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The ability of probiotic bacteria to bind to human intestinal mucus

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Abstract

Human mucus was isolated from faecal samples of newborns, two and six month old infants and adults. The adhesion to this mucus by the bacteria mentioned below was assessed in vitro. Depending on the age group: 44–46% of the applied *Lactobacillus* GG, 23–30% of *Bifidobacterium lactis* Bb-12, 9–14% of *Lactobacillus johnsonii* LJ-1, 3–10% of *Lactobacillus salivarius* LM2-118, *Lactobacillus crispatus* M247, *Lactobacillus paracasei* F19 and 2% of *L. crispatus* Mu5 adhered. All the strains adhered better to the mucus of adults than to that of infants. With some of the strains significant differences between the infant age groups were also observed. In conclusion, the age of the target group may be worthy of consideration when planning a schedule for probiotic or functional food therapy.

Keywords: Adhesion; Age effect; Intestinal mucus; *Bifidobacterium*; *Lactobacillus*; Probiotic bacteria

1. Introduction

Probiotics are microbial food supplements, which beneficially affect the host's health. They are used to treat disturbed intestinal microflora and increased gut permeability which commonly occur in children with acute rotavirus diarrhoea, people with food allergies or colonic disorders and in patients undergoing pelvic radiotherapy [1]. One of the main criteria for selecting probiotic strains is their ability to adhere to intestinal surfaces. Attachment to mucosa prolongs the time probiotics can influence the gastrointestinal immune system and microbiota of the host. Thus the ability to adhere to intestinal surfaces is thought to correspond to the efficacy of the probiotic strain. The antibody titres detected from the serum of people treated with probiotic bacteria has been shown to be directly correlated with the adherence ability of the used strain [2]. Bacterial adhesion is initially based on non-specific physical interactions between two surfaces, which then enable specific interactions between adhesins (usually proteins) and complementary receptors [3]. Studying bacterial adhesion in vivo is difficult and in vitro models with intestinal cell lines are widely adapted methods for this assessment [4].

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The mucus covering the epithelial cells is the initial surface that ingested micro-organisms confront in the human gut and is considered an important site for bacterial adhesion and colonisation [5]. Mucus is continually subjected to degradation, conversely new mucin glycoproteins (the major components of mucus) are constantly secreted. Thus, bacteria able to adhere to mucus but unable to reach the epithelial cells might be dislodged from the mucosal surface with the degraded mucin and washed away with the luminal contents. This may partly explain the transient pattern of colonisation characteristic for most probiotic bacteria. On the basis of these remarks, an in vitro evaluation of the bacterial adhesion to human intestinal mucus provides a good additional model for studying the ability of probiotics to adhere to intestinal surfaces.

It has been shown previously that the composition and degradation of intestinal mucin is altered during postnatal development and in response to changes in diet [6,7]. In many animal studies, the number of receptors available for some pathogens, in intestinal mucus and enterocytes, have been shown to increase postnatally [8–11]. In addition, the intestinal *Lactobacillus* population has been shown to change during infancy [5,12]. These data suggest that the ability of probiotic bacteria to adhere and colonise the intestinal mucosa, and therefore their efficacy to balance the endogenous microflora and to modulate the gastrointestinal immune system, may be dependent on the age of the host under probiotic therapy.

In the present study, we examined a number of probiotic strains for their ability to adhere to intestinal mucus isolated from human faeces. The objective was to evaluate whether the age of the subject (especially at the important stages of intestinal maturation such as the beginning and the end of weaning) is a determinant influencing the action of probiotic bacteria and thus should be considered when selecting the strain and dose for most effective probiotic treatment.

2. Materials and methods

2.1. Micro-organisms and culture conditions

All the bacterial strains used in the study were prepared by Christian Hansen Ltd. (Denmark) and are included in the Probdemo project (FAIR CT96-1028) of the European Union. For the Probdemo project, the strains were selected mainly on the basis of their technological and in vitro putative probiotic properties as shown in individual scientific studies [13]. The strains and their original suppliers (in brackets) were as follows: *Bifidobacterium lactis* Bb-12 (Chr. Hansen Ltd), *Lactobacillus crispatus* M247 (Prof. Lorenzo Morelli, U.C.S.C., Italy), *L. crispatus* Mu5 (Morelli), *Lactobacillus* GG (*L. rhamnosus* ATCC 53103) (Valio Ltd, Finland), *L. johnsonii* LJ-1 (Nestec, Switzerland), *L. paracasei* F19 (Arla, Sweden) and *L. salivarius* LM2-118 (University College Cork, Ireland). *Bb. lactis* was cultured for 2 days and all the lactobacilli for 18–22 h at 37°C in de Man, Rogosa and Sharpe broth (MRS; Merck, Germany) containing 10 μl ml^{-1} of tritiated thymidine (5′-^{3}H, 117 Ci $mmol^{-1}$; Amersham International, UK) for radiolabelling. After cultivation, bacteria were harvested by centrifugation ($2000 \times g$ for 10 min), washed three times and resuspended in Hanks's balanced salt solution HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-2-[ethane sulfonic acid]) (HH; 10 mM HEPES; pH 7.4). The concentration of each bacterial suspension was adjusted to correspond to an absorbance of 0.25 ± 0.01 at 600 nm.

2.2. Human intestinal mucus

Mucus was isolated from the faeces by extraction and dual ethanol precipitation according to the method of Miller and Hoskins [14]. Faecal samples were collected from healthy newborns ($n = 28$), 2 month and 6 month old infants ($n = 11$ and 17, respectively) and adults ($n = 14$; age: 25–52 years). Equal amounts of mucus from individuals in each group were pooled and dissolved in HH at a concentration of 0.5 mg ml^{-1}. Any particulate material was removed from the suspension by centrifugation ($2000 \times g$ for 10 min).

2.3. The in vitro adhesion assay

The quantitation of the bacterial adhesion to the intestinal mucus was determined according to a procedure based on that described by Cohen and Laux

[15]. First, a solid phase mucus layer was prepared by incubating 100 μl of the clarified mucus suspension for 15–20 h at 4°C on polystyrene microtitre plate wells (Maxisorp; Nunc, Denmark). To remove unbound mucus components, the wells were washed twice with 200 μl of HH. The immobilised mucus was then covered with 100 μl of radioactively labelled bacteria, the plates incubated at 37°C for 1 h and the wells washed twice with 200 μl of HH to remove any unbound bacteria. To release and lyse the adhered bacteria, 250 μl of 1% SDS-0.1 M NaOH was added to each well and the plates were incubated at 60°C for 1 h. The lysate was removed from the wells, mixed with scintillation liquid (OptiPhase 'HiSafe 3'; Wallac, UK) and finally the radioactivity was measured by liquid scintillation counting. The proportion of adhered bacteria was assessed as the percentage of radioactivity recovered from the wells as compared to 100 μl of the radiolabelled bacterial suspension.

2.4. Statistical analysis

The results are presented as averages from four independent experiments, which were performed in triplicate. Two-factor analysis of variance was used to evaluate the statistical significance of the differences among tested strains and different mucus isolates. The paired *t*-test was then used in order to evaluate the statistical significance of the differences between two strains or two mucus preparations (i.e. age groups).

3. Results

3.1. Age dependency of the adhesion ability of probiotics to faecal mucus

All the bacterial strains studied tended to adhere in higher numbers to the mucus of adults than to that of infants (by 0.3–6.5%) (Table 1). With the strains *L. johnsonii*, *L. paracasei* and *L. salivarius* this difference reached statistical significance with all infant age groups. Within the infant age groups, the effect of age seemed strain specific: *L. johnsonii* and *L. salivarius* adhered significantly better to the mucus of 6 than 2 month old infants (by 1.2–2.4%), while the opposite was observed with *Lactobacillus* GG. In addition, *L. salivarius* adhered significantly better to newborn than to 2 month old infant mucus (by 2.1%).

3.2. Strain dependency of the adhesion properties

The ability to bind to intestinal mucus varied significantly between the different strains. Depending on the mucus type: 44.1–46.0% of *Lactobacillus* GG, 23.2–29.9% of *Bb. lactis*, 9.4–14.4% of *L. johnsonii*, 2.5–9.7% of *L. salivarius*, *L. crispatus* M247, *L. paracasei* and 1.5–2.1% of the applied *L. crispatus* Mu5 adhered. The spontaneous mutant of *L. crispatus* M247, strain *L. crispatus* Mu5, adhered in significantly fewer numbers ($P < 0.037$) to all the mucus isolates than any of the other strains. Conversely, *Lactobacillus* GG and *Bb. lactis* adhered significantly better to any infant mucus than the rest of the strains ($P < 0.048$).

Table 1
Adhesion of the radioactively labelled bacterial strains to the mucus of newborns, two month old infants, six month old infants and adults

Bacterial strain/Age group	Adhesion % (standard deviation)			
	Newborns	Two month olds	Six month olds	Adults
Bifidobacterium lactis Bb-12	26.3 (5.1)	23.2 (8.9)	26.9 (6.0)	29.8 (13.0)
Lactobacillus crispatus Mu5	1.8 (0.4)	1.5 (0.5)[d]	1.7 (0.7)[d]	2.1 (0.5)[b,c]
Lactobacillus crispatus M247	6.2 (2.2)[d]	6.7 (2.1)[d]	7.2 (2.3)	10.4 (4.0)[a,b]
Lactobacillus GG	45.3 (5.5)	45.7 (5.9)[c]	44.1 (7.2)[b]	46.0 (3.8)
Lactobacillus johnsonii LJ-1	9.6 (3.6)[d]	9.4 (2.5)[c,d]	11.8 (2.9)[b,d]	14.4 (5.4)[a,b,c]
Lactobacillus paracasei F19	6.6 (2.1)[d]	6.0 (2.5)[d]	6.2 (2.2)[d]	9.7 (4.4)[a,b,c]
Lactobacillus salivarius LM2-118	4.6 (1.5)[b,d]	2.5 (0.7)[a,c,d]	3.7 0.7)[b,d]	7.7 (3.4)[a,b,c]

[a,b,c,d]Significantly different ($P < 0.05$) to adhesion to mucus from [a]newborns, [b]two month old infants, [c]six month old infants, [d]adults.

4. Discussion

In this study, *Lactobacillus* GG and *Bb. lactis* adhered in greater numbers to human intestinal mucus than the other strains tested. The ability of *Lactobacillus* GG to adhere in high numbers to intestinal surfaces is supported by the studies of Elo et al. (1991) [16] and Lehto and Salminen (1997) [4] in which *Lactobacillus* GG was shown to adhere well to Caco-2 cells. Conversely, in the study by Schiffrin and co-workers [17] *Bb. lactis* was shown to have weak adhering abilities in in vitro systems, but *L. johnsonii* adhered well to the Caco-2 cell line. In our study *Bb. lactis* was shown to adhere in higher numbers to the intestinal mucus than *L. johnsonii*, in all age groups. These results suggest that the receptors for bacterial adhesins on mucus may be different, or present in different numbers than on enterocytes. It is possible that some strains adhering well to intestinal cells are valuable in treating hosts with a damaged mucus layer allowing adhesion to the epithelial cells. In nutritional or therapeutical perspective these strains may not be as suitable for healthy subjects or for patients with extensive mucus secretion (can be caused by many enteric infections and overgrowth of the endogenous bacteria in contaminated small bowel syndrome) in which cases the mucus layer may prevent the bacteria reaching the epithelia. In the future, it may be possible to plan probiotic treatments, in which the microflora balancing effects are targeted individually for bacterial populations in the mucus or in the epithelial cells. However, more information on these different subpopulations and their interaction with probiotic bacteria in vivo is still needed.

The strain *L. crispatus* Mu5 is a spontaneous non-hydrophobic mutant of *L. crispatus* M247, which shares the same fermentation pattern, drug resistance and ribotyping, but lacks the autoaggregative pattern of growth, characteristic of the wild-type. In our study, *L. crispatus* M247 showed a moderate ability to adhere to human intestinal mucus, whereas *L. crispatus* Mu5 can be considered non-adhering. This is in agreement with the previous finding by Morelli et al. 1997 [18] that *L. crispatus* Mu5, unlike its parental strain, does not adhere in vitro to immobilised human intestinal glycoproteins and to their suggestion that the aggregation phenotype promotes colonisation.

The age related differences noted in this study were small but reproducible. All the strains tested adhered better to the mucus of adult than of infant origin. This may be due to the intestinal environment of infants being immature and providing less sites for adherence. Our finding is in agreement with a recent study by Bolte and coworkers 1998 [19] who showed that in comparison to newborns, the brush border membranes of adult rats demonstrated higher food protein binding capacities. We observed age related differences in the bacterial adherence also within the infant age groups but no general trend was found. The more the mucus composition differs between two mucus preparations the more variation is likely to occur between the ability of bacteria to adhere to these two isolates. In the present study, the mucus degradation was not characterised but the results of the adhesion tests seem to correlate well with previously reported age related changes in mucus degradation. Norin and coworkers [20] showed that a significant breakdown of mucin starts only during the second year of life but Midvedt et al. [7] reported a positive correlation between increasing age and increased degradation of mucin also between birth and 1 month and between 6 and 9 months. An important factor influencing the mucus composition is the endogenous microflora and thus its establishment is of interest when studying the bacterial adhesion to intestinal mucus. The mucus of newborns has not been challenged by bacteria, but during the first week of life, bacterial strains known to be able to produce mucin oligosaccharide degrading enzymes, *Bacteroides* and *Bifidobacteria* in particular, reach the immature intestine in proportionally high populations [5]. Relatively, however, the bacterial array that confronts the intestinal mucus during the first two months of life is small. At the time of weaning and introduction of solid foods, a 6 month old child is challenged with a myriad of new bacterial agents. At this point, of the mucus degrading strains, *Bacteroides* and *Clostridia* can be detected from the faeces [5]. Great differences in the ability of probiotic bacteria to adhere to the intestinal mucus are expectable between 6 month old infants and adults because in comparison to 6 month old infants adults have

more mature microflora and full scale mucin degradation.

In conclusion, with the strains included in this study the age related differences in the adhesion to intestinal mucus were small. However, the age effect seemed to be dependent on the bacterial strain and thus with some probiotic strains it is worth considering whether the age of the target group should be taken into account when planning a schedule for probiotic or functional food therapy. For future studies it is important to characterise strain specific adhesion factors and to understand the relation between intestinal integrity, mucus adhesion and the development of the intestinal barrier.

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