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The effect of calcium ions on adhesion and competitive exclusion of *Lactobacillus* ssp. and *E. coli* O138

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Abstract

The adhesion abilities of 11 strains of *Lactobacillus* were determined *in vitro* using the IPEC-J2 cell line as a model system. Bacteria cultures included the probiotic strains *L. rhamnosus* GG, *L. reuteri* ATCC 55730, *L. johnsonii* NCC 533 and *L. reuteri* DSM 12246, and new isolates of *Lactobacillus* ssp. Adhesion was quantified by scintillation counting of radiolabelled bound bacteria. The highest adhesion of 38%, was determined for *L. reuteri* DSM 12246 followed by *L. plantarum* Q47 with an adhesion level of 24%. Other strains showed moderate to low binding of less than 16%. Competitive adhesion experiments on IPEC-J2 cells demonstrated that strongly adhesive strains, as *L. reuteri* DSM 12246 and *L. plantarum* Q47, significantly reduced the attachment of the less adhesive strains, such as *L. rhamnosus* GG and *L. johnsonii* NCC 533, both under condition of co-incubation and in displacement assays, indicating that bacteria may share the same binding sites for attachment to intestinal cells. Furthermore, it was revealed that calcium ions significantly increased the binding of tested lactobacilli to IPEC-J2 cells; and therefore, added calcium may be useful in enhancing the adhesion of normally weakly adhesive probiotic cultures. In contrast, no significant change in adhesion of lactobacilli was observed in the presence of Mg and Zn ions. Displacement assays performed with pathogenic *E. coli* O138 showed that all tested *Lactobacillus* strains reduced the attachment of *E. coli* O138 to IPEC-J2 by more than 2-fold both in the presence and the absence of calcium ions. The strains of *Lactobacillus* did not differ significantly in the extent of their inhibition of *E. coli* O138 adhesion, indicating that the reduced adhesion of *E. coli* O138 was due to steric hindrance of the binding sites rather than to specific interactions.

Keywords: Adhesion; *Lactobacillus* species; Divalent ions; Probiotic

1. Introduction

Lactobacillus species are desirable members of intestinal microbiota and act as probiotic bacteria in the gut. The beneficial effects of *Lactobacillus* on health are well-documented and include stabilization of the indigenous microflora, prevention and treatment of diarrhoea, alleviation of lactose intolerance, increased nutritional value of foods, stimulation of immune system, and reduction of serum cholesterol levels (Isolauri et al., 1994; Hooper et al., 1999; Isolauri, 2001).

An important step in the successful colonization and execution of probiotic effects is the ability of bacterial strains to adhere to intestinal epithelium. High adherence is commonly used as a selection criterion for probiotic strains (Bernet et al., 1994; Hudault et al., 1997; Blum et al., 1999). Several *in vitro* models for assessing the adhesive abilities of bacteria, based on tissue-cultured cells and intestinal mucus preparations, have been developed. For example, the epithelial-like tumor cell lines, HT-29 and Caco-2, have been used extensively to select for adhesive strains. The porcine intestinal epithelial cell line IPEC-J2 is a more recently developed and so far less extensively used cell line which has been reported as a relevant *in vitro* model system for intestinal cell-to-cell interactions (Schierack et al., 2005). IPEC-J2 cells can differentiate in culture and exhibit enterocytic features, like microvilli, tight junctions and glycocalyx-bound mucin. As shown by numerous studies, adhesion in different *in vitro* models varies even within the same strain, indicating that bacterial structures involved in binding to epithelial cells and to mucus may be different.

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Furthermore, the experimental evidence shows that adhesion may be favoured by such factors as a particular medium, temperature and pH (Jones et al., 1976; Tuomola et al., 2001). It has also been suggested that divalent ions, as e.g., calcium, have an influence on bacterial attachment (Zarate et al., 2002). Calcium is known to promote non-specific interactions such as neutralization of the electrical double layer between the cells as well as specific adhesive interactions with protein and polysaccharide adhesin molecules at the cell surface (Deman et al., 1974; Geesey et al., 2000). However, the effects of calcium on adhesion of probiotic bacteria have not been thoroughly investigated. A few reported experiments have shown that adherence of lactobacilli to Caco-2 and intestine mucus might be either calcium promoted or not influenced by calcium, depending on the strain (Kleeman and Klaenhammer, 1982; Chauviere et al., 1992; Bernet et al., 1994; Gusils et al., 2003).

Adhesive probiotics are generally considered to be more effective in competitively excluding pathogenic bacteria when compared to non-adhesive strains (Vesterlund et al., 2004). The activity of probiotics in exclusion of pathogens may be due to the stimulation of the immune system or to direct competition with pathogens for the same binding sites on epithelial cells. One widespread pathogen, enteropathogenic *Escherichia coli* (EPEC), belongs to the coliform faecal group, restricted to organisms that grow in the gastrointestinal tract of humans and warm-blood animals (Giammanco et al, 1996; Go and Cunha, 2004). EPEC causes gastroenteritis in human and domestic animals, traveller's diarrhoea and sporadic cases of hemorrhagic colitis characterized by bloody diarrhoea. Several probiotic strains of *Lactobacillus* and *Bifidobacteria* were reported to inhibit adhesion of enteropathogenic *E. coli* to intestinal mucosa (Collado et al., 2005) and to Caco-2 cell line (Lee et al., 2000; Forestier et al., 2001; Gopal et al., 2001; Lee and Puong, 2002; Gagnon et al., 2004). However, according to the other publications, no competitive exclusion of pathogenic *E. coli* by lactobacilli was observed in similar assays (Ouwehand et al., 2001; Parassol et al., 2005).

The aim of the present study was to investigate the adhesion of both known strains and new isolates of probiotic *Lactobacillus,* incubated separately and in competition using IPEC-J2 cell line model. Possible inhibition of attachment of enteropathogenic *E. coli* O138 to IPEC-J2 by lactobacilli was also studied. In addition, we examined the effect of divalent ions on the adhesion of *Lactobacillus* ssp. and *E. coli* incubated alone and in competitive assays on IPEC-J2 cells.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich A/S (Denmark) or Merck (Damstadt, Germany) unless indicated otherwise.

2.2. Bacterial strains and growth media

The *Lactobacillus* strains examined in this study are listed in Table 1. The strains were maintained in de Man, Rogosa and Sharpe broth (MRS; Difco Laboratories; Becton, Dickinson and Co, USA) containing 15% (vol/vol) glycerol at −80 °C.

Table 1
Origin and source of investigated *Lactobacillus* strains

Strain	Origin	Source [a]
L. reuteri DSM 12246	Pig faeces	DSM, Germany
L. reuteri ATCC 55730	Human intestine	BIOGAIA, Sweden
L. reuteri DC 20	Human isolate	Danisco A/S, Denmark
L. plantarum Q47	Biopsy of human adults	KVL, Denmark
L. plantarum DC 13	Raw ham	Danisco A/S, Denmark
L. plantarum M.1.1	Faeces of human babies	KVL, Denmark
L. rhamnosus GG	Human adult faeces	LGG® products, Valio Ltd., Finland
L. johnsonii NCC 533	Human intestine	NESTEC LTD, Switzerland
L. paraplantarum D14	Human adult faeces	KVL, Denmark
L. acidophilus X37	Biopsy of human adults	KVL, Denmark
L. crispatus LMG 18191	Chicken faeces	LMG, Belgium

[a] DSM — Deutsche Sammlung von mikroorganismen und Zellkulturen Gmb H, Braunschweig, Germany; LMG — Laboratorium voor Microbiologie (Co-ordinated Collections of Micro-organisms), Universiteit Gent, Belgium; ATCC — The American Type Culture Collection, Manassas, Virginia, USA.

E. coli O138 (strain no. 99–10502–1) is a Shiga-like toxins producing strain and was isolated from pigs with post-weaning diarrhoea. The strain was kindly donated by the Danish Institute for Food and Veterinary Research (Copenhagen, Denmark) and maintained in Luria–Bertani (LB) broth in 15% (vol/vol) glycerol.

2.3. IPEC-J2 cell line

The piglet jejunal epithelial cell line IPEC-J2 was kindly provided by Professor Anthony Blikslager, North Carolina State University, USA. The cells were cultivated in tissue culture flasks (TPP, Switzerland) in a 1:1 mixture of Dulbecco's Modified Eagle Medium (DMEM) and F12 solution supplemented with 100 mg/l streptomycin (Fluka Chemie GmbH, Steinheim, Switzerland), 100 mg/l penicillin, 2 mM L-glutamine, 1 mM pyruvate and 10% (vol/vol) fetal bovine serum (Cambrex Bio Science, Verviers, Belgium). The cells were routinely grown at 37 °C in a 95% air — 5% CO_2 atmosphere in humidified incubator. The culture medium was replaced every other day.

2.4. In vitro adhesion assays on IPEC-J2

Adhesion assays were performed essentially according to the method of Kühle et al. (2006). Briefly, 2 mL of IPEC-J2 cell suspension in DMEM were added in 12 wells tissue culture plates (Greiner Bio-One, Germany) at a concentration of approximately 5.0×10^5 cells/mL and grown as described above 4–5 days until confluence. Prior adhesion assays IPEC-J2 monolayers were washed twice with DMEM without antibiotics (streptomycin and penicillin). Bacterial strains were cultivated in MRS broth containing 2.5 μCi/mL of L-[methyl-^{3}H] methionine (1 mCi/mL; Amersham Bioscience, Sweden) under micro-aerophilic conditions at 37 °C. Cells were harvested at the stationary phase after 18 h of incubation at an

amount corresponding to approximately 10^8 colony forming units (CFU)/well and washed three times in phosphate buffered saline PBS (9.0 g NaCl, 0.144 g K_2PO_4 and 0.795 g Na_2HPO_4). The volume of the culture with the required number of CFU was determined by measuring optical density (OD) at 600 nm in the sample and using a conversion factor determined for each strain in the prior experiments. The CFU were determined by plating serial dilutions of *Lactobacillus* suspensions on MRS agar plates and incubating the plates in micro-aerophilic conditions at 37 °C overnight. In the following procedure DMEM without antibiotics was used. Bacterial cells were re-suspended in DMEM and added into the wells coated with IPEC-J2 monolayer in the total volume of 1 mL medium. Non-viable cultures were prepared immediately before application on IPEC-J2 by treatment of the cell suspensions in DMEM with gentamycin (1 mg/mL, Life Technologies, Gibco, Rockville, MD, USA) at 37 °C for 1 h. Cultures were considered non-viable if no colonies were produced after the culture was plated onto MRS agar and incubated in micro-aerophilic conditions at 37 °C overnight. In some experiments divalent ions in form of $CaCl_2$, $MgCl_2$ and $ZnCl_2$ and tri-natriumcitrate-dihydrate were dissolved in DMEM, each at the concentration of 10 mM, and pH was adjusted to 7.5. Plates were incubated at 37 °C for 1 h. Bacterial suspension was discarded and IPEC-J2 monolayers washed three times with 1 mL DMEM to remove the non-attached bacteria. The monolayers with adhered bacteria were solubilized by incubation overnight with 500 μL of NaOH/SDS solution (0.1 M NaOH, 0.1% w/vol SDS (Amersham Bioscience) at room temperature. Samples of the solubilized cells and of the original suspensions added to the wells were mixed with OptiPhase scintillation liquid (Fisher Chemicals, Loughsborough Leisc., UK) and their radioactivity was measured by scintillation counting (Wallac 1414 WinSpectral, Turku, Finland). The adhesion of bacterial strains was determined by comparing the counts in the adhered cells with the counts in the added samples. The experiment was performed in triplicate.

2.5. Competitive adhesion assay

Competitive adhesion assays were performed for the following combinations of bacteria strains: *L. reuteri* DSM 12246 with *L. johnsonii* NCC 533, *L. reuteri* DSM 12246 with *L. rhamnosus* GG (LGG), and *L. plantarum* Q47 with *L. johnsonii* NCC 533. The cells of *L. reuteri* DSM 12246 and *L. plantarum* Q47 were labelled with L-[methyl-^{3}H] methionine as described above. The cells of *L. johnsonii* NCC 533 and *L. rhamnosus* GG were labelled during incubation in MRS broth containing 2.5 μCi/mL of L-[^{35}S] methionine (15 mCi/mL; Amersham Bioscience, Sweden) at the same conditions. Changing of the radio-active label did not affect the adhesion results (data not shown). The strains were either added onto the IPEC-J2 monolayer either simultaneously and co-incubated (co-incubation assay) as described above, or one of the strains was applied on IPEC-J2 before another (displacement assay). In the displacement assays, one of the strains was pre-incubated on IPEC-J2 at the concentration of approximately 10^8 CFU/well for 30 min at 37 °C, followed by the addition of another strain at the same concentration and further co-incubation for the next 30 min. Washing and solubilization of monolayers with adhered bacteria and measurements of radioactivity was performed as *in vitro* adhesion assays. Competitive adhesion was assessed in two independent experiments performed in triplicate in the absence and the presence of Ca ions.

2.6. Adhesion inhibition of E. coli O138

The cell suspensions of *Lactobacillus* strains in DMEM were added onto the IPEC-J2 monolayer at the concentration of approximately 10^8 CFU/well, pre-incubated for 30 min at 37 °C. Suspensions were then removed and the monolayers washed three times with DMEM. Afterwards, *E. coli* O138 suspensions in DMEM were applied into the wells at the same concentration and incubated further for 30 min. CFU of *E. coli* O138 cell suspensions were determined by plating serial dilutions on LB-agar and incubation the plates for 24 h at 37 °C. The inhibition of *E. coli* O138 adhesion was quantified by comparison of CFU counts of adhered bacteria to the counts in the control experiments without lactobacilli.

2.7. Statistical analysis

Statistical analysis of adherence data was performed by using the Paired Student's *t* test ($P<0.05$) after one-way analysis of variance (ANOVA) provided by SAS statistic program (release 8.2, NC, USA).

3. Results

3.1. Adhesion of Lactobacillus ssp. to IPEC-J2

The adhesion study to IPEC-J2 cell line was carried out on 11 strains of *Lactobacillus* as presented in Table 2. The binding ability varied significantly between the strains. The strongest adherence of 38% in average exhibited *L. reuteri* DSM 12246 followed by *L. plantarum* Q47 with adhesion levels of 24%.

Table 2
Adhesion of *Lactobacillus* strains to IPEC-J2 cells

Strain	Adhesion (±SD) [a]
	%
L. reuteri DSM 12246	38.0±6.0
L. reuteri ATCC 55730	3.5±2.7
L. reuteri DC 20	4.2±0.9
L. plantarum Q47	24.1±3.8
L. plantarum DC 13	8.7±2.8
L. plantarum M.1.1	1.8±1.7
L. rhamnosus GG	10.9±2.6
L. johnsonii NCC 533	4.0±2.3
L. paraplantarum D14	15.8±4.7
L. acidophilus X37	3.9±1.2
L. crispatus LMG 18191	2.8±1.7

[a] Adhesion is expressed as percentage of the radiolabelled bacteria adhered to IPEC-J2 compared to the total counts of radioactivity in the added samples. Means of three independent determinations±standard deviation are presented.

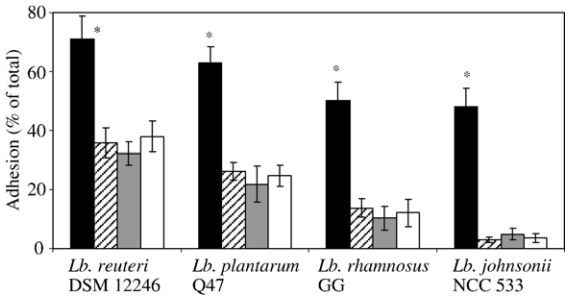

Fig. 1. The effect of divalent ions on adhesion of *L. reuteri* DSM 12246, *L. plantarum* Q47, *L. rhamnosus* GG, and *L. johnsonii* NCC 533 to the IPEC-J2 cell line: (■) — Ca ions; (▨) — Mg ions; (▩) — Zn ions; (□) — controls without addition of divalent ions. Additives were added in form of chloride salts at the concentration of 10 mM. Cultures were labelled with L-[methyl-^{3}H] methionine and incubated on IPEC-J2 monolayers at 37 °C for 1 h. The extent of adhered bacteria was determined by scintillation counts. Adhesion levels significantly different ($P<0.054$) from the controls are denoted with asterisks.

Moderate to low binding of 8–16% was demonstrated for *L. paraplantarum* D14, *L. rhamnosus* GG and *L. plantarum* DC 13. Adhesion of the remaining strains, including *L. johnsonii* NCC 533, *L. reuteri* ATCC 55730, *L. plantarum* M.1.1, *L. reuteri* DC20 and *L. crispatus* LMG 18191, was less than 5%.

3.2. Effect of divalent ions on adhesion of Lactobacillus ssp. to IPEC-J2 cells

The effect of divalent ions on adherence of *L. reuteri* DSM 12246, *L. plantarum* Q47, *L. rhamnosus* GG, and *L. johnsonii* NCC 533 to IPEC-J2 is shown in Fig. 1. Addition of Ca ions significantly enhanced ($P<0.05$) the binding of all tested strains. The largest effect was registered for *L. johnsonii* NCC 533 and for *L. rhamnosus* GG, for which adhesion was increased by more than 10-fold and 5-fold, respectively, as compared to the controls without calcium. Adhesion of bacteria was reduced to the original levels upon removal of Ca ions by addition of natriumcitrate in the reaction medium (data not shown). The similar effect of calcium was established in the binding experiments on IPEC-J2 with non-viable cultures obtained by treatment with gentamycin (results not shown). No significant change in attachment of lactobacilli was observed upon addition of Mg and Zn ions.

3.3. Competitive adhesion of Lactobacillus ssp.

The results of competitive adhesion assays carried out with and without calcium are presented in Table 3. Strongly adhesive strains, *L. reuteri* DSM 12246 and *L. plantarum* Q47, were used in combination with the less adhesive *L. rhamnosus* GG and *L. johnsonii* NCC 533. Adhesion of bacteria to IPEC-J2 cells was examined when the strains were either incubated simultaneously (co-incubation assays; Table 3) or one of the tested lactobacilli was applied on IPEC-J2 before another (displacement assays; Table 3). Results of competitive assays were compared to the corresponding adhesion data obtained when the strains were incubated alone with and without calcium, at the same experimental conditions (controls; Table 3).

Under conditions of co-incubation, binding of the highly adhesive strains, *L. reuteri* DSM 12246 and *L. plantarum* Q47, to IPEC-J2, was not found to be significantly different from the controls. On the contrary, attachment of *L. johnsonii* NCC 533 and *L. rhamnosus* GG was significantly ($P<0.05$) decreased in the co-incubation experiments in the Ca-dependent manner. The lowest adhesion of about 5% for LGG and to the non-detectable levels for *L. johnsonii* NCC 533 was observed in the absence of Ca ions. In the presence of calcium, adherence of *L. johnsonii* NCC 533 was reduced by approximately 2-fold as compared to the control assays with calcium, reaching 26% upon co-incubation with *L. reuteri* DSM 12246 and 22% while co-incubated with *L. plantarum* Q47. Similarly, adhesion of LGG was reduced from 50% when incubated alone with calcium to 35% under co-incubation with *L. reuteri* DSM 12246.

In displacement assays (Table 3) the strains *L. reuteri* DSM 12246 and *L. plantarum* Q47 showed significantly reduced binding ($P<0.05$) both in the presence and the absence of calcium ions when applied on IPEC-J2 after the weakly adhesive strains. The largest reduction in adhesion of these strains, by about 2-fold compared to the controls, was observed in the presence of Ca ions.

Table 3
Adhesion of *Lactobacillus* ssp. to IPEC-J2 cells in co-incubation assays and in displacement assays assessed in the absence of Ca ions (−Ca) and the presence of Ca ions (+Ca)

Assays	*Lactobacillus* strains	Adhesion (±SD), %[a]	
		−Ca	+Ca
Co-incubation:			
L. reuteri DSM 12246 and	DSM 12246	39.0±5.1	63.5±5.0
L. johnsonii NCC 533	NCC 533	0*	26.1±6.9*
L. plantarum Q47 and	Q47	23.0±2.7	57.9±3.0
L. johnsonii NCC 533	NCC 533	0*	21.8±5.6*
L. reuteri DSM 12246 and	DSM 12246	44.2±4.8	68.0±5.8
L. rhamnosus GG	LGG	4.6±1.3*	34.7±4.5*
Displacement:			
L. reuteri DSM 12246 applied	DSM 12246	41.7±3.9	62.7±2.4
before *L. johnsonii* NCC 533	NCC 533	0*	0*
L. johnsonii NCC 533 applied	DSM 12246	27.1±3.5*	32.8±6.0*
before *L. reuteri* DSM 12246	NCC 533	0*	33.6±3.7*
L. plantarum Q47 applied	Q47	24.9±2.5	55.1±5.3
before *L. johnsonii* NCC 533	NCC533	0*	1.8±1.0*
L. johnsonii NCC 533 applied	Q47	18.4±1.7*	35.5±2.0*
before *L. plantarum* Q47	NCC533	0*	37.1±5.3*
L. reuteri DSM 12246 applied	DSM 12246	43.2±3.1	65.1±8.4
before *L. rhamnosus* GG	LGG	0*	6.7±2.9*
L. rhamnosus GG applied	DSM 12246	26.1±3.3*	30.6±2.4*
before *L. reuteri* DSM 12246	LGG	13.6±4.0	33.4±6.0*
Controls:			
L. reuteri DSM 12246	DSM 12246	38.0±5.2	70.9±7.9
L. johnsonii NCC 533	NCC 533	3.5±1.5	48.2±6.2
L. plantarum Q47	Q47	24.7±3.6	63.0±5.5
L. rhamnosus GG	LGG	12.1±4.7	50.2±6.3

[a] Adhesion is expressed as percentage of the radiolabelled bacteria adhered to IPEC-J2 compared to the total counts of radioactivity in the added samples. Means of three independent determinations±standard deviation are presented. Adhesion values of the strains incubated separately at the similar conditions were used as controls. Asterisks denote the values significantly different ($P<0.05$) from the controls.

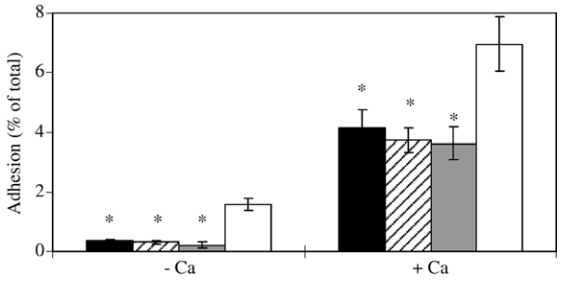

Fig. 2. Adhesion of *E. coli* O138 to IPEC-J2 cells in the inhibition assays with *L. reuteri* DSM 12246 (■), *L. plantarum* Q47 (▨), *L. rhamnosus* GG (▩) and in the control experiments without probiotic strains (□). The cultures were pre-incubated on IPEC-J2 monolayer for 30 min at 37 °C, followed by addition of *E. coli* O138 and further incubation for 30 min. Assays were carried out in the absence and the presence of Ca ions (−Ca and +Ca) added in form of $CaCl_2$ at the concentration of 10 mM. Adhesion values were determined by CFU counts as an extent of adhered bacteria to the loaded number of bacteria. Asterisks denote adhesion values significantly different ($P<0.05$) from the controls.

Attachment of the poorly adhesive *L. rhamnosus* GG and *L. johnsonii* NCC 533 in displacement assays was greatly affected by the order of strain application on IPEC-J2 and by addition of $CaCl_2$. Thus, when the highly adhesive strains were applied first, adhesion of *L. johnsonii* NCC 533 was significantly decreased ($P<0.05$) up to non-detectable levels in all the assays, while the binding of LGG was negligible in the absence of Ca and reduced to 6.7% in the presence of Ca ions. In case of application of the weakly adhesive strains prior their highly adhesive counterparts, attachment of *L. johnsonii* NCC 533 in the absence of calcium was close to non-measurable values, in contrast to *L. rhamnosus* GG which binding in the similar assays was not significantly different from the controls. In the same experiments performed in the presence of calcium ions, adhesion of *L. johnsonii* NCC 533 and LGG, though significantly reduced ($P<0.05$) compared to controls, was however considerably higher than without calcium ions. Namely, adhesion of *L. johnsonii* NCC 533 decreased from 48% in the controls to 33.6% and 37.1% when incubated before *L. reuteri* DSM 12246 and *L. plantarum* Q47, respectively. In the same way, LGG demonstrated reduction in adherence from 50.2% in control experiments to approximately 33.4% when followed by *L. reuteri* DSM 12246.

3.4. Adhesion inhibition of E. Coli O138 by Lactobacillus ssp.

Adhesion of pathogenic strain *E. coli* O138 to IPEC-J2, assessed in inhibition assays with probiotic bacteria *L. reuteri* DSM 12246, *L. plantarum* Q47 and *L. rhamnosus* GG in the presence and the absence of Ca ions, is shown in Fig. 2. Addition of Ca ions in the reaction medium resulted in considerably higher binding of *E. coli* O138. Thus, attachment of *E. coli* to IPEC-J2 increased from 1.6% to 7.0% in Ca-containing medium when *E. coli* was incubated alone without probiotics. Probiotic strains had a strong inhibitory effect on adhesion of *E. coli* O138 in the strain independent manner. As seen from Fig. 2, adhesion was significantly reduced ($P<0.05$) as compared to the controls from 1.6% to less than 0.5% in the absence of Ca and from 7% to 4% in the experiments with calcium.

4. Discussion

We have assessed the *in vitro* binding abilities of 11 *Lactobacillus* isolates. Among the tested strains were probiotic bacteria with well-documented effects, including *L. rhamnosus* GG (Arvola et al., 1999), *L. reuteri* ATCC 55730 (Valeur et al., 2004; Dobrogosz, 2005), *L. johnsonii* NCC 533 (Ibnou-Zekri et al., 2003; Bernet et al., 1994) and *L. reuteri* DSM 12246 (Rosenfeldt et al., 2002; Rosenfeldt et al., 2003) and new isolates of potential probiotic strains of *Lactobacillus*. The porcine epithelial IPEC-J2 cell line was used as an *in vitro* model for the human intestinal epithelium. Although not exactly mimicking natural conditions, IPEC-J2 cell line provide a broad variety of cell functions of intestinal epithelia and is considered to be a good model system for studies of bacterial-host interactions in the gut (Schierack et al., 2005).

The tested strains of *Lactobacillus* differed considerably in their binding abilities to IPEC-J2 cells. The highest adhesion of about 40% was observed for *L. reuteri* DSM 12246, a probiotic strain previously characterised as being strongly adhesive to Caco-2 cells (Jacobsen et al., 1999). *L. plantarum* Q47, a new isolate from human biopsies, was another strain which displayed a high adhesiveness (of 25%). The adhesion of 15% that was observed for *L. rhamnosus* GG is in good agreement with the results on Caco-2 published by Tuomola and Salminen (1998). However, it is less than the values reported for LGG by Kirjavainen et al. (1998) and Ouwehand et al. (1999) in the experiments with humane mucus models, probably due to deficiency of mucins secretion by IPEC-J2. Binding of *L. johnsonii* NCC 533 was found to be lower than that of LGG which is also in agreement with the previous findings (Jacobsen et al., 1999; Ouwehand et al., 1999; Ouwehand et al., 2002). Unexpectedly, *L. johnsonii* NCC 533 and *L. reuteri* ATCC 55730, extensively studied and commonly used for their probiotic-associated activities, were among the poorly adhesive strains.

The attachment of *L. reuteri* DSM 12246, *L. plantarum* Q47, *L. rhamnosus* GG and *L. johnsonii* NCC 533 to IPEC-J2, was significantly increased upon addition of Ca ions, but not affected by divalent ions Mg and Zn; this is in accordance with prior findings (Deman et al., 1974; Enriquez-Verdugo et al., 2004). Concentration of calcium in adhesion assays was of 10 mM that corresponds to calcium content in milk, pointing to the importance of calcium as an ingredient of milk-based probiotic foods. The effect of Ca was demonstrated both in viable cultures and antibiotic-treated non-viable cultures. These results suggest that intracellular events, such as Ca-dependent signal transduction, are not among the mechanisms that affect the adhesion.

The recent developments in probiotic foods are directed towards mixed cultures formulations. The competition experiments on IPEC-J2 showed that attachment of *L. rhamnosus* GG and *L. johnsonii* NCC 533 was strongly reduced by *L. reuteri* DSM 12246 and *L. plantarum* Q47 both in co-incubation and displacement assays. It indicates that probiotic cultures share the same binding sites for attachment to intestinal cells. Consequently, poorly adhesive strains might be out-competed by strongly adhesive strains at natural conditions as well after intake of complex probiotic formulations, which is not

desirable, since all the probiotic components are supposed to exert a beneficial effect. Furthermore, it was revealed that in the presence of calcium ions, binding of poorly adhesive strains was considerably increased as compared to the same assays without Ca, particularly when the strains were applied before or simultaneously with strongly adhesive strains. In this context, the control of calcium content in probiotic formulations seems to be especially important as it might influence the attachment of weakly adhesive probiotic strains to the intestine.

Displacement assays with pathogenic *E. coli* O138 showed that all tested *Lactobacillus* strains reduced the attachment of *E. coli* to IPEC-J2 by more than 2-fold both in the presence and the absence of calcium ions. These results are supported by prior research and indicate a significant effect of lactobacilli on adhesion of different pathogenic strains (Lehto and Salminen, 1997; Mack et al., 1999; Tuomola et al., 1999; Lee et al., 2000). Furthermore, the strains were not significantly distinguished in the extent of inhibitory effect, indicating that adhesion inhibition of *E. coli* O138 was most likely due to steric hindrance rather than to involvement of the specific binding sites. The ability of probiotics to displace other microorganisms is important for prevention of the colonization of the intestine by harmful bacteria.

In conclusion, in this study a large variation between the strains of *Lactobacillus* in their adhesion abilities to IPEC-J2 cells was demonstrated. Knowledge of adhesive characteristics is important for selection of probiotic strains, as high adhesive properties might enable lactobacilli to establish themselves in the intestine and to displace the weakly adhesive strains. All tested strains of *Lactobacillus* were found to be effective in reducing adhesion of *E. coli* O138 to IPEC-J2, indicating on their possible protective role against infection of *E. coli*. The positive effect of Ca on adhesion of lactobacilli was established in this research and might be useful for development of probiotic foods.

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