In Vitro Evaluation of *Lactobacillus gasseri* Strains of Infant Origin on Adhesion and Aggregation of Specific Pathogens

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ABSTRACT

Numerous *Lactobacillus* species are members of the normal healthy human intestinal microbiota, and members of the *Lactobacillus* family predominate among the current marketed probiotic strains. Most of the current commercial probiotic strains have not been selected for specific applications but rather have been chosen based on their technological properties. Often the ability of such strains to temporarily colonize the gastrointestinal tract may be lacking, and the interactions with intestinal microbiota are few. Furthermore, the competitive exclusion properties of potential probiotic bacteria are strain specific and vary greatly. Thus, it is highly desirable that new candidate probiotic isolates originate from the healthy target population. In this study, seven newly isolated strains of *Lactobacillus gasseri* originating from feces of a healthy newborn child were evaluated for their ability to adhere to intestinal mucus, to autoaggregate and coaggregate with the model pathogens *Cronobacter sakazakii* (ATCC 29544) and *Clostridium difficile* (1296). All the bacterial strains, single or in combination, in viable and nonviable forms, were able to autoaggregate. The coaggregation with *C. sakazakii* or *C. difficile* was higher (P < 0.05) in nonviable than in the viable forms. Single *L. gasseri* strains showed similar adhesion abilities to intestinal colon mucus. The seven *L. gasseri* strains when combined were also able to significantly compete with, displace, and inhibit the adhesion of *C. sakazakii* and *C. difficile* in the mucus model. This study demonstrates that the studied *L. gasseri* strains fulfill the basic adhesion and aggregation properties for probiotics and could be considered for potential future use in children.

Specific probiotics have been used in pediatric studies and have gained acceptance for modulating infant and even preterm (born at less than 37 weeks gestational age) infant microbiota development (17, 26, 29). Lactobacilli in general are members of the healthy human intestinal microbiota, and the *Lactobacillus* genus predominates among the currently used probiotics. Among other benefits, the *Lactobacillus* microbiota creates a positive environment for bifidobacteria and helps in establishing a barrier against pathogens. By promoting the healthy succession of microbiota in the newborn child they have a role in the maturation of the child’s immune system (13). With the increasing interest in foods that decrease the risk of disease, specific probiotics have become feasible alternatives to be used in pediatric gastroenterology and nutrition. Most lactobacilli are considered safe, and strains should be well identified and characterized with desirable characteristics targeted to this age group. Human breast milk provides complete nutrition for the infant and is always a preferred choice for infant nutrition; breast milk contains many protective nutrients, including viable bacteria and oligosaccharides, which act as specific and selective substrates for the bacteria. In many situations, breast feeding may not be possible and alternative feeding regimens should be provided. Until recently, formula-fed children have often been less resistant to infections. It is known that the use of specific probiotics may prevent morbidity and death in premature, low-birthweight infants (17). Thus, in newborn children, it is feasible that probiotic microorganisms could become primary colonizers, which modify the colonization process in the infant (10, 31). Pathogen inhibition by lactic acid bacteria may provide significant human health benefits through protection against pathogen infection as a natural barrier against pathogen exposure in the gastrointestinal tract (11). The adhesion ability of lactic acid bacteria to intestinal mucus and to enterocytes is among the desirable traits of potential probiotic strains. An adhesive probiotic strain may provide, for example, a better protection to the host because it facilitates colonization and antagonism against pathogens (12). Studies on adhesive properties of potential pathogens and probiotic bacteria suggest that this property is strain dependent (14, 19, 28). It has been demonstrated that single or combined specific probiotic strains are able to counteract adhesion of the pathogen to the mucus (2–4, 14, 28). Furthermore, aggregation properties of probiotics with pathogens are of importance for both food preservation and the therapeutic impact of food on intestinal microbiota.
Some reports attest that this coaggregation ability might enable the probiotic strains to form a barrier that prevents colonization by pathogenic bacteria (1, 5, 22–24). In this study, we evaluated seven strains of Lactobacillus gasseri, recently isolated from newborn infant feces, for autoaggregation and coaggregation with the model pathogens Cronobacter sakazakii and Clostridium difficile in viable and nonviable forms. The strains were also evaluated for their adhesion ability in the human intestinal mucus model, single or in combination, and for the ability of the combined strains to counteract the adhesion of C. sakazakii and C. difficile.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** Altogether seven L. gasseri strains (designated in the official culture collection of the Federal University of Vigo [UFVCC] as UFVCC 1083, 1091, 1099, 1103, 1105, 1108, and 1111) were isolated from the feces of breast-fed newborn children and maintained at UFVCC, Minas Gerais, Brazil. These strains were previously tested for bile salts and antibiotic tolerance, artificial gastric juice, and pathogen antagonism and identified as L. gasseri through sequencing of 16S rDNA (7). They were maintained in de Man Rogosa Sharpe broth (MRS; Oxoid Ltd., Basingstoke, Hampshire, England) with 30% glycerol solution, at −80°C. Before analysis, they were subcultured three consecutive times in MRS broth and incubated at 37°C. The pathogens evaluated included C. sakazakii (ATCC 29544) and C. difficile (DSM 1296). They were grown in Gifu anaerobic medium broth (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) at 37°C under anaerobic conditions (10% H2, 10% CO2, and 80% N2; Concept 400 anaerobic chamber, Ruskin Technology, Leeds, UK).

**Auto- and coaggregation assays.** Autoaggregation assay was performed according to Mathara and coworkers (18) with minor modifications. Bacteria were grown for 18 h at 37°C in MRS broth. The bacterial cells were harvested after centrifugation at 5,000 × g for 15 min, washed twice, and resuspended in phosphate-buffered saline (PBS). Bacterial absorbance (A600 nm) was adjusted to 0.25 ± 0.05 to standardize the concentration (106 cells per ml). Four-milliliter aliquots of the bacterial cell suspensions were mixed by vortexing for 10 s, and autoaggregation was determined during 2 h of incubation without agitation at 37°C. After incubation, 0.1 ml of the upper suspension was placed into another tube with 0.9 ml of PBS, and absorbance was measured at 600 nm. The viable and nonviable (heat inactivated in 95°C for 10 min) L. gasseri cells were used to study autoaggregation and coaggregation. The autoaggregation percentage was expressed as 1 − (A1/A0) × 100, where A1 represents the absorbance at time t = 2 h and A0 the absorbance at t = 0. Autoaggregation was determined in three independent experiments, and each assay was performed in triplicate to calculate intra-assay variation. Seven L. gasseri strains were tested for coaggregation ability with C. sakazakii and C. difficile. The strains were cultivated separately in broth. Overnight cultures were mixed together in equal amounts and were incubated at 37°C without agitation, and coaggregation tests were performed as described previously. In short, equal amounts of the Lactobacillus strain, single or in strain combination, and the pathogen, single or in combination, were mixed together to a volume of 4-ml aliquots. Absorbance was determined for the mix and for the bacterial suspensions alone. Coaggregation percentage was determined during 2 h of incubation at 37°C, as the difference of the pathogen strains, with and without the lactobacilli, single or combined, following the same calculations as indicated previously for the autoaggregation study. This evaluation was determined in three independent experiments, and each assay was performed in triplicate.

**In vitro assay of adhesion to human intestinal mucus.** Human intestinal mucus was obtained as described elsewhere (20) from human colon. Colonic mucus was dissolved (0.5 mg of protein/ml) in N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)–Hanks (HH) buffer (10 mM HEPES, pH 7.4). For adhesion, competitive exclusion, displacement, and inhibition assays the strains were grown for 18 h, harvested, and then washed twice with PBS buffer. All microorganisms were metabolically labeled by addition to the media of 10 μl/ml tritiated thymidine (5-3H-thymidine, 1.0 mCi/ml; Amersham Biosciences, Little Chalfont, UK). Radiolabeled A600 nm was adjusted to 0.25 ± 0.05 to standardize the bacterial concentration (106 cells per ml). The adhesion assessment of L. gasseri strains and bacterial pathogens was carried out as previously described (4). 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 triplicate to calculate intra-assay variation.

**Exclusion by inhibition assay.** To test the ability of the L. gasseri strains to inhibit the adhesion of pathogens, the procedure described previously (2) was used. In brief, unlabeled strains (106 cells per ml) were added to the wells and incubated for 1 h at 37°C; they were then removed by washing with HH buffer. Radiolabeled pathogens (106 cells per ml) were then added to the wells and incubated at 37°C for 1 h. Thereafter the wells were washed, and bound bacteria were recovered after lysis. Radioactivity was measured by liquid scintillation. The percentage of adhesion inhibition was calculated as the difference between the adhesion of the pathogen in the absence and presence of the different strains. Inhibition was determined in three independent experiments, and each assay was performed in triplicate.

**Exclusion by displacement assay.** The ability of the studied L. gasseri strains to displace pathogens already adhered was assessed according to Collado and colleagues (2). Radiolabeled pathogens were added to the wells containing mucus. After washing and removal of unbound pathogens, nonradiolabeled strains were added. Wells were incubated and washed, bound bacteria were recovered after lysis, and radioactivity was measured. Displacement of pathogens was calculated as the difference between adhesion of pathogens before and after the addition of the L. gasseri strains. At least three independent experiments were carried out. Each assay was performed in triplicate to calculate intra-assay variation.

**Exclusion by competition assay.** Competitive inhibition of the model pathogens by the studied L. gasseri strains was determined as previously described (2). For the competition test, equal quantities of a given bacterial suspension of strains and radiolabeled pathogens were mixed and then added to intestinal mucus and incubated as previously indicated. The cells of the pathogen bound to the mucus were then removed and adhesion calculated as described above. Competition was determined in three independent experiments, and each assay was performed in triplicate.

**Statistical analysis.** Statistical analysis was conducted using SPSS 11.0 software (SPSS Inc., Chicago, IL). Data were subjected to one-way analysis of variance (ANOVA) and, when appropriate,
the Student-Newman-Keuls test was used for comparison of the
means. P ≤ 0.05 was considered statistically significant.

RESULTS

Auto- and coaggregation assays. All the L. gasseri
strains, single and in combination, in viable and nonviable
forms, showed autoaggregation abilities. The autoaggre-
gation of L. gasseri strains varied from 19.0% (strain UFVCC
1091) to 33.5% (strain UFVCC 1103) and from 16.8% (strain UFVCC
1111) to 37.3% (strain UFVCC 1083) for viable and nonviable
forms, respectively (Table 1).

Two L. gasseri strains, designated UFVCC 1083 and
UFVCC 1091, showed significant difference (P < 0.05) in
the autoaggregation of viable versus nonviable forms
(21.9 versus 37.3% and 19.0 versus 31.6%, respectively)
(Table 1). All the L. gasseri strains and their combination
were able to coaggregate with model pathogens C. sakazakii
and C. difficile (Tables 2 and 3). The coaggregation of all L.
gasseri strains, single and in combination with C. sakazakii
and C. difficile, was highly dependent on the viability of the
strains used; autoaggregation was significantly higher (P < 0.05) for all the L. gasseri strains tested when
they were used in nonviable versus viable form (Tables 2
and 3).

In vitro adhesion assay to intestinal human mucus.
All tested L. gasseri strains showed adhesion abilities to
intestinal colon mucus, and the adhesion varied from 5.3%
(strain UFVCC 1103) to 7.2% (strain UFVCC 1099) (Fig. 1).
No significant difference could be found in the
adhesion percentages among the tested L. gasseri strains
(Fig. 1). The presence of six other L. gasseri strains
significantly increased the adhesion percentage of strain
UFVCC 1085 (adhesion single 6.0% versus adhesion in
combination 10.2%, P < 0.05; Fig. 1). All the other L.
gasseri strains showed no significant difference in the
adhesion of single versus in combination with other L.
gasseri strains. The adhesion percentage of L. gasseri strain
UFVCC 1085 in the presence of six other L. gasseri strains
was also reported as the highest adhesion percentage among

TABLE 1. Aggregative abilities of viable and nonviable L. gasseri strains at 2 h

<table>
<thead>
<tr>
<th>L. gasseri strain (UFVCC)</th>
<th>Viable (%)</th>
<th>Nonviable (%)</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1083</td>
<td>21.9 ± 14.26</td>
<td>37.3 ± 15.91 A</td>
<td>0.012</td>
</tr>
<tr>
<td>1091</td>
<td>19.0 ± 10.83</td>
<td>31.58 ± 15.69 A</td>
<td>0.018</td>
</tr>
<tr>
<td>1099</td>
<td>25.82 ± 18.13</td>
<td>23.29 ± 17.78 B</td>
<td>0.995</td>
</tr>
<tr>
<td>1103</td>
<td>33.52 ± 25.11</td>
<td>19.87 ± 11.19 B</td>
<td>0.215</td>
</tr>
<tr>
<td>1105</td>
<td>26.44 ± 23.48</td>
<td>21.98 ± 14.36 B</td>
<td>0.827</td>
</tr>
<tr>
<td>1108</td>
<td>19.48 ± 17.23</td>
<td>15.21 ± 11.68 B</td>
<td>0.713</td>
</tr>
<tr>
<td>1111</td>
<td>24.86 ± 21.76</td>
<td>16.78 ± 8.65 B</td>
<td>0.434</td>
</tr>
<tr>
<td>MIX</td>
<td>21.42 ± 20.01</td>
<td>20.45 ± 9.39 B</td>
<td>0.862</td>
</tr>
</tbody>
</table>

ANOVA P value:

|                  | 0.770 | 0.003 | — |

a Values are means ± standard deviations.

b ANOVA analysis comparing viable and nonviable data for each individual strain. Differences were considered significant when P values were <0.05.

c ANOVA analysis comparing autoaggregation data among strains of each group (viable and nonviable). Differences were considered significant when P values were <0.05. Differences obtained by post hoc Student-Newman-Keuls test were shown by letters A and B.

TABLE 2. Coaggregative abilities of viable and nonviable L. gasseri strains with pathogenic C. sakazakii after 2 h of incubation at 37°C

<table>
<thead>
<tr>
<th>L. gasseri strain + C. sakazakii</th>
<th>Viable (%)</th>
<th>Nonviable (%)</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1083</td>
<td>11.62 ± 5.75 AB</td>
<td>43.95 ± 10.10 AB</td>
<td>0.0001</td>
</tr>
<tr>
<td>1091</td>
<td>9.01 ± 3.62 A</td>
<td>48.90 ± 3.70 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>1099</td>
<td>17.65 ± 2.28 B</td>
<td>34.92 ± 5.61 A</td>
<td>0.0001</td>
</tr>
<tr>
<td>1103</td>
<td>17.60 ± 3.74 B</td>
<td>36.91 ± 10.45 A</td>
<td>0.0001</td>
</tr>
<tr>
<td>1105</td>
<td>13.46 ± 3.74 AB</td>
<td>36.18 ± 5.73 A</td>
<td>0.0001</td>
</tr>
<tr>
<td>1108</td>
<td>15.65 ± 6.18 AB</td>
<td>32.57 ± 5.54 A</td>
<td>0.0001</td>
</tr>
<tr>
<td>1111</td>
<td>11.18 ± 4.50 AB</td>
<td>35.60 ± 14.60 A</td>
<td>0.002</td>
</tr>
<tr>
<td>MIX</td>
<td>10.25 ± 2.35 A</td>
<td>38.30 ± 4.98 A</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

ANOVA P value:

|                  | 0.003 | 0.002 | — |

a Values are means ± standard deviations.

b ANOVA analysis comparing viable and nonviable data for each individual strain. Differences were considered significant when P values were <0.05.

c ANOVA analysis comparing autoaggregation data among strains of each group (viable and nonviable). Differences were considered significant when P values were <0.05. Differences obtained by post hoc Student-Newman-Keuls test were shown by letters A and B.
TABLE 3. Coaggregative abilities of viable and nonviable L. gasseri strains with pathogenic C. difficile at 2 h

<table>
<thead>
<tr>
<th>L. gasseri strain + C. difficile</th>
<th>Viable</th>
<th>Nonviable</th>
<th>ANOVA P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1083</td>
<td>16.82 ± 5.37 AB</td>
<td>28.31 ± 13.70 A</td>
<td>0.042</td>
</tr>
<tr>
<td>1091</td>
<td>18.12 ± 5.73 AB</td>
<td>41.41 ± 9.81 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>1099</td>
<td>23.63 ± 5.56 A</td>
<td>39.72 ± 4.68 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>1103</td>
<td>15.38 ± 2.89 AB</td>
<td>35.90 ± 6.75 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>1105</td>
<td>16.66 ± 6.16 AB</td>
<td>38.57 ± 6.82 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>1108</td>
<td>14.77 ± 6.39 AB</td>
<td>38.41 ± 6.23 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>1111</td>
<td>12.85 ± 5.56 B</td>
<td>40.02 ± 10.10 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>MIX</td>
<td>18.93 ± 5.02 AB</td>
<td>43.60 ± 6.17 B</td>
<td>0.0001</td>
</tr>
<tr>
<td>ANOVA P valuec</td>
<td>0.052</td>
<td>0.001</td>
<td>—</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations.

b ANOVA analysis comparing viable and nonviable data for each individual strain. Differences were considered significant when P values were <0.05.

c ANOVA analysis comparing autoaggregation data among strains of each group (viable and nonviable). Differences were considered significant when P values were <0.05. Differences obtained by post hoc Student-Newman-Keuls test were shown by letters A and B.

seven L. gasseri strains tested, single and in combination (Fig. 1). Among the pathogenic strains tested, the most marked ability to adhere to colonic mucus was detected for C. difficile, 4.4% ± 0.36% (versus 3.3% ± 0.68% for C. sakazakii).

In vitro exclusion mechanisms. When the exclusion mechanisms were tested, the seven L. gasseri strains were all combined (mixed) and their effect on inhibition of, displacement of, and competition with C. sakazakii and C. difficile was assessed. The seven L. gasseri strains in combination were able to inhibit (12.8% ± 2.84%), displace (24.0% ± 5.16%), and compete with (41.7% ± 5.01%) the adhesion of C. sakazakii, and the difference among the three exclusion mechanisms was reported to be significant (P = 0.003, Fig. 2). C. difficile adhesion was also successfully reduced during pathogen exposure to the seven L. gasseri strains. There was no significant difference (P = 0.053) among the percentages of adhesion exclusion mechanisms: inhibition (27.1% ± 9.40%) versus displacement (12.9% ± 2.1%) versus competition (28.9% ± 1.88%) of C. difficile by L. gasseri strains (Fig. 2). Inhibition and displacement mechanisms of the mixture of L. gasseri to the pathogen strains were not significantly different (P = 0.176 and P = 0.063, respectively); however, the competition mechanism was significantly different (P = 0.014). The mixture of L. gasseri was more efficient (P = 0.014) in the reduction of C. sakazakii adhesion (Fig. 2).

DISCUSSION

Our results demonstrated the ability of adhesion and aggregation of the potentially probiotic strains of L. gasseri. These strains were originated from a breast-fed infant with a known health history. The strains, either single or in combination, have shown adhesion properties and significant antagonism against the model pathogens studied. C. sakazakii is an opportunistic pathogen in very young children (<12 months), prevalent among neonates (<28 days of age) and immunocompromised children, especially those with very low birth weight or who are premature (8, 10, 25). This pathogen has been associated with contamination of infant formula. Because of this, the European Food Safety Authority and the World Health Organization (9, 30) have recommended the introduction of a performance objective for powdered infant formula and follow-up formula designed to eliminate C. sakazakii from infant dehydrated formula. If fortified with a specific probiotic, these formulas could improve the gut microbiota composition, thus increasing the ability of the newborn to fight infections (21). C. difficile is also a strictly anaerobic opportunistic pathogen, commonly involved, among others, with necrotizing enterocolitis and nosocomial diarrhea in children (27, 31). Both C. difficile and C. sakazakii are more hazardous in newborn children, in whom a commensal microbiota has not yet been established. Pathogen inhibition by lactic acid bacteria may provide significant health benefits through an enhanced natural barrier against pathogens such as the model pathogens used in this study. The two factors contributing to this protection include (i) health factors also known as probiotic factors and (ii) adaptation factors. Probiotic factors include maintenance of intestinal microbiota balance, epithelial protection, and immune modulation; while adaptation factors include stress resistance, active metabolism adapted to the host environment, and adherence to the intestinal mucus and epithelium (16). Thus, adhesion, aggregation, and coaggregation are phenotypic traits that potentially provide microbial colonization advantage within the intestinal tract. They are strain specific; therefore, newly isolated candidate probiotic cultures should be characterized with these properties in mind. All seven L. gasseri strains studied in this work in viable and nonviable form, single or in combination, displayed some degree of autoaggregation and/or coaggregation with the model pathogens C. difficile and C. sakazakii as well as adhesion to colon mucus, displacement of, and competition with the pathogens studied. The autoaggregation of the L. gasseri strains varied from 16.8% (UFVCC 1111) to 37.3% (UFVCC 1083), corrob-
orating the variability among strains of the same species. In a previous work of Collado and coworkers (6), seven lactobacilli species were evaluated among others (Lactobacillus acidophilus NCFM, L. casei Shirota, L. fermentum ME-3, L. plantarum Lp-115, L. rhamnosus GG, L. rhamnosus LC-705, and L. salivarius Ls-33) for their ability to coaggregate with C. sakazakii ATCC 29544. After 2 h of incubation at 37°C, the coaggregation varied from 0% (L. rhamnosus GG) to 27% (L. fermentum ME-3). In our study, in the same time point evaluation, the percentage of coaggregation with C. sakazakii ranged from 9.0% (UFVCC 1091) to 15.7% (UFVCC 1108) in the assay with viable cells and from 32.6% (UFVCC 1108) to 48.9% (UFVCC 1091) for nonviable cells. This corroborates the diversity of this phenotype among strains of the same species, isolated from the same subject. Interestingly, the strains with the lowest and highest coaggregation percentages demonstrated the opposite behavior whether in the viable or nonviable state. The capacity of the nonviable cells to keep coaggregative abilities could be considered a phenotype advantage, enabling these strains to be kept functional in harsh conditions such as those related to the drying processing. Also, it is known that aggregation enables probiotic strains to reach the cell mass necessary for the achievement of their functionality. Thus, aggregative and coaggregative abilities of Lactobacillus species might enable them to establish an intestinal barrier that prevents colonization with pathogenic bacteria (23). Evaluating adherence in feces mucin of healthy and rotavirus-infected infants, Juntenon and coworkers (15) verified adherence of probiotics ranging from 1 to 34% in healthy subjects as indicated: L. rhamnosus GG, 34%; B. lactis Bb12, 31%; L. acidophilus LA5, 4%; L. paracasei F19, 3%; and L. casei Shirota, 1%. The rotavirus diarrhea did not influence the overall pattern of probiotic adherence. The adhesion of Bb12 in the presence of GG increased from 31 to 39% in healthy infants (P = 0.018) and in episodes of diarrhea increased from 26 to 44% (P = 0.001). The authors stated that the combination of probiotic strains might synergistically favor adherence. In our work, all single L. gasseri strains have shown similar adhesion abilities to intestinal colon mucus. The seven L. gasseri strains, when combined, were also able to significantly (P < 0.05) compete with (41.7%), displace (24.0%), and inhibit (10.8%) the adhesion of C. sakazakii. Although not significantly (P > 0.05), the combined L. gasseri strains successfully compete with (28.8%), displace (10.2%), and inhibit (19.9%) C. difficile. Due to the variability of these phenotypes among strains of the same species, the best combinations should be known in order to benefit from the synergistic effect provided by the selected strains. Taken together, our results demonstrate that all the evaluated L. gasseri strains autoaggregared in variable degrees and have shown coaggregative abilities with C. sakazakii and C. difficile. The adhesion properties, as well as the ability to displace C. difficile and C. sakazakii, were strain dependent. The present study suggests that L. gasseri strains, single or in combination, meet the criteria of adhesion and competitive exclusion to be further assessed for use as future probiotics.

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