Potential Probiotic Characteristics of *Lactobacillus* and *Enterococcus* Strains Isolated from Traditional Dadih Fermented Milk against Pathogen Intestinal Colonization

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MS 06-424: Received 7 August 2006/Accepted 20 October 2006

**ABSTRACT**

Traditional fermented buffalo milk in Indonesia (dadih) has been believed to have a beneficial impact on human health, which could be related to the properties of the lactic acid bacteria (LAB) involved in its fermentation process. In previous studies, it was discovered that strains of dadih lactic isolates possessed some beneficial properties in vitro. In the present study, the adhesion capacity of specific LAB isolates from dadih to intestinal mucus was analyzed. Further, the ability to inhibit model human pathogens and displace them from mucus was assessed. The adhesion of tested LAB strains was strain-dependent and varied from 1.4 to 9.8%. The most adhesive *Lactobacillus plantarum* strain was IS-10506, with 9.8% adhesion. The competition assay between dadih LAB isolates and pathogens showed that a 2-h preincubation with *L. plantarum* at 37°C significantly reduced pathogen adhesion to mucus. All tested LAB strains displaced and inhibited pathogen adhesion, but the results were strain-specific and dependent on time and pathogen strains. In general, *L. plantarum* IS-10506 showed the best ability against pathogen adhesion.

Specific selected lactic acid bacteria (LAB) strains from the genera *Lactobacillus* and *Bifidobacterium* have been increasingly introduced and characterized as probiotics for functional food products. A probiotic has been defined as a “live microorganism which when administered in adequate amounts confers a health benefit on the host” (11). Many criteria have been suggested for the selection of probiotics, including safety, tolerance to gastrointestinal conditions, ability to adhere to the gastrointestinal mucosa, and competitive exclusion of pathogens (7, 9, 22). Adhesion to the intestinal mucosa would allow colonization, although transient, of the human intestinal tract (2) and has been related to the ability to modulate the immune system, especially during its development (29). Thus, adhesion is one of the main selection criteria for new probiotic strains (13, 26). In spite of the lack of definitive evidence, a relationship between in vitro adhesion and in vivo colonization has been reported (6, 10). In addition, adhesion to and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients (34) or immune modulation (26). Moreover, it has been shown that certain lactobacilli share carbohydrate-binding specifcites with some enteropathogens (19), and inhibition of pathogen adhesion by steric hindrance has been reported (15). This provides the rationale for the use of probiotics to prevent infection by inhibiting the adhesion of pathogens by competitive exclusion.

The LAB strains used in this study were isolated from the Indonesian traditional fermented buffalo milk dadih. Dadih is fermented milk in bamboo tubes by natural LAB, and the resulting product is thought to be beneficial to human health. The benefits may be a result of the presence of the indigenous LAB involved in dadih fermentation. Our interest in indigenous dadih LAB strains is based on their tolerance to acid and bile, their antimicrobial activity against pathogenic bacteria, and their antimutagenic properties (30, 31). The present study was designed to characterize both the adhesive properties of the specific LAB strains and their ability to inhibit the adhesion or displacement of model pathogens. This study provides a basis for the selection of natural probiotics with the ability to exclude, displace, and inhibit intestinal pathogens in the human intestinal mucus system (21, 22).

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** The indigenous dadih LAB strains were *Lactobacillus plantarum* IS-10506 and IS-20506 and *Enterococcus faecium* IS-27526, IS-23427, and IS-16183. All strains were isolated from dadih fermented milk (30) and were identified by 16S rRNA gene sequencing as *L. plantarum* (GenBank accession no. DQ860148 and DC860149) and *E. faecium* (GenBank accession no. EF068251, EF068250, and EF068249). All strains were kept in the University of Turku culture collection. The LAB isolates were cultured in deMan Rogosa Sharpe (MRS) broth (Scharlau Microbiology, Barcelona, Spain) for 48 h at 37°C, harvested by centrifugation, and freeze-dried and stored in the Functional Foods Forum Culture Collection, University of Turku, Finland. For assays with freshly grown bacteria, the bacteria were cultured in MRS broth for 18 h at 37°C under aerobic conditions, harvested by centrifugation (3,200 × g, 4°C, 20 min), and washed twice with phosphate-buffered saline (PBS;
The bacterial pathogens used in this study were \textit{Bacteroides vulgatus} DSM 1447, \textit{Clostridium histolyticum} DSM 627, \textit{Escherichia coli} K-2, \textit{Salmonella enterica} serovar Typhimurium ATCC 12028, and \textit{Staphylococcus aureus} DSM 20231. Bacterial pathogens were grown in Gifu anaerobic medium (Nissui Pharmaceutical, Tokyo, Japan) under anaerobic conditions (10% H₂, 10% CO₂, and 80% N₂; Concept 400 anaerobic chamber, Ruskin Technology, Leeds, UK).

Bacteria were metabolically labeled by the addition of titrated thymidine at 10 μM [³H]thymidine, 120 Ci/mmole; Amer sham Biosciences, Buckinghamshire, UK). All bacterial cultures were incubated at 37°C.

Mucus adhesion assay. Human intestinal mucus was isolated from the healthy part of resected colonic tissue as described earlier (20, 22). Before use, the protein concentration was determined by a modification of the method of Lowry et al. (17) as described by Miller and Hoskins (18), with bovine serum albumin (Sigma, St. Louis, Mo.) as the standard. Human mucus was dissolved (0.5 mg of protein per ml) in HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid)–Hanks buffer (HH; 10 mM HEPES, pH 7.4), and 100 μl of the solution was immobilized into polystyrene microtiter plate wells (Maxisorp, Nunc, Denmark) by overnight incubation at 4°C by the method of Collado et al. (7).

Radiolabeled bacteria were harvested after overnight incubation and washed twice with HH buffer. The A₅₀₀ value was adjusted to 0.25 ± 0.05 to standardize the number of bacteria (approximately 10⁸ CFU/ml), and 100 μl of this suspension was added to the wells and incubated for 1 h at 37°C. Subsequently, the wells were washed twice with 200 μl of HH to remove unattached bacteria. Adhered bacteria were released and lysed with 1% (wt/vol) sodium dodecyl sulfate (SDS) in NaOH (0.1 mol/liter, 200 μl per well) by incubation at 65°C for 1 h. The contents of the wells were transferred to microfuge tubes containing scintillation liquid (OptiPhase “HiSafe 3,” Wallac, Turku, Finland), and radioactivity was measured by liquid scintillation. Adhesion was expressed as the percentage of radioactivity recovered after adhesion relative to the radioactivity of the bacterial suspension added to the immobilized mucus. Adhesion was determined in three independent experiments, and each assay was performed in quadruplicate to calculate intra-assay variation.

Inhibition of pathogen adhesion. To test the ability of the LAB strains to inhibit the adhesion of pathogens, the procedure described by Collado et al. (7) was used. In brief, unlabeled probiotic bacteria (100 μl, 10⁶ CFU/ml) were added to the wells and incubated for 1 h at 37°C. Unbound LAB cells were removed by washing twice with HH buffer, and radiolabeled pathogens (100 μl, 10⁸ CFU/ml) were added to the wells and incubated at 37°C for 1 h. Then, unbound labeled bacteria were washed, and the bound bacteria were released and lysed with 1% (wt/vol) SDS in 0.1 M NaOH at 65°C for 1 h. Radioactivity was assessed by liquid scintillation. Results were expressed as the percentage of radioactivity recovered after adhesion relative to the radioactivity of the bacterial suspension added to the wells. The percentage of adhesion inhibition was calculated as the difference between the adhesion of the pathogen in the absence and presence of lactobacilli. Inhibition was determined in three independent experiments, and each assay was performed in quadruplicate.

Displacement of pathogens. The ability of the probiotic strains to displace previously adhered pathogens was assessed following the methodology described elsewhere (7). Radiolabeled pathogens were added to the wells and incubated for 1 h at 37°C; after the removal of unbound pathogens by washing twice with HH buffer, nonradiolabeled probiotics (100 μl, 10⁸ CFU/ml) were added to the plates and incubated for 1 h at 37°C. Then, the wells were washed again, and the bound bacteria were released and lysed as described above. Radioactivity was measured as previously described. Displacement of pathogens was calculated as the difference between the adhesion of pathogens before and after the addition of the LAB strains. At least three independent experiments were carried out. Each assay was performed in quadruplicate to calculate intra-assay variation.

Competitive exclusion of the pathogens by tested probiotics was determined as described previously (25). Competitive exclusion was calculated as the percentage of pathogens bound after the combination with LAB relative to the pathogens bound in the absence of LAB (control). At least three independent experiments were carried out for each probiotic strain.

For the competition test, a suspension of 50% probiotic and 50% radiolabeled pathogenic cells (10⁸ CFU/ml) was added simultaneously to intestinal mucus (0.5 mg of protein per ml) and incubated for 120 min at 37°C. Also, we analyzed the influence of the previous incubation in the competition test; for this, probiotic and pathogen strains were mixed in the same proportions and were incubated for 120 min at 37°C. After incubation, the mix was added to the intestinal mucus, and competitive adhesion was assessed following the methodology described elsewhere (7). After a total reaction time of 120 min for each condition, the cells of the pathogen bound to the mucus were removed, and the adhesion ratio (percent) was calculated as described above. For each condition, wells with PBS only, instead of nonradiolabeled adhesive cells, were included as controls, and the percentage of the pathogenic cells bound was considered the reference value (0%).

Statistical analysis. Statistical analysis was done by SPSS 11.0 software (SPSS Inc., Chicago, Ill.). Data were subjected to a one-way analysis of variance.

RESULTS

Adhesion of strains. The results of bacterial adhesion to human intestinal mucus are shown in Figure 1. The adhesion of LAB strains varied from 1.4 to 9.8%, depending on the strain. The most adhesive \textit{L. plantarum} strain was IS-10506, with 9.8% adhesion, whereas the most adhesive \textit{E. faecium} strain was IS-16183, with 4.9% adhesion. With regard to the pathogenic bacteria, \textit{C. histolyticum} DSM 627, \textit{E. coli} K-2, and \textit{S. aureus} DSM 20231 showed the highest adhesion values (11.6 to 12.6%). The least adhesive pathogen was \textit{S. enterica} serovar Typhimurium ATCC 12028, which showed only 0.6% adhesion to human intestinal mucus.

Inhibition of the adhesion of pathogens. Inhibition of the adhesion of pathogenic microorganisms by the studied LAB strains depended on each strain and the pathogen assayed (Table 1). All LAB strains isolated from dadih fermented milk were able to significantly reduce (P < 0.05) the adhesion levels of all the pathogens tested. The reduction levels were highest for the five pathogens included in this study. In general, \textit{L. plantarum} strains showed better inhibition abilities than \textit{E. faecium} strains, but the differences were not significant (P > 0.05). The data showed
FIGURE 1. Adherence of probiotic strains (A) and pathogen strains (B) to human intestinal mucus. Results were expressed as average percent adhesion to mucus ± standard deviation.

that *L. plantarum* IS-10506 and *E. faecium* IS-27526 had the highest inhibition abilities.

*B. vulgatus* was the pathogen that was easiest to be inhibited by LAB strains isolated from dadih fermented milk. The inhibition of *L. plantarum* strains ranged from 44.0 to 47.4%, whereas the inhibition of *E. faecium* strains ranged from 24.5 to 37.2%. The best LAB strain to inhibit *B. vulgatus* was *E. faecium* IS-16183, which significantly decreased (*P* < 0.05) pathogen adhesion by 37.2%.

*C. histolyticum* was the pathogen that was difficult to be inhibited by LAB strains. Inhibition ranged from 25.5 to 29.7% for all LAB strains. The best LAB strain to inhibit *B. vulgatus* was *L. plantarum* IS-10506, which significantly decreased (*P* < 0.05) pathogen adhesion by 29.7%.

All the other LAB strains were able to inhibit the adhesion of at least one of the pathogenic strains tested. The inhibition of *S. aureus* ranged from 29.5 to 40.1% for all LAB strains, *Salmonella* inhibition ranged from 22.0 to 27.2%, and *E. coli* inhibition ranged from 29.5 to 35.5%.

Displacement of pathogens. The results of pathogen displacement by LAB strains are shown in Table 2. All pathogens were displaced by natural strains from dadih, although differences were found among strains. Every LAB strain was able to displace (*P* < 0.05) all pathogens tested in this study.

*B. vulgatus* was the pathogen that was easiest to be displaced by LAB strains isolated from dadih fermented milk (*P* < 0.05), and the percentage of displacement ranged from 60.7 to 66.2%. The best LAB strain to displace *B. vulgatus* was *E. faecium* IS-16183 (66.2%). *C. histolyticum* and *S. aureus* were the pathogens that were difficult to be displaced by all LAB strains tested, and the displacements ranged from 18.4 to 22.5% and 16.4 to 31.0% for *C. histolyticum* and *S. aureus*, respectively. The best LAB strain to inhibit *C. histolyticum* was *E. faecium* IS-16183, and for *S. aureus*, it was *L. plantarum* IS-20506. All the other LAB strains were able to inhibit the adhesion of at least three of the pathogenic strains tested. The displacement of adhered *S. enterica* serovar Typhimurium ranged from 53.3 to 61.5%, and the displacement of *E. coli* was from 59.9 to 64.6%.

Comparison between pathogens and LAB strains. Results of competitive exclusion studies between pathogens and LAB strains with and without preincubation at 37°C for 2 h are presented in Table 3. The results show that previous incubation at 37°C for 2 h decreased the pathogen adhesion to intestinal mucus, but this behavior depended on each LAB strain and the pathogen assayed.

All tested LAB strains could compete and displace

![Figure 1](https://example.com/f1.png)

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**TABLE 1. Inhibition of pathogen adhesion by LAB strains isolated from dadih fermented milk**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Bacteroides vulgatus</th>
<th>Clostridium histolyticum</th>
<th>Escherichia coli</th>
<th>Salmonella Typhimurium</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecium</em></td>
<td>IS-16183</td>
<td>37.2 ± 11.9*</td>
<td>29.5 ± 6.5*</td>
<td>29.5 ± 1.5*</td>
<td>27.2 ± 5.3*</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>IS-23427</td>
<td>24.5 ± 0.7*</td>
<td>27.6 ± 8.7*</td>
<td>35.5 ± 0.9*</td>
<td>22.0 ± 0.0*</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>IS-27526</td>
<td>34.2 ± 1.9*</td>
<td>25.5 ± 5.3*</td>
<td>32.7 ± 2.2*</td>
<td>37.6 ± 14.6*</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>IS-20506</td>
<td>44.0 ± 11.5*</td>
<td>27.5 ± 9.5*</td>
<td>34.7 ± 10.5*</td>
<td>22.8 ± 2.0*</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>IS-10506</td>
<td>47.4 ± 8.8*</td>
<td>29.7 ± 9.4*</td>
<td>34.0 ± 2.8*</td>
<td>24.5 ± 6.3*</td>
</tr>
</tbody>
</table>

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* Results are expressed as average percent adhesion inhibition ± standard deviation.

* Changes in the adhesion of pathogens in the absence of LAB (control) were assigned a value of 0%.

* Asterisks denote values significantly different from the control 0% (*P* < 0.05).
TABLE 2. Inhibition of pathogens by LAB strains isolated from dadih fermented milk

<table>
<thead>
<tr>
<th>Strains</th>
<th>Bacteroides vulgatus</th>
<th>Clostridium histolyticum</th>
<th>Escherichia coli</th>
<th>Salmonella Typhimurium</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecium</td>
<td>IS-16183</td>
<td>66.2 ± 4.9*</td>
<td>22.5 ± 1.9*</td>
<td>61.7 ± 2.7*</td>
<td>61.5 ± 3.1*</td>
</tr>
<tr>
<td>E. faecium</td>
<td>IS-23427</td>
<td>65.7 ± 5.4*</td>
<td>20.3 ± 8.9*</td>
<td>59.9 ± 2.7*</td>
<td>57.9 ± 9.0*</td>
</tr>
<tr>
<td>E. faecium</td>
<td>IS-27526</td>
<td>60.7 ± 9.4*</td>
<td>19.3 ± 2.9*</td>
<td>64.6 ± 8.8*</td>
<td>53.3 ± 3.3*</td>
</tr>
<tr>
<td>L. plantarum IS-20506</td>
<td>61.6 ± 9.8*</td>
<td>21.7 ± 1.5*</td>
<td>63.8 ± 1.5*</td>
<td>52.3 ± 9.3*</td>
<td>31.0 ± 6.2*</td>
</tr>
<tr>
<td>L. plantarum IS-10506</td>
<td>62.1 ± 8.5*</td>
<td>18.4 ± 4.2*</td>
<td>62.3 ± 0.9*</td>
<td>57.3 ± 15.3*</td>
<td>16.4 ± 7.3*</td>
</tr>
</tbody>
</table>

a Results are expressed as average percent pathogen displaced ± standard deviation.
b Changes in the adhesion of preadhered pathogens following the addition of buffer without LAB strains (control) were assigned a value of 0%.
c Asterisks denote values significantly different from the control (P < 0.05).

The natural, indigenous LAB observed in dadih could be derived from bamboo tubes, buffalo milk, or banana leaves involved in milk fermentation. Previous studies on the probiotic properties of indigenous strains isolated from dadih fermented milk have demonstrated that it exhibits antimutagenic tolerance to gastrointestinal conditions (acid and bile) as well as antipathogenic properties (8, 30, 31), and all of these properties justified the selection of these LAB strains. In the present study, we examined the adhesion of these strains to human intestinal mucus and their abilities against the in vitro colonization of selected pathogens (S. aureus, S. enterica serovar Typhimurium, C. histolyticum, E. coli, and B. vulgatus) to immobilized mucus extracted from the human intestine. In this study, the adhesion of these strains to human intestinal mucus and their ability to inhibit adhesion, displace previously adhered pathogens, and compete for adhesion sites in intestinal human mucus were tested. Adhesion to intestinal mucosa has been reported for most of the current probiotic strains with demonstrated clinical efficacy. Adhesion may allow colonization by the specific strain of the human gastrointestinal tract (2). We used an immobilized human intestinal mucus model

TABLE 3. Competence between pathogens and LAB strains isolated from dadih fermented milk to adhere to intestinal mucus with and without preincubation for 2 h at 37°C

<table>
<thead>
<tr>
<th>Strains</th>
<th>Bacteroides vulgatus</th>
<th>Clostridium histolyticum</th>
<th>Escherichia coli</th>
<th>Salmonella Typhimurium</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecium</td>
<td>IS-16183</td>
<td>19.7 ± 4.1*</td>
<td>−13.5 ± 4.3*</td>
<td>43.0 ± 3.8*</td>
<td>29.8 ± 10.2*</td>
</tr>
<tr>
<td>E. faecium</td>
<td>IS-23427</td>
<td>5.0 ± 1.1*</td>
<td>−13.5 ± 0.6*</td>
<td>43.2 ± 10.0*</td>
<td>32.9 ± 17.0*</td>
</tr>
<tr>
<td>E. faecium</td>
<td>IS-27526</td>
<td>28.4 ± 8.7*</td>
<td>−9.0 ± 0.7*</td>
<td>41.3 ± 1.1*</td>
<td>12.4 ± 0.5*</td>
</tr>
<tr>
<td>L. plantarum IS-20506</td>
<td>38.1 ± 3.6*</td>
<td>4.9 ± 1.8*</td>
<td>41.4 ± 9.8*</td>
<td>18.0 ± 4.3*</td>
<td>24.0 ± 4.8*</td>
</tr>
<tr>
<td>L. plantarum IS-10506</td>
<td>−33.5 ± 6.5*</td>
<td>−9.9 ± 7.0</td>
<td>39.5 ± 7.2*</td>
<td>30.4 ± 2.0*</td>
<td>27.3 ± 4.6*</td>
</tr>
</tbody>
</table>

% pathogen inhibition without preincubation

<table>
<thead>
<tr>
<th>Strains</th>
<th>Bacteroides vulgatus</th>
<th>Clostridium histolyticum</th>
<th>Escherichia coli</th>
<th>Salmonella Typhimurium</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecium</td>
<td>IS-16183</td>
<td>62.0 ± 9.6*</td>
<td>28.5 ± 6.1*</td>
<td>60.8 ± 4.2*</td>
<td>42.1 ± 11.1*</td>
</tr>
<tr>
<td>E. faecium</td>
<td>IS-23427</td>
<td>66.8 ± 9.2*</td>
<td>23.1 ± 9.7*</td>
<td>62.5 ± 3.2*</td>
<td>56.0 ± 6.9*</td>
</tr>
<tr>
<td>E. faecium</td>
<td>IS-27526</td>
<td>44.4 ± 12.8*</td>
<td>14.4 ± 9.5*</td>
<td>64.7 ± 3.3*</td>
<td>32.8 ± 6.0*</td>
</tr>
<tr>
<td>L. plantarum IS-20506</td>
<td>46.2 ± 12.2*</td>
<td>34.0 ± 4.0*</td>
<td>63.5 ± 6.3*</td>
<td>39.1 ± 7.2*</td>
<td>58.2 ± 8.6*</td>
</tr>
<tr>
<td>L. plantarum IS-10506</td>
<td>46.1 ± 12.5*</td>
<td>32.8 ± 5.0*</td>
<td>57.7 ± 5.4*</td>
<td>37.5 ± 3.3*</td>
<td>58.4 ± 6.1*</td>
</tr>
</tbody>
</table>

% pathogen inhibition with preincubation

a Results are expressed as average percent reduction ± standard deviation.
b Changes in the adhesion of pathogens in the presence of dadih isolates. The control (pathogen adhesion) was assigned a value of 0%.
c Asterisks denote values significantly different from the control (P < 0.05).
to simulate intestinal conditions. Good correlations have been previously reported between this model and the enterocyte-like Caco-2 model (1, 21, 33).

The ability to exclude and displace pathogens from mucus by specific probiotic strains has been reported in other studies (7, 16). The difficulties involved in the analysis of bacterial adhesion in vivo have led to the development of in vitro model systems for the selection of potentially adherent strains. Many different intestinal mucosa models have been used to assess the adhesive ability of probiotics; among them, adhesion to human intestinal mucus has been widely used (7, 14, 23, 25, 32). This study has also used a reproducible and sensitive human intestinal mucus model (33).

The adhesion levels obtained for LAB strains isolated from dadih fermented milk ranged from 1.4 to 9.8%. Some of the strains tested showed higher percentages of adhesion than the commercial LAB strains used in other studies (12, 14). Interestingly, all the pathogens tested showed a high adherence to intestinal mucus, with the exception of S. enterica serovar Typhimurium (adherence = 0.6%). Pathogens have the capacity to bind to intestinal mucus, which improves pathogen colonization in human intestinal mcosa. Natural, indigenous probiotic microorganisms that prevent and decrease pathogen adhesion are important and relevant for the development of future probiotic foods from natural strains. Five pathogens, i.e., S. enterica serovar Typhimurium, E. coli, B. vulgatus, S. aureus, and C. histolyticum, were selected, given their importance in human diseases, to assess the ability of indigenous dadih strains to inhibit the adhesion of pathogens, to displace adhered pathogens from mucus, and to compete with them in mucus sites.

All tested LAB strains reduced pathogen adhesion ($P < 0.05$), and they could also displace preadhered pathogens from intestinal mucus ($P < 0.05$). However, great differences were observed, and adhesion was clearly demonstrated to be a strain-dependent property. Adhesion was also dependent on the pathogen strain tested. Some of the LAB strains showed high inhibition ability, with inhibition values of over 40% for some of the pathogens tested. In accordance with our results, it has been previously reported that certain probiotic bacteria are able to inhibit the mucosal adhesion of enteropathogenic E. coli and Salmonella by competitive exclusion (3, 7).

The displacement of preadhered pathogens was also found to be strain- and pathogen-dependent, and, as in the inhibition of pathogen adhesion, no direct correlation was found between the adhesion of LAB strains and the displacement of pathogens. Interestingly, no association was found between the results obtained for the adhesion inhibition and the displacement of pathogens, indicating that different mechanisms are involved in the processes. The displacement of preadhering pathogens was also found to be strain- and pathogen-dependent, and, as in the inhibition of pathogen adhesion, no direct correlation was found between the adhesion of LAB strains and the displacement of pathogens. Nevertheless, adhesion would appear to be one of the factors involved. In this sense, the highly adhesive L. plantarum IS-10506 was, in general, the most effective strain against the pathogens tested. Interestingly, no relation was found between adhesion inhibition and displacement of pathogens, indicating that different mechanisms are involved in these processes.

On the other hand, most LAB strains enhanced the adhesion of pathogen strains during competition for mucus sites; for example, the presence of most of the dadih isolates improved C. histolyticum ($P < 0.05$) adhesion to mucus to between 9.0 and 13.5%. B. vulgatus adhesion increased by 33.5% in the presence of L. plantarum IS-10506. Although the biological importance of these increases in pathogen adhesion is unknown, the enhanced adhesion should be considered a risk factor associated with those strains, and further evaluation of the competitive exclusion properties should be conducted. Interestingly, when the pathogen and probiotic mixture was incubated for 2 h at 37°C prior to mucus adhesion, all LAB strains significantly reduced pathogen adhesion. In most cases, the reduction of pathogen adhesion was more than 50% greater than without preincubation. In general, preincubation with LAB strains reduced pathogen adhesion. This is consistent with the coaggregation between pathogen and LAB strains shown in previous studies (8). These results suggest that coaggregation mechanisms between pathogen and LAB strains are involved in the reduction of pathogen adhesion to mucus. Several reports show that the coaggregation abilities of LAB strains may enable the formation of a barrier to prevent colonization by pathogens (24, 27, 28). Also, adhesion of bacterial cells is usually related to cell surface characteristics (4, 5). However, as reported earlier (4), no correlation was found between adhesion and the pathogen inhibition ability of the LAB strains tested, indicating that, in addition to the adhesion to mucus, other factors, such as coaggregation with the pathogen, are involved.

Natural wild strains isolated from dadih typical Indonesian fermented milk show inhibitory, competitive, and displacing properties against pathogens, and they are promising candidates for future probiotics. However, considering the high specificity of these processes, it is very important to characterize the properties of the strains and strain combinations in order to select the best strain or strain combination for the prevention or treatment of specific infections. For best results, selection of the right food matrix may also be of importance. Taken together, our results suggest that the procedures used are applicable to the selection and characterization of new probiotic strains for human use, which is relevant to future probiotic food development from natural strains. In this study, L. plantarum IS-10506 and E. faecium IS-27526 appear to have the best properties of the assessed isolates. As there are concerns about the use of enterococci for human probiotic purposes, only the L. plan-
tarum strain remains a possible strain for further studies, which should characterize the strain properties for food and feed applications.

ACKNOWLEDGMENTS

This study was supported by the Academy of Finland, Research Council for Biosciences and Environment (decision no. 210309 to Åbo
REFERENCES


