Disease-Dependent Adhesion of Lactic Acid Bacteria to the Human Intestinal Mucosa

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Their adhesion to the intestinal mucosa is considered one of the main reasons for the beneficial health effects of specific lactic acid bacteria (LAB). However, the influence of disease on the mucosal adhesion is largely unknown. Adhesion of selected LAB to resected colonic tissue and mucus was determined in patients with three major intestinal diseases (i.e., diverticulitis, rectal carcinoma, and inflammatory bowel disease) and compared to healthy control tissue. All strains were observed to adhere better to immobile mucus than to whole intestinal tissue. Two strains (Lactobacillus rhamnosus strain GG and L. reuteri) were found to exhibit disease-specific adhesion to intestinal tissue. All tested strains, with the exception of L. rhamnosus strain GG, displayed disease-specific adhesion to intestinal mucus. These results suggest that strains with optimal binding characteristics for a particular intestinal disease can be selected.

Specific lactic acid bacteria (LAB) and bifidobacteria have been scientifically shown to be useful for balancing the intestinal microflora and for the management of microflora dysfunctions (20). For many of the health effects, adhesion to the intestinal mucosa is considered important, i.e., antagonism against pathogens (3), transient colonization (1, 15), modulation of the immune system (32), and enhanced healing of damaged intestinal mucosa (4). Adhesion to the intestinal mucosa is therefore considered one of the main properties of the beneficial health effects of selected LAB (13).

Fecal levels of lactobacilli and bifidobacteria have been shown to be reduced in patients with inflammatory bowel diseases (IBD) such as Crohn’s disease (CD) and ulcerative colitis (UC) (6, 8, 12) as well as in allergy (16), thus suggesting a role for these bacteria in the prevention of these diseases. For a number of gastrointestinal diseases, specific LAB strains have been used successfully. The duration of rotavirus diarrhea has been shown to be reduced by selected lactobacilli (10, 33). Some, but not all, epidemiological studies suggest that the consumption of milk products fermented with LAB may have some protective effect against changes associated with the occurrence of colon cancer (29). Consumption of individual strains of LAB may promote positive changes in humans during CD (17), juvenile chronic arthritis (18), irritable bowel syndrome (22), UC (34), diverticulitis (7), and chronic pouchitis (9). Disturbances in the composition of the intestinal microflora during these diseases can be counteracted with selected LAB (5, 8, 31). As stated above, adhesion to the intestinal mucosa is considered an important component of the beneficial health effects of selected LAB. However, the influence of the disease on the adhesion of LAB is largely unknown.

There are a few reports that indicate that the specificities of different LAB may prove to be different in the gut mucosa of patients suffering from different intestinal diseases (1, 19), and these properties can be related to the adhesive capacity of the strains in question. Therefore, we assessed the ability of probiotic and proposed-probiotic LAB to adhere to human intestinal mucosa or intestinal mucus from patients with IBD (CD and UC), diverticulitis, or rectal carcinoma, and we compared these with LAB adhesion to healthy tissue. With this method, disease- and strain-specific differences in the adhesion properties can be identified and will provide new methods for selecting probiotics for specific intestinal dysfunctions.

MATERIALS AND METHODS

Bacteria. Six LAB strains were used in the present study: Lactobacillus rhamnosus GG (ATCC 53103), L. rhamnosus LC 705 (Valio Ltd., Helsinki, Finland), L. rhamnosus E-800 (VTT, Espoo, Finland), L. reuteri (University of Helsinki, Helsinki, Finland), L. reuteri ING1 (INGman, Söderkulla, Finland), and Bifidobacterium lactis Bb12 (Chr. Hansen, Harsholm, Denmark). The bacteria were grown from stocks stored at −75°C in 40% glycerol (1% inoculum). The lactobacilli were grown in a de Man, Rogosa, and Sharp broth mixture (Merck, Darmstadt, Germany), while L. reuteri strain Bb12 was grown in Griffin Anaerobic Medium (Nissui Seiyaku Co., Tokyo, Japan). Ten microliters of tritiated thymidine ([methyl-1,2-3H]thymidine, 120 Ci/mmol) per milliliter was added to the medium to metabolically radio label the bacteria. After overnight growth (16 h) under anaerobic conditions, the bacteria were harvested by centrifugation (2,000 × g), washed twice with phosphate-buffered saline (PBS [pH 7.2]; 10 mM phosphate) and resuspended in PBS. The absorbance (at 600 nm) was adjusted to 0.25 ± 0.02 in order to standardize the number of bacteria in the suspension (107 to 108 CFU/ml) and to avoid saturation of the substrata in the adhesion assay (see below).

Tissue and mucus preparation. The joint ethics committee of the University of Turku and Turku University Central Hospital approved the use of resected human intestinal material. Tissue samples were obtained from five diverticulitis patients (from the area exhibiting active infection), five patients with rectal carcinoma (from areas adjacent to the tumor), and six patients with IBD (four with CD and two with UC, from areas exhibiting active inflammation). Control samples were obtained from four patients who had undergone operations for reasons other than inflammation or malignancy. The patient data are presented in Table 1. The intestinal material was processed as described previously (25, 26). In short, after resection the contents were removed and the tissues were gently washed in PBS containing 0.01% gelatin. From the tissue, 9-mm circular pieces (i.e., 64 mm2) were cut out. The pieces were stored at −70°C in PBS with 40%
Bound bacteria were released and lysed with 1% sodium dodecyl sulfate in PBS, and the tissue pieces were fixed with 3% glutaraldehyde in PBS for 45 min at 4°C and 1 h at 37°C. Nonbound bacteria were removed by washing with 0.5 mg of protein per ml. Radiolabeled bacteria were added to the wells and the adhesion was expressed as the percentage of radioactivity recovered after adhesion relative to the radioactivity in the bacterial suspensions added to the immobilized mucus.

**Adhesion assay.** The adhesion of the radioactively labeled bacteria to immobilized colonic mucus was determined as described previously (23, 24). In short, mucus from part of the tissue was collected into a small amount of 70% perchloric acid in glass scintillation vials at temperatures ranging from −20°C to −70°C until use. Freezing and thawing of the material was not found to influence the adhesion (26).

Mucus from part of the tissue was collected into a small amount of N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES)-Hanks buffer (pH 7.4; 10 mM HEPES) by gently scraping the mucosa with a rubber spatula. The collected mucus was centrifuged for 10 min at 13,000 × g to remove cell debris and bacteria. The mucus was stored at −70°C until use.

**RESULTS**

The adhesion to the colonic tissue pieces of the tested strains was found to range from 1.4% for *L. rhamnosus* LC 705 to diverticulitis tissue to 9.7% for *L. rhamnosus* GG to tissue from patients with IBD (Table 2). *L. rhamnosus* GG was found to adhere significantly better to the intestinal tissue in general than all other tested strains (*P < 0.05*), while *L. rhamnosus* LC 705 was found to adhere significantly less to the intestinal tissue than all other tested strains (*P < 0.05*). The other tested strains did not differ in their ability to adhere to the intestinal tissue. *L. rhamnosus* GG adhered significantly less (*P < 0.05*) to control tissue (4.9%) and diverticulitis tissue (3.3%) than to IBD tissue (9.7%) and rectal carcinoma tissue (6.5%). *L. reuteri* ING1 bound significantly better (*P < 0.05*) to tissue from IBD (8.7%) than to tissue from control samples (3.7%) and patients with rectal carcinoma (5.3%) and diverticulitis (2.9%). There was a trend for *L. rhamnosus* E-800 to also bind better to IBD tissue (8.9%) than to rectal carcinoma tissue (4.2%). However, this did not reach statistical significance (*P = 0.08*). No differences were observed in the adhesion to tissue from patients with UC or CD; these observations were therefore grouped as IBD.

The tested strains exhibited a much wider range of adhesion abilities to the immobilized mucus, ranging from 1.9% for *L. rhamnosus* LC 705 to control tissue to 26.3% for *L. rhamnosus* GG to diverticulitis tissue (Table 3). Here too, *L. rhamnosus* GG was observed to adhere significantly better than all other tested strains (*P < 0.05*), and *L. rhamnosus* LC 705 was found to adhere significantly less (*P < 0.01*). *L. rhamnosus* LC 705 was observed to adhere significantly better to mucus from patients with diverticulitis (3.0%; *P = 0.0339*) and rectal carcinoma (3.2%; *P = 0.0339*) than to mucus from control tissue (1.9%) and IBD tissue (2.2%). Also *L. reuteri* ING1 was found to adhere better to mucus from the diseased tissues than to

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**TABLE 1. Details of patients**

| Subject no. | Gender | Age | Diagnosis          |
|-------------|--------|-----|-------------------|
| 1           | M      | 52  | Diverticulitis    |
| 2           | M      | 57  | Diverticulitis    |
| 3           | F      | 50  | Diverticulitis    |
| 4           | F      | 59  | Diverticulitis    |
| 5           | M      | 67  | Diverticulitis    |
| 6           | F      | 55  | Rectal carcinoma  |
| 7           | M      | 61  | Rectal carcinoma  |
| 8           | M      | 55  | Rectal carcinoma  |
| 9           | M      | 62  | Rectal carcinoma  |
| 10          | F      | 65  | Rectal carcinoma  |
| 11          | M      | 37  | CD                |
| 12          | M      | 57  | CD                |
| 13          | F      | 32  | CD                |
| 14          | F      | 72  | CD                |
| 15          | M      | 45  | UC                |
| 16          | F      | 65  | UC                |
| 17          | M      | 62  | Control           |
| 18          | F      | 70  | Control           |
| 19          | M      | 45  | Control           |
| 20          | F      | 50  | Control           |

**TABLE 2. Adhesion (%)a of LAB to tissue from patients with healthy colon (control), rectal carcinoma, diverticulitis, and IBD**

| Lactic acid bacteria strain | Control | Rectal carcinoma | Diverticulitis | IBDb |
|----------------------------|---------|------------------|----------------|------|
| *L. rhamnosus* GG          | 4.9 ± 0.4de | 6.5 ± 0.6de | 3.3 ± 1.5de | 9.7 ± 4.5 |
| *L. rhamnosus* LC 705      | 1.6 ± 0.3 | 2.1 ± 1.0 | 1.4 ± 0.7 | 2.9 ± 1.3 |
| *L. rhamnosus* E 800       | 8.3 ± 2.4 | 4.2 ± 2.7 | 9.1 ± 10.9 | 8.9 ± 4.4 |
| *L. breve* PEL1            | 2.0 ± 0.6 | 4.2 ± 2.7 | 2.0 ± 1.7 | 4.6 ± 1.8 |
| *L. reuteri* ING1          | 3.7 ± 0.8de | 5.3 ± 3.0de | 2.0 ± 0.6de | 8.7 ± 0.7 |

a Mean ± standard deviation.
b CD and UC.

Significantly smaller than inflammatory bowel disease (*P < 0.05*).

d Significantly smaller than rectal carcinoma (*P < 0.05*).
mucus from the control tissue ($P < 0.05$). However, *B. lactis* Bb12 adhered significantly less ($P < 0.01$) to mucus from diseased tissue than to mucus from the control tissue. *L. breve* PEL1 was found to adhere better to mucus from patients with rectal carcinoma (18.8%) and IBD (17.4%) than to mucus from control tissue (6.1%).

With the exception of *L. rhamnosus* LC 705, all strains were found to adhere significantly better to intestinal mucus than to the respective tissue ($P < 0.05$). A positive correlation between adhesion to mucus and tissue was observed only for *L. breve* strain PEL1 ($P = 0.0445$).

No differences were observed in levels of LAB adhesion to tissue from different sexes ($P > 0.05$).

**DISCUSSION**

Adhesion to the intestinal mucosa is considered the main property of the health-promoting effects of specific LAB strains (6). The results of the present study show that LAB adhere to human intestinal tissue and mucus in a strain-dependent manner. Strain-specific adhesion to intestinal mucus has been observed previously (23). However, we also show here the disease-specific adhesion of *L. reuteri* ING1 and *L. rhamnosus* GG to colonic tissue and of *L. rhamnosus* LC 705, *L. rhamnosus* E800, *L. breve* PEL1, *L. reuteri* ING1, and *B. lactis* Bb12 to colonic mucus. This could indicate that each strain exhibits varying effects in the intestines of patients with different intestinal diseases.

Although the intestinal mucosa exhibits mucus to the lumen, there was a significant difference in adhesion to immobilized mucus and mucosal tissue for most of the LAB strains tested. These differences may relate to the presence of the normal intestinal microbiota on the mucosa, which is not present in immobilized mucus. The disease-specific adhesion to the intestinal tissue may therefore be caused by changes in the microbiota present (7, 20)—though qualitative (28) and quantitative (30) changes in the intestinal mucus may also be responsible for the observed differences. This is also indicated by the observed disease-specific adhesion to mucus of most of the tested strains, which may indicate differences in composition of the intestinal mucus and which affects the availability of adhesion sites. The presence of inflammatory markers such as immunoglobulins and cytokines on the tissue may affect the adhesion of the tested LAB. During the progression of disease, changes in the physiology of the intestinal epithelium may also affect the adhesive ability of the tested LAB.

Decreased numbers of lactobacilli and bifidobacteria have been observed especially during IBD (5, 8, 27) but also in association with colon cancer (31). Supplementation with appropriate LAB that, among others, exhibit good adhesive abilities, may alleviate and reduce the risk of IBD and colon cancer. Oral administration of adhesive lactobacilli has also been shown to increase fecal bifidobacteria levels, thus further promoting intestinal integrity through microbiota changes (2).

*L. rhamnosus* strain GG has been observed to have a positive effect in children with CD by improving their intestinal integrity (11, 17). It could be posited that this improvement relates to the preferential binding of *L. rhamnosus* strain GG to tissue from IBD patients over that of tissue from diverticulitis, rectal carcinoma, or control tissue. The same may apply for *L. reuteri*. In a similar manner, *L. breve* appeared to adhere best to the mucus from patients with rectal carcinoma than to mucus from IBD or diverticulitis patients.

The intra-assay variation in adhesion was in general small (coefficient of variation, <10%) (26); the variation that was observed is therefore likely to represent true person-to-person variation. Such variations have also been observed previously in vivo (35).

In the present study, only the adhesion to colonic tissue was determined. However, it is also important that the adhesion to other parts of the intestine be investigated, since the intestinal microbiota differs quantitatively and qualitatively along the length of the gastrointestinal tract—with accompanying differences in the microbes adhering to the intestinal mucus lining and directly interacting with the epithelial cells (21).

In conclusion, despite the relatively small number of subjects, this study shows that a disease-specific adhesion exists for selected LAB. This information may provide background for selecting disease-specific therapeutic LAB strains and warrants further investigations that will use larger numbers of subjects.

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