGESTIBLE CARBOHYDRATES

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Introduction and Objectives: Prebiotic activities have been ascribed to carbohydrates inducing by fermentation specific changes in the gastrointestinal microflora that confers benefits upon host well-being and health. Among these changes, an increase in *Lactobacillus sp.* is of importance as it is considered as beneficial for the host. Screening the prebiotic potential of novel indigestible carbohydrates is a challenge and in vitro models are increasingly used for such purposes. However previous works indicate a low development of *Lactobacilli* in such models. As recently extracellular binding proteins responsible for the adherence to intestinal mucus were described for several species belonging to this group, we added mucus in the model and evaluated the evolution of the Lactobacillus genus in the bottles during the fermentation.

Materials and Methods: Mucin-covered microcosms were prepared as described by Van den Abbeele et al. (2012, Microbial Biotechnology, 5, 106-115), and introduced in the gas fermentation bottles with an inoculum prepared from fresh faeces from 3 sows mixed with a nutritive buffer solution. Fermentation was performed at 39°C, using 200 mg of inulin and cellulose as substrates, 30 ml of inoculum and 0 or 6 mucin-covered microcosms, yielding approx. 20 mg mucin each, in 140 ml glass bottles. After 8, 24 and 72h, microcosms were collected in 3 bottles per treatment to perform *Lactobacillus* numeration on MRS plates and the fermentation broth was centrifuged (13,000 x g, 5 min). The broth from 3 bottles of each treatment without microcosms was also centrifuged. The pellet was used for DNA extraction and quantification by real-time PCR.

Results and Discussion: *Lactobacillus* grew fast on the microcosms, with a population reaching 7 log cfu/100 mg of mucin after 8h of fermentation with both substrates as well as in the control bottles without substrate, showing the role played by mucin on the development of this group. With inulin, the evolution over the time of the *Lactobacillus* population in presence of microcosms was consistent with the fermentation kinetics, the *Lactobacillus* population increased with the fermentation (from 7.09 at 0h to 8.15 log cfu/ml after 8h, $P < 0.001$) and decreased ($P < 0.05$) after 8h when the substrate was depleted. In the bottles without mucin *Lactobacillus* population grew less, remained stable ($P>0.05$) until 24h and then decreased. With cellulose, no increase of *Lactobacillus* population was observed during the fermentation even with the microcosms. These results indicate a better development of *Lactobacillus* in the bottles containing the mucin only when the substrate shows prebiotic properties. We may conclude that the addition of mucin-covered microcosms in the *in vitro* gas fermentation method will allow a better assessment of the prebiotic potential of new indigestible carbohydrates.