**Lactobacillus plantarum DSM 2648 is a potential probiotic that enhances intestinal barrier function**

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**Abstract**

The aim of this research was to identify bacterial isolates having the potential to improve intestinal barrier function. *Lactobacillus plantarum* strains and human oral isolates were screened for their ability to enhance tight junction integrity as measured by the transepithelial electrical resistance (TEER) assay. Eight commercially used probiotics were compared to determine which had the greatest positive effect on TEER, and the best-performing probiotic strain, *Lactobacillus rhamnosus* HN001, was used as a benchmark to evaluate the isolates. One isolate, *L. plantarum* DSM 2648, was selected for further study because it increased TEER 135% more than *L. rhamnosus* HN001. The ability of *L. plantarum* DSM 2648 to tolerate gastrointestinal conditions and adhere to intestinal cells was determined, and *L. plantarum* DSM 2648 performed better than *L. rhamnosus* HN001 in all the assays. *Lactobacillus plantarum* DSM 2648 was able to reduce the negative effect of *Escherichia coli* [enteropathogenic *E. coli* (EPEC)] O127:H6 (E2348/69) on TEER and adherence by as much as 98.75% and 80.18%, respectively, during simultaneous or prior coculture compared with EPEC incubation alone. As yet, the precise mechanism associated with the positive effects exerted by *L. plantarum* DSM 2648 are unknown, and may influence its use to improve human health and wellness.

**Introduction**

Probiotics are defined as ‘live microorganisms which, when administered in adequate amounts, confer a health benefit onto the host’ (Guarner & Schafsma, 1998). Most probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*, and are often selected for their ability to grow in dairy products, survive gastrointestinal conditions and adhere to intestinal epithelial cells (Dunne et al., 2001; Delgado et al., 2008). Although these properties are important to the delivery of viable probiotics to the site of action, greater emphasis should be placed on selecting probiotics based on their specific health benefits to target particular consumer groups or health ailments (Gueimonde & Salminen, 2006). Probiotics can have a number of different mechanisms by which they are proposed to improve health, such as inhibition of pathogenic bacteria, improving epithelial and mucosal barrier function and altering the host’s immune response.

Despite the known association between impaired intestinal barrier function, gastrointestinal disorders (Barbara, 2006; Bruewer et al., 2006; Gutman et al., 2006) and illnesses in other parts of the body (Liu et al., 2005; Maes, 2008; Maes & Leunis, 2008; Sandek et al., 2008; Vaarala et al., 2008), few studies have focused on selecting probiotics based on their ability to enhance intestinal barrier function. However, using the transepithelial electrical resistance (TEER) assay as a measure of the integrity of the tight junctions between intestinal epithelial cells, studies have shown that some bacteria can enhance intestinal barrier function. A number of these studies used strains of *Lactobacillus plantarum*. For example, *L. plantarum* CGMCC 1258 was able to lessen the negative impact of enteroinvasive *Escherichia coli* ATCC 43893 serotype O124:NM on TEER (Qin et al., 2009), *L. plantarum* 299v mitigated the TNF-α-induced decrease in TEER (Ko et al., 2007) and *L. plantarum* MF1298 attenuated the decrease in TEER induced by *Listeria monocytogenes* 6896 (Klingberg et al., 2005).
The aim of this research was to identify lactobacilli isolates, with an emphasis on L. plantarum, that enhance TEER and therefore have the potential to be used as probiotics targeted at improving intestinal barrier function. Eight commercially used probiotics were compared to determine which had the greatest positive effect on TEER across intestinal epithelial cell layers, and then the best probiotic was used as a benchmark to evaluate several isolates, including four L. plantarum strains and 15 human oral isolates. The oral cavity was chosen as a source of potential probiotics because evidence suggests that lactobacilli found in human faeces, and therefore present in the intestines, originate from the oral cavity (Dal Bello & Hertel, 2006; Maukonen et al., 2008). The isolate with the greatest positive effect on TEER was further investigated to evaluate its suitability for use as a probiotic, including its ability to tolerate gastrointestinal conditions, to adhere to intestinal epithelial cells and affect adherence and TEER of enteropathogenic E. coli (EPEC) O127:H6 (E2348/69), a known enteric pathogen (Baldini et al., 1983), during coculture.

Materials and methods

Bacterial strains

The source of the bacterial strains used in this study is described in Table 1. Eight commercially used probiotics were chosen on the basis that there were published data showing their efficacy in various in vitro and in vivo models (Table 1). Further strains were either L. plantarum obtained from the Deutsche Sammlung von Mikroorganismen (DSM) or human oral lactobacilli isolates. Human oral isolates were obtained from the mouth lining, tongue and teeth of volunteers using sterile tooth picks, which were incubated individually in 10 mL of Man, Rogosa and Sharpe (MRS) broth overnight at 37 °C (5% CO2) to select for lactic acid bacteria. Cultures were diluted in phosphate-buffered saline (PBS, pH 7.2), plated onto Rogosa agar and incubated in 5% CO2 at 37 °C for 48 h to select for lactobacilli. Putative L. plantarum strains with large white colonies similar to those of known L. plantarum strains were subcultured onto fresh Rogosa agar and incubated at 37 °C (5% CO2) for 48 h. Sample colonies were stored as glycerol stocks at −85 °C.

Isolates were identified based on their 16S rRNA gene sequences. From the isolated genomic DNA, 16S rRNA gene was amplified using FD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and RD1 (5'-AAGGAGGTGATCCTGGCTCAG-3') primers and PCR Supermix (Invitrogen) using a Thermo Hybaid PX2 thermocycler, and purified using a QIAquick PCR purification kit. Sequencing was performed at the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand), and traces were aligned using CONTIGEXPRESS VECTOR NTI and the 16S rRNA gene sequences were compared with known bacterial sequences using the NCBI BLAST database.

The EPEC O127:H6 (E2348/69) was obtained from Dr Roberto La Ragione at Veterinary Laboratories Agency, Weybridge, UK.

TEER assay

Caco-2 cells (human colorectal adenocarcinoma cell line; ATCC HTB-37) were used as a model of the intestinal epithelial barrier because they differentiate spontaneously into polarized intestinal cells possessing apical brush borders and tight junctions. Caco-2 cells were seeded onto collagen membrane inserts (Collagen™ Discs CD-24, MP Biomedicals, OH) and incubated in 12-well plates in M199 with 10% v/v foetal bovine serum, 1% v/v nonessential amino acids (MEM nonessential amino acids 100× solution and 1% v/v penicillin–streptomycin) (10 000 U penicillin G sodium salt and 10 000 μg streptomycin sulphate in

### Table 1. Summary of the effect of commercially used probiotics (lactobacilli and bifidobacteria) on TEER across confluent undifferentiated Caco-2 monolayers (5 days old) over 12 h

<table>
<thead>
<tr>
<th>Probiotic strain</th>
<th>Company</th>
<th>References for efficacy</th>
<th>Mean change in TEER (± SEM) % compared with the control media (n=4) expressed relative to L. plantarum MB 452 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus rhamnosus</em> HN001</td>
<td>Danisco</td>
<td>Gill et al. (2000), Cross et al. (2002)</td>
<td>222 (19)*</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> 299</td>
<td>Probi AB</td>
<td>Adlerberth et al. (1996)</td>
<td>158 (33)*</td>
</tr>
<tr>
<td><em>Bifidobacterium lactis</em> Bb12</td>
<td>Chr Hansen Biosk</td>
<td>Ruiz et al. (2005)</td>
<td>148 (31)*</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> MB 452</td>
<td>VSL Pharmaceuticals</td>
<td>Unpublished data</td>
<td>100</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> 299v</td>
<td>Probi AB</td>
<td>Mangell et al. (2002), Schultz et al. (2002)</td>
<td>66 (32)</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em> Shirota</td>
<td>Yakult</td>
<td>Herias et al. (2005)</td>
<td>14 (57)*</td>
</tr>
<tr>
<td>Medium 199 control</td>
<td>Control medium</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> GG</td>
<td>Valio</td>
<td>Gosselink et al. (2004), Roselli et al. (2006)</td>
<td>−11 (79)*</td>
</tr>
<tr>
<td><em>Bifidobacterium lactis</em> HN019</td>
<td>Danisco</td>
<td>Gill et al. (2000), Shu et al. (2001)</td>
<td>−115 (34)*</td>
</tr>
</tbody>
</table>

*P value < 0.05 compared with *Lactobacillus plantarum* MB 452.

1P value < 0.05 compared with the control medium.
CO2 for 5 days until confluent (undifferentiated) for the screening assays. Undifferentiated Caco-2 cells were used for the initial screening because of the ease of preparing undifferentiated Caco-2 cells compared with differentiated Caco-2 cells. This was necessary because of the high volume of assays that were carried out during the screening. The TEER assay measures the integrity of the tight junctions between epithelial cells, and as these tight junctions are already formed when Caco-2 cell monolayers reach confluence (5 days), undifferentiated Caco-2 cells are often used to assess tight junction integrity. An additional TEER assay was carried out using differentiated Caco-2 cells (18 days old) to confirm the positive effects of the best selected isolates.

Caco-2 monolayers were prepared the day before the TEER assay by removing the media, washing with PBS (pH 7.2) and adding M199 with 1% v/v nonessential amino acids (without foetal bovine serum and penicillin–streptomycin). In each experiment, control media (M199 with 1% nonessential amino acids) and a positive bacterial strain (either L. plantarum MB452 for commercially used probiotic strain testing or Lactobacillus rhamnosus HN001 for isolate testing) were included as controls. Overnight cultures of bacterial cells (MRS broth, 37°C, 5% CO2) were collected by centrifugation (20000 g for 5 min) and resuspended in M199 with 1% v/v nonessential amino acids to an OD600 nm of 0.9. After the initial resistance readings, the media were removed from the Caco-2 monolayers and replaced with treatment solutions. Each bacterial strain was tested in quadruplicate.

The resistance across each cell monolayer was measured every 2 h in an electrode chamber (ENDOHM-12 tissue culture chamber; World Precision Instruments, FL) using a voltohmmeter (EVOM Epithelial Tissue Voltohmmeter; World Precision Instruments). The TEER was calculated from the resistance using Eqn.(1), where the background resistance was 14Ω and the membrane area was 1.54 cm². The change in TEER for each insert was calculated using Eqn.(2). Treatments were compared in GENSTAT (version 11) using residual maximum likelihood (REML) analysis with an unstructured covariance model to take into account the repeated measures.

\[
\text{TEER} = \frac{(\text{resistance} - \text{background resistance}) \times \text{membrane area}}{(1)
\]

\[
\text{Change in TEER} = (\text{TEER} - \text{initial TEER}) - 100
\]

**Acid and bile tolerance**

Bacterial cultures were grown overnight in MRS broth at 37°C with 5% CO2. Each culture was vortexed, and separate 10-mL aliquots were collected by centrifugation (3800 g for 20 min). Cell pellets were suspended to an approximate cell concentration of 10⁸–10¹⁰ CFU mL⁻¹ in the following test solutions: MRS broth control, MRS broth adjusted to pH 2.0, MRS broth adjusted to pH 4.0, 0.5% w/v bile and 1% w/v bile. The time points (2 and 4 h) were chosen to represent the time it takes to pass through the human upper gastrointestinal system to the lower intestinal tract. The concentrations of bile (0.5% and 1%) and pH values (pH 2.0 and 4.0) were chosen to represent the range of these variables found in the human stomach. Bacterial viability was assessed after 2 and 4 h with triplicate 20-μL dilution spots on Luria–Bertani (LB) agar plates. Values were log-transformed before REML analysis using an unstructured covariance model.

**Bacterial cell adherence to Caco-2 epithelial cells**

Quantitative analysis of bacterial adherence to both confluent undifferentiated (5 days) and differentiated (18 days) Caco-2 cells was tested as described previously (Donnenberg & Nataro, 1995). Bacterial strains were grown overnight in MRS broth and approximately 10⁷ CFU (10 μL) were added to each well, with each strain being assessed for adherence (3 and 6 h) in triplicate. Lactobacilli were enumerated on LB agar plates as described previously. Values were log-transformed before ANOVA analysis.

**Lactobacillus plantarum DSM 2648 and EPEC coculture**

The effect of coculture of L. plantarum DSM 2648 and EPEC O127:H6 (E2348/69) was examined in both the TEER and the cell adherence assay. The TEER assay was performed with two hourly readings for 10 h as described previously with overnight cultures of L. plantarum DSM 2648 prepared from MRS broths. The EPEC strain was grown aerobically overnight at 37°C in LB broth, with shaking at 100 r.p.m. EPEC cells were collected by centrifugation (20000 g for 5 min) and suspended in M199 with 1% v/v nonessential amino acids to an OD600 nm of 0.1. TEER coculture experiments also included both bacterial strains individually to assess separate effects for control purposes. For adherence to Caco-2 cell monolayers, both the L. plantarum DSM 2648 and the EPEC strain were grown in MRS and LB broth and inoculated into tissue culture wells containing undifferentiated Caco-2 cells as described previously. The EPEC strain was incubated alone or simultaneously cocultured with L. plantarum DSM 2648 for 3 or 6 h. The effects of a 3-h preincubation of L. plantarum DSM 2648 with Caco-2 cells before the addition of the EPEC strain and a 3-h preincubation of the EPEC strain with Caco-2 cells before the addition of L. plantarum DSM 2648 were also evaluated. EPEC were enumerated selectively on sorbitol MacConkey agar plates incubated aerobically at 37°C for 18 h. EPEC adherence during coincubation with L. plantarum DSM 2648 was calculated as a percentage of the adherence of the EPEC strain during 3- and 6-h incubations, respectively.
Treatments were compared using a paired-samples t-test (two tails).

Results and discussion

Identification of bacterial strains

The activity of four *L. plantarum* strains obtained from DSM and 15 human oral lactobacilli isolates was compared with eight commercially used probiotics chosen on the basis of published data showing their efficacy in various *in vitro* and/or *in vivo* models. Fifteen human oral bacteria were isolated with the intention of obtaining novel *L. plantarum* strains; however, based on 16S rRNA gene sequencing, only one was *L. plantarum* (Table 2). The most commonly isolated species were *L. rhamnosus* and *Lactobacillus fermentum*, of which four and five strains were isolated, respectively. The other isolates were strains of *Lactobacillus*

Table 2. Summary of the effect of *Lactobacillus* isolates on the TEER across confluent undifferentiated Caco-2 monolayers (5 days old) over 12 h

<table>
<thead>
<tr>
<th>Experimental strain</th>
<th>Source</th>
<th>Best strain matches to the 16S rRNA gene sequence*</th>
<th>Mean change in TEER (± SEM) % compared with the control media (n=4) expressed relative to <em>L. rhamnosus</em> HN001 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. plantarum</em> DSM 2648</td>
<td>Silage</td>
<td>99% <em>Lactobacillus fermentum</em> SFCB2-6c (DQ486144)</td>
<td>235 (14)†</td>
</tr>
<tr>
<td><em>L. fermentum</em> AGR1485</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus paracasei</em> ATCC 53103 (AY370682)</td>
<td>114 (9)†</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> HN001</td>
<td>Best commercially used</td>
<td>100% <em>Lactobacillus rhamnosus</em> GG ATCC 53103 (AY370682)</td>
<td>100</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> AGR1500</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus rhamnosus</em> GG ATCC 53103 (AY370682)</td>
<td>62 (3)†</td>
</tr>
<tr>
<td><em>L. plantarum</em> DSM 20205</td>
<td>Corn silage</td>
<td>99% <em>Lactobacillus paracasei</em> (DQ199664)</td>
<td>56 (18)†</td>
</tr>
<tr>
<td><em>L. paracasei</em> AGR1491</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus paracasei</em> (DQ199664)</td>
<td>49 (12)†</td>
</tr>
<tr>
<td><em>L. oris</em> AGR1493</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus oris</em> (X94229)</td>
<td>48 (16)†</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> AGR1499</td>
<td>Unknown</td>
<td>99% <em>Lactobacillus rhamnosus</em> GG ATCC 53103 (AY370682)</td>
<td>47 (28)†</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> AGR1514</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus rhamnosus</em> MCRF-412 (AY299488)</td>
<td>45 (3)†</td>
</tr>
<tr>
<td><em>L. plantarum</em> DSM 12028</td>
<td>Dry fermented sausage</td>
<td>99% <em>Lactobacillus rhamnosus</em> MCRF-412 (AY299488)</td>
<td>42 (8)†</td>
</tr>
<tr>
<td><em>L. helveticus</em> AGR1517</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus surnotreus</em> LH5 (AY675251)†</td>
<td>32 (21)†</td>
</tr>
<tr>
<td><em>L. plantarum</em> AGR1492</td>
<td>Human oral isolate</td>
<td>98% <em>Lactobacillus plantarum</em> L5 (DQ239698)</td>
<td>31 (24)†</td>
</tr>
<tr>
<td><em>L. gasseri</em> AGR1515</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus gasseri</em> ATCC 33323 (AF519171)</td>
<td>24 (16)†</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> AGR1523</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus rhamnosus</em> MCRF-412 (AY299488)</td>
<td>14 (8)†</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>Medium 199</td>
<td>Control medium</td>
<td>5 (38)†</td>
</tr>
<tr>
<td><em>L. fermentum</em> AGR1512</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus fermentum</em> SFCB2-6c (DQ486144)</td>
<td>0</td>
</tr>
<tr>
<td><em>L. fermentum</em> AGR1502</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus fermentum</em> SFCB2-6c (DQ486144)</td>
<td>−11 (38)†</td>
</tr>
<tr>
<td><em>L. jensenii</em> AGR1519</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus jensenii</em> KC36b (AF243159)</td>
<td>−60 (11)†</td>
</tr>
<tr>
<td><em>L. fermentum</em> AGR1489</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus fermentum</em> AF302116</td>
<td>−166 (80)†</td>
</tr>
<tr>
<td><em>L. fermentum</em> AGR1487</td>
<td>Human oral isolate</td>
<td>99% <em>Lactobacillus fermentum</em> AF302116</td>
<td>−231 (17)†</td>
</tr>
</tbody>
</table>

*Results are given as percentage match, strain name (GeneBank accession number).*

†P value < 0.05 compared with *Lactobacillus rhamnosus* HN001.

‡P value < 0.05 compared with the control medium.

*Lactobacillus suntoreus* was reclassified to *Lactobacillus helveticus* (Naser et al., 2006).
paracasei, Lactobacillus oris, Lactobacillus helveticus, Lactobacillus gasseri and Lactobacillus jensenii.

Effect of commercially used probiotics on TEER

The commercially used probiotics were screened in the TEER assay to assess their effect on the integrity of the tight junctions between the intestinal confluent undifferentiated Caco-2 monolayers (5 days old). Lactobacillus plantarum MB452 was used to normalize between assays, because it has a consistently positive effect on TEER (unpublished data). Lactobacillus plantarum 299, L. rhamnosus HN001 and Bifidobacterium lactis Bb12 were the three commercially used probiotics that had the greatest positive effect on TEER measurements and induced increases compared with the control media of 158%, 222% and 148%, respectively (Table 1). Only L. rhamnosus HN001 positively enhanced the overall TEER more than L. plantarum MB452 ($P < 0.05$ compared with L. plantarum MB452).

Lactobacillus rhamnosus HN001 was selected as the benchmark for isolate comparison because it had the greatest positive effect on TEER at all time points and the smallest

Fig. 1. Change in the TEER across confluent Caco-2 monolayers over time. The change in TEER is the percentage change compared with the initial TEER for each monolayer. The values plotted are the means for four monolayers and the error bars show the SEM. *$P < 0.05$ compared with the control media.

- (a) Confluent undifferentiated Caco-2 cells in the presence of the three commercially used probiotics that had the greatest positive effect on TEER.
- (b) Confluent undifferentiated Caco-2 monolayers in the presence of Lactobacillus plantarum DSM 2648 and Lactobacillus rhamnosus HN001.
- (c) Differentiated Caco-2 monolayers in the presence of L. plantarum DSM 2648 and L. rhamnosus HN001.
- (d) Confluent undifferentiated Caco-2 monolayers in the presence of L. plantarum DSM 2648 and Escherichia coli O127:H6 alone and in combination.
variation between replicates (Fig. 1a). Lactobacillus rhamnosus HN001 reduces the severity of pathogen infections (Gill et al., 2001; Shu & Gill, 2002) and stimulates the immune response in rodents (Gill et al., 2000; Gill & Rutherfurd, 2001a, b; Cross et al., 2002), and this study shows that it is also able to enhance tight junction integrity.

Effect of isolates on TEER

The 19 bacterial isolates were screened in the TEER assay using confluent undifferentiated Caco-2 monolayers (5 days old) to determine whether any isolates were able to enhance TEER to a greater extent than the commercially used probiotic benchmark, L. rhamnosus HN001. Nine isolates positively enhanced TEER compared with the control media (Table 2; P < 0.05). Of these, one isolate, L. plantarum DSM 2648, caused a 235% increase in TEER, which was greater than the benchmark probiotic (P < 0.05 compared with the control media and L. rhamnosus HN001) (Fig. 1b). Lactobacillus plantarum DSM 2648 also had a similar effect on TEER when tested using differentiated Caco-2 monolayers (18 days old) (Fig. 1c).

This study demonstrates the strain-dependent effects of lactobacilli on intestinal barrier function and that all strains of the same species should not be assumed to have similar health-promoting properties. Lactobacillus plantarum are effective in enhancing TEER, with three out of the five L. plantarum isolates tested having a positive effect on TEER compared with the control media. A number of human oral isolates were also effective in enhancing TEER compared with the control media. Three out of four L. rhamnosus isolates, the L. paracasei isolate and the L. oris isolate had a positive effect on TEER. However, several of the human oral isolates had a negative effect on TEER; three out of five L. fermentum isolates and the L. jensenii isolate induced a decrease in TEER compared with the control media. In contrast, one isolate of L. fermentum induced an increase in TEER compared with the control media.

Ability of L. plantarum DSM 2648 to tolerate gastrointestinal conditions and adhere to intestinal cells

Lactobacillus plantarum DSM 2648 was chosen for further investigation because it had a greater positive effect on TEER
compared with the benchmark, *L. rhamnosus* HN001, over the 12-h test period. Acid and bile tolerance (2 and 4 h) of *L. plantarum* DSM 2648 was compared with that of *L. rhamnosus* HN001 (Fig. 2). Both bacterial strains were able to tolerate acidic conditions (pH 4 for 4 h) without the loss of cell viability; however, both strains had a reduced viability of 6–7 log units under conditions of pH 2 for 4 h. The viability of *L. rhamnosus* HN001 decreased by 2 log units in the presence of 0.5% bile and by 5 log units in the presence of 1% bile, whereas the viability of *L. plantarum* DSM 2648 only reduced by 2 log units by 1% bile.

The ability of *L. plantarum* DSM 2648 to adhere to intestinal cells (3 and 6 h) was also compared with that of the benchmark strain, *L. rhamnosus* HN001 (Fig. 3). *Lactobacillus plantarum* DSM 2648 adhered in higher numbers (10 times more) to both confluent undifferentiated and differentiated Caco-2 cells compared with *L. rhamnosus* HN001 (*P < 0.05 at 3 and 6 h*). *Lactobacillus plantarum* DSM 2648 displayed better *in vitro* tolerance to gastrointestinal conditions compared with *L. rhamnosus* HN001, which has been detected in human faeces after ingestion (Tannock *et al.*, 2000); thus, it is possible that *L. plantarum* DSM 2648 may also survive passage through the human gastrointestinal tract.

**Action of *L. plantarum* DSM 2648 on EPEC-induced TEER changes and bacterial adherence**

*Lactobacillus plantarum* DSM 2648 was also able to prevent the deleterious EPEC-induced TEER changes observed when the EPEC strain was incubated alone (Fig. 1d); however, the action of *L. plantarum* DSM 2648 was transient, lasting for at most 8 h. The action of *L. plantarum* DSM 2648 on EPEC interactions with Caco-2 cells was further explored using coculture adherence experiments. *Lactobacillus plantarum* DSM 2648 was found to have the greatest inhibitory effect on the adherence of EPEC (80.18% reduction) to Caco-2 cells when added before the enteric pathogen, but had no effect when added 3 h after the addition of the EPEC strain (Fig. 4). In contrast to the study by Michail & Abernathy (2002), where coincubation of the *L. plantarum* 299v with the EPEC strain did not result in any statistically significant reduction in EPEC adherence, when the *L. plantarum* DSM 2648 was added simultaneously with the EPEC in this study, EPEC adherence to the Caco-2 cells was reduced by 65.5% (3 h) and 55.9% (6 h), respectively (Fig. 4).
Conclusions

This study showed that *L. plantarum* DSM 2648 has a number of characteristics desirable for a probiotic selected specifically for its ability to enhance intestinal barrier function. These data warrant further investigation to determine whether the promising *in vitro* results correspond with *in vivo* efficacy and to understand the mechanism by which it exerts the positive effects on Caco-2 cells alone and a reduction in the deleterious effects of EPEC during coinubcation. The ability of *L. plantarum* DSM 2648 to survive passage through the gastrointestinal system could be investigated by monitoring viability in the faeces of humans consuming the bacterium. If proven to be effective, *L. plantarum* DSM 2648 could be used as a probiotic to benefit humans with a range of conditions as well as for general well-being.

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References


