Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli

Yuan-Kun Lee,¹ Kim-Yoong Puong,¹ Arthur C. Ouwehand²
and Seppo Salminen²

Correspondence

Yuan-Kun Lee

micleeyk@nus.edu.sg

¹Department of Microbiology, Faculty of Medicine, National University of Singapore, 5 Science Drive 2, Singapore 117597

²Department of Biochemistry and Food Chemistry, University of Turku, Fin-20014 Turku, Finland

Competition, competitive exclusion and displacement of eight strains of *Escherichia coli* and *Salmonella* spp. by *Lactobacillus rhamnosus* GG and *Lactobacillus casei* Shirota from adhesion on human intestinal mucus glycoproteins and Caco-2 cell surfaces were studied. Lactobacilli were able to compete with, exclude and displace pathogenic gastrointestinal (GI) bacteria when they were incubated together, but the degree of inhibition of adhesion was bacterial strain-dependent.

Competition and exclusion profiles of GI bacteria by lactobacilli were similar. Displacement profiles of GI bacteria were different from those of competition and exclusion and the process was relatively slow: displacement equilibrium took more than 2 h. These findings are important for development, selection and *in vitro* assessment of target- and function-specific probiotics.

Received 24 June 2002

Accepted 24 December 2002

INTRODUCTION

Observations have been made that *Lactobacillus* supplementation in food may not significantly alter the intestinal microbiota, including potential pathogens, in the gastrointestinal (GI) tract of human subjects (Millar *et al.*, 1993; Apostolou *et al.*, 2001). There are, however, reports that probiotic lactobacilli protect the human host (especially infants) against GI infections (Gorbach *et al.*, 1987; Isolauri *et al.*, 1994; Saavedra *et al.*, 1994; Grönlund *et al.*, 2000). These health-effects of probiotic bacteria have been attributed to non-competitive exclusion mechanisms, such as modulation of immune responses (O'Halloran *et al.*, 1998; Pelto *et al.*, 1998), enhancement of mucosal repair (Elliott *et al.*, 1998; Kirjavainen *et al.*, 1999) and non-specific proliferation enhancement of intestinal anaerobes (Apostolou *et al.*, 2001). Probiotics have been reported to alter enzymic activities in the human intestine, which may require alteration of intestinal microflora (Goldin & Gorbach, 1984; Ling *et al.*, 1992). The ability of probiotic bacteria to compete with pathogens for adhesion sites on the intestinal mucosal surface is still under investigation. Ability to adhere to the surface of epithelial cells is a key pathogenic factor of intestinal pathogens (Levine, 1987; Alam *et al.*, 1996; Weinstein *et al.*, 1998; Scaletsky *et al.*, 2002). Lactobacilli have been shown to possess surface adhesins similar to those on bacterial pathogens (Neeser *et al.*, 2000). The current *in vitro* study of competition for adhesion to human intestinal

mucus glycoproteins and enterocyte-like Caco-2 cell surfaces was designed to examine and characterize mechanisms of adhesion. It is hoped that these will provide a mechanistic basis for the development of selection criteria for optimal probiotics that can competitively exclude GI pathogens.

METHODS

Bacterial strains. The two probiotic lactobacilli studied are *Lactobacillus casei* strain Shirota (Yakult; Singapore) and *Lactobacillus rhamnosus* strain GG (ATCC 53103). Both bacterial strains have probiotic properties that have been demonstrated clinically (Lee *et al.*, 1999). Lactobacilli were cultured in MRS broth (BBL) at 37 °C in an atmosphere of 5% (v/v) CO₂ in air for 18–20 h before the study.

Escherichia coli O157, *E. coli* ATCC 11775, *Salmonella choleraesuis* subsp. *choleraesuis* serotype *typhimurium* (*Salmonella typhimurium*) ATCC 14028 and *S. choleraesuis* subsp. *choleraesuis* serotype *enteritidis* (*Salmonella enteritidis*) ATCC 13076 were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). *S. typhimurium* E10 (NCTC 8391) was obtained from the National Collection of Type Cultures (NCTC; Colindale, UK). *E. coli* TG1 (Gibson, 1984) was obtained from the collection of our department, whereas *S. typhimurium* E12 and '*Salmonella bellurup*' E23 are faecal isolates provided by the National University Hospital. These bacteria were grown in Luria–Bertani broth (BBL) at 37 °C for 18–20 h before use.

To label bacteria, methyl (1',2'-³H)-thymidine was added to the medium at a concentration of 10 µl ml⁻¹ (117 Ci mmol⁻¹). After growth, the lactobacillus strains were washed twice with sterile acetate buffer (pH 5.0) and resuspended in the same buffer. The other eight potential pathogens were washed once with acetate buffer (pH 5.0) that contained 0.1% (w/v) sodium azide to avoid bacterial invasion. These GI bacteria were then washed once more with acetate buffer and resuspended in the same buffer.

Abbreviations: GI, gastrointestinal; LCS, *Lactobacillus casei* Shirota; LGG, *Lactobacillus rhamnosus* GG.

Intestinal cell culture. Caco-2 cell cultures were used in the adhesion assay (Fogh *et al.*, 1977). This human colon adenocarcinoma cell-line was obtained from the ATCC. Cells were cultured in Dulbecco's modified Eagle's minimal essential medium (Gibco-BRL) that contained 25 mM glucose, 20 % (v/v) heated inactivated fetal calf serum (Gibco-BRL) and 1 % non-essential amino acids (Gibco-BRL). Cells were grown at 37 °C in an atmosphere of 5 % v/v CO₂ in air. For the adhesion assay, monolayers of Caco-2 cells were prepared in 24-well tissue-culture dishes (Falcon type 3047; Becton Dickinson) by inoculating 1 × 10⁵ viable cells per well in 1.0 ml culture medium. Medium was replaced every 2 days.

Intestinal mucus. Human intestinal mucus glycoproteins were isolated from faeces of healthy adult volunteers by extraction and dual ethanol precipitation (Ouwehand *et al.*, 2001). In short, faecal extracts were prepared by homogenizing faeces in PBS (pH 7.4) that contained protease inhibitors and sodium azide and centrifuging the suspension at 15 000 g. Mucus was isolated from the clear faecal extract by dual ethanol precipitation; the crude mucus was further lyophilized and stored at 4 °C.

Adhesion assay

(i) On Caco-2 cells. Fifteen-days-post-confluent Caco-2 cell monolayers were washed once with 1 ml sterile acetate buffer (pH 5.0) before the adhesion assay. Bacteria at concentrations between 1×10⁸ and 1×10⁹ c.f.u. ml⁻¹ were added to each well in 1.0 ml (total volume) acetate buffer (pH 5.0) and incubated at 37 °C in an atmosphere of 5 % (v/v) CO₂ in air with gentle rocking. After 60 min incubation, monolayers were washed three times with sterile acetate buffer (pH 5.0) to remove free bacterial cells. Concentration of adhered bacterial cells was estimated from radioactivity, which was assayed by liquid scintillation (Ouwehand *et al.*, 2001).

(ii) On immobilized mucus. Study of adhesion of micro-organisms to mucus glycoproteins was performed as described previously (Ouwehand *et al.*, 1999). In short, 100 µl human intestinal mucus (0.5 mg ml⁻¹) in HEPES–Hanks buffer (10 mmol HEPES l⁻¹, pH 7.4) was immobilized in polystyrene microtitre plate wells (MaxiSorp; Nunc) by overnight incubation at 4 °C. Wells were washed once with 200 µl acetate buffer (pH 5.0). Bacterial suspension (100 µl) at concentrations between 1.0 × 10⁸ and 5.0 × 10⁸ c.f.u. ml⁻¹ was added

to the wells, followed by incubation at 37 °C for 1.5 h. Radioactivity was assessed by liquid scintillation.

In the study of competition exclusion for adhesion on both Caco-2 cells and mucus, lactobacillus and the respective GI bacterium were added simultaneously or sequentially. In the latter case, free cells of the first type of bacterium were removed by washing with acetate buffer (pH 5.0) before the second type of bacterium was added.

Statistics. Differences between treatments were examined for significance by Student's *t*-test after analysis of variance. *P* > 0.05 was considered to be statistically insignificant.

RESULTS

Adhesion of *L. rhamnosus* GG (LGG) and GI bacteria on human mucin

Competition for adhesion on the surface of mucin was observed when equal concentrations (1 × 10⁸ cells ml) of GI bacteria and LGG were incubated together (Table 1). The degree of competitive inhibition of adhesion of GI bacteria was, however, strain-dependent. *E. coli* O157 showed no inhibition by LGG, whereas *E. coli* ATCC 11775 showed the highest degree of inhibition (83.68 %) within 1 h incubation.

In the exclusion study (Table 1), LGG was allowed to adhere to the mucin surface first and each of the other GI bacteria was added subsequently. The data showed that LGG adhered on the mucin surface was able to exclude all GI bacteria except *E. coli* O157 (no exclusion was observed), with *E. coli* ATCC 11775 showing the highest degree of inhibition (77.73 %) by LGG.

When GI bacteria were allowed to adhere to the mucin first and LGG was added subsequently, low degrees of displacement of the GI bacteria (0–14 %) were observed (Table 1). Adhesion of *E. coli* O157 was enhanced.

Table 1. Inhibition of adhesion of GI bacteria by LGG on human mucin

Changes in adhesion of GI bacteria to the surface of human mucin in the absence of LGG were assigned to 0 % (control).

GI bacterium	Change in adhesion, mean ± SD (%)		
	Competition	Exclusion	Displacement
<i>E. coli</i> O157	3.72 ± 9.94	19.89 ± 45.52	53.93 ± 37.02*
<i>E. coli</i> ATCC 11775	−83.68 ± 1.07*	−77.73 ± 5.29*	−7.23 ± 4.74
<i>E. coli</i> TG-1	−74.20 ± 1.42*	−69.13 ± 2.20*	4.40 ± 0.17
<i>S. enteritidis</i> ATCC 13076	−21.29 ± 10.02*	−35.74 ± 11.77*	−13.04 ± 10.35
<i>S. typhimurium</i> ATCC 14028	−61.60 ± 6.95*	−55.01 ± 9.71*	−10.19 ± 8.74
<i>S. typhimurium</i> E10	−70.01 ± 3.44*	−68.55 ± 3.60*	−1.08 ± 6.53
<i>S. typhimurium</i> E12	−25.11 ± 15.26*	−51.02 ± 5.01*	−14.25 ± 8.52
' <i>S. bellurup</i> ' E23	−13.52 ± 3.27*	−14.38 ± 4.92*	−9.74 ± 5.73

*Significantly different from control (Student's *t*-test: *P* < 0.05).

Adhesion of LGG and GI bacteria on Caco-2 cells

Competition of LGG and GI bacteria, except *E. coli* O157, for adhesion on the surface of Caco-2 cells was observed (Table 2). *E. coli* TG1 showed the highest degree of inhibition (48.97 %) by LGG.

As shown in Table 2, adhered LGG was able to exclude most GI bacteria, except '*S. bellurup*' E23 and *S. typhimurium* E10, with *E. coli* TG1 showing the highest degree of exclusion (37.38 %).

Free LGG was able to displace *S. typhimurium* E10 (45.02 %), *E. coli* ATCC 11775 (31.05 %), *S. enteritidis* (8.87 %) and *S. typhimurium* ATCC 14028 (27.49 %), but not the other GI bacteria (*E. coli* TG1, *S. typhimurium* E12, '*S. bellurup*' E23 or *E. coli* O157) within 1 h incubation together (Table 2). Extension of the incubation time of LGG with adhered *S. typhimurium* ATCC 14028 for another hour (2 h in total) showed a higher degree of displacement (from 27.49 to 36.13 %, $P < 0.05$) (Fig. 1).

Adhesion of *L. casei* Shirota (LCS) and GI bacteria on human mucin

LCS was able to compete with most GI bacteria (except for '*S. bellurup*' E23) for adhesion on the mucin surface (Table 3). *E. coli* TG1, *S. typhimurium* E10, *E. coli* O157, *E. coli* ATCC 11775 and *S. typhimurium* ATCC 14028 showed higher degrees of inhibition.

Degrees of exclusion of GI bacteria by LCS ranged between +20 and -36 %, with *S. typhimurium* E10 and *S. typhimurium* ATCC 14028 showing higher degrees of exclusion by LCS (Table 3).

Degrees of displacement of adhered GI bacteria by LCS were generally low (< 23 %) (Table 3). No displacement was observed with *E. coli* TG1, *S. typhimurium* E10 or *E. coli* O157.

Fig. 1. Displacement study: changes in adhesion of *E. coli* ATCC 11775 and *S. typhimurium* ATCC 14028 when incubated with respective lactobacillus for different incubation time-periods. ♦, LGG+*E. coli* ATCC 11775; ■, LGG+*S. typhimurium* ATCC 14028; ▲, LCS+*S. typhimurium* ATCC 14028.

Adhesion of LCS and GI bacteria on Caco-2 cells

LCS was able to achieve up to 46 % competitive inhibition of the GI bacteria tested (Table 4). Higher degrees of inhibition (> 30 %) were observed for *E. coli* TG1, *S. typhimurium* E10, *E. coli* ATCC 11775 and *S. typhimurium* ATCC 14028.

Higher degrees of exclusion were observed for *E. coli* TG1, *S. typhimurium* E10, *E. coli* O157, *E. coli* ATCC 11775 and *S. typhimurium* ATCC 14028 (Table 4).

Only ≤28 % displacement of GI bacteria by LCS was observed after 1 h incubation (Table 4). *E. coli* TG1 (15.78 %), *E. coli* O157 (no displacement), *E. coli* ATCC 11775 (no displacement) and *S. enteritidis* ATCC 13076 (5.22 %) could not be effectively displaced by LCS within 1 h incubation. When the incubation time of LCS with adhered *E. coli* ATCC 11775 and *S. typhimurium* ATCC 14028 on Caco-2 cells was extended to 2 h, more GI bacteria were

Table 2. Inhibition of adhesion of GI bacteria by LGG on Caco-2 cells

Changes in adhesion of GI bacteria to Caco-2 cells in the absence of LGG were assigned to 0 % (control).

GI bacterium	Change in adhesion, mean ± SD (%)		
	Competition	Exclusion	Displacement
<i>E. coli</i> O157	39.99 ± 44.34	-7.95 ± 3.83	-1.17 ± 0.33
<i>E. coli</i> ATCC 11775	-25.15 ± 12.58*	-27.39 ± 2.08*	-31.05 ± 3.33*
<i>E. coli</i> TG-1	-48.97 ± 2.06*	-37.38 ± 4.35*	6.89 ± 0.91
<i>S. enteritidis</i> ATCC 13076	-41.59 ± 3.20*	-27.53 ± 5.05*	-8.87 ± 3.40
<i>S. typhimurium</i> ATCC 14028	-47.16 ± 2.34*	-28.20 ± 0.23*	-27.49 ± 3.72*
<i>S. typhimurium</i> E10	-18.36 ± 9.24	-4.89 ± 4.04	-45.02 ± 3.41*
<i>S. typhimurium</i> E12	-48.15 ± 5.75*	-29.74 ± 4.55*	-15.35 ± 8.40
' <i>S. bellurup</i> ' E23	-20.99 ± 6.21	9.18 ± 6.99	-4.96 ± 1.32

*Significantly different from control (Student's *t*-test: $P < 0.05$).

Table 3. Inhibition of adhesion of GI bacteria by LCS on human mucin

Changes in adhesion of GI bacteria to the surface of human mucin in the absence of LCS were assigned to 0 % (control).

GI bacterium	Change in adhesion, mean $\pm$ SD (%)		
	Competition	Exclusion	Displacement
<i>E. coli</i> O157	-56.59 $\pm$ 30.77	-4.09 $\pm$ 17.63	2.62 $\pm$ 6.28
<i>E. coli</i> ATCC 11775	-57.14 $\pm$ 3.54*	4.20 $\pm$ 2.81	-6.11 $\pm$ 1.74
<i>E. coli</i> TG-1	-48.62 $\pm$ 3.40*	-1.73 $\pm$ 2.69	-0.55 $\pm$ 7.38
<i>S. enteritidis</i> ATCC 13076	-30.70 $\pm$ 14.36*	19.72 $\pm$ 12.90	-13.04 $\pm$ 10.35
<i>S. typhimurium</i> ATCC 14028	-42.96 $\pm$ 10.16*	-36.28 $\pm$ 6.61*	-10.56 $\pm$ 6.07
<i>S. typhimurium</i> E10	-62.41 $\pm$ 6.39*	-27.35 $\pm$ 3.75*	0.32 $\pm$ 2.70
<i>S. typhimurium</i> E12	-24.23 $\pm$ 9.32*	-15.86 $\pm$ 8.73*	-22.62 $\pm$ 8.19
' <i>S. bellurup</i> ' E23	8.67 $\pm$ 5.89	-12.80 $\pm$ 6.78	-18.90 $\pm$ 5.44

*Significantly different from control (Student's *t*-test: $P < 0.05$).

Table 4. Inhibition of adhesion of GI bacteria by LCS on Caco-2 cells

Changes in adhesion of GI bacteria to Caco-2 cells in the absence of LCS were assigned to 0 % (control).

GI bacterium	Change in adhesion, mean $\pm$ SD (%)		
	Competition	Exclusion	Displacement
<i>E. coli</i> O157	-19.92 $\pm$ 7.82	-23.44 $\pm$ 7.50*	-11.13 $\pm$ 4.24
<i>E. coli</i> ATCC 11775	-31.07 $\pm$ 4.06*	-28.63 $\pm$ 6.25*	-1.92 $\pm$ 11.89
<i>E. coli</i> TG-1	-45.97 $\pm$ 1.62*	-25.82 $\pm$ 8.26*	-15.78 $\pm$ 4.91
<i>S. enteritidis</i> ATCC 13076	-4.73 $\pm$ 20.19	-13.35 $\pm$ 2.59	-5.22 $\pm$ 3.08
<i>S. typhimurium</i> ATCC 14028	-34.59 $\pm$ 6.55*	-30.55 $\pm$ 7.41*	-14.13 $\pm$ 4.52*
<i>S. typhimurium</i> E10	-40.40 $\pm$ 5.52*	-24.82 $\pm$ 2.75*	-24.89 $\pm$ 3.77*
<i>S. typhimurium</i> E12	-6.76 $\pm$ 8.10	-18.62 $\pm$ 1.71*	-27.65 $\pm$ 0.21*
' <i>S. bellurup</i> ' E23	-19.85 $\pm$ 3.80	1.74 $\pm$ 0.30	-15.79 $\pm$ 6.53*

*Significantly different from control (Student's *t*-test: $P < 0.05$).

displaced (*E. coli* ATCC 11775, from 1.92 to 20.18 % displacement; *S. typhimurium* ATCC 14028, from 14.13 to 38.49 % displacement) (Fig. 1).

DISCUSSION

When incubated together, lactobacilli were able to compete with the eight GI bacteria studied for adhesion on human mucin glycoprotein and the surface of Caco-2 cells. The degree of competition was strain-dependent and was probably determined by the affinity of adhesins on respective bacterial surfaces for the stereo-specific receptors that they are competing for, or their relative positions in the case of steric hindrance (Lee & Puong, 2002). The data clearly demon-

strated that each lactobacillus could only compete with a limited range of GI bacteria (including GI pathogens) for adhesion sites. For example, LGG was not able to compete with the enteropathogenic *E. coli* strain O157, whereas LCS failed to compete with '*S. bellurup*' E23. Adhered lactobacilli were able to exclude GI bacteria to different degrees. As expected, the profile of exclusion of GI bacteria by LGG and LCS was similar to that of competition. This confirms that the mechanisms of competition inhibition and exclusion of GI bacteria by LGG and LCS are similar.

Displacement profiles for the GI bacteria by lactobacilli were, however, very different from those of competition and exclusion. Degrees of displacement were generally much lower than the degree of inhibition achieved by competition

and exclusion. Many GI bacteria could not be displaced within 1 h incubation. When the incubation time was extended to 2 h, higher degrees of displacement were observed. These results suggest that displacement of GI bacteria by LGG and LCS is a very slow process. Resident time of food material in the small intestine is about 2 h, whereas liquid beverage stays for an even shorter period of time (Kutchai, 1988). This may not allow sufficient time for incoming LGG and LCS to displace adhered GI bacteria (including pathogens) on the intestinal surface. In the large intestine, the resident time of faecal material is much longer (up to the time it is discharged); however, most probiotic bacteria travelling along the large GI tract are probably trapped in viscous faecal material. Bacterial concentration in faecal water is much lower.

Slow displacement of adhered GI bacteria by LGG could be understood as follows. Adhesion of LGG to the mucosal surface occurs mainly via hydrophobic interactions (Lee & Puong, 2002); thus, competition for a specific receptor that binds GI bacteria is due to steric hindrance. LGG would not be able to competitively displace an adhered GI bacterial cell unless this cell detaches from the receptor and the binding of LGG hinders the reattachment of the bacterium to the receptor. A GI bacterium with high affinity for the receptor would not detach and would reattach readily.

LCS was shown to possess multiple surface adhesins and up to four adhesins could bind to the mucosal surface at any time (Lee *et al.*, 2000). Such an arrangement is effective for competition and exclusion interactions, as one LCS is able to out-compete up to four pathogens and an adhered LCS could exclude up to four pathogens. However, there is only a low probability that an LCS would displace four adhered pathogens simultaneously. One-to-one competition between lactobacillus and pathogen would have a better chance for the former to displace the latter.

This study has suggested that the method of delivery of probiotics to the host is an important parameter to consider in the preparation of probiotic products. A desirable carrier for probiotic bacteria should allow sufficient time and frequency for interaction between bacteria and adhesion sites on the intestinal surface. Selection of probiotics that compete directly with pathogens, as well as their ability to elicit local immunological responses and enhance recovery of damaged mucosal surfaces, would be a logical approach to the selection and development of probiotics for therapeutic treatment of GI infectious diseases.

ACKNOWLEDGEMENTS

This study was made possible by the visit of Y.-K. L. to the laboratory of S. S. under the Overseas Research Programme supported by the Office of Research, National University of Singapore.

REFERENCES

Alam, M., Miyoshi, S., Yamamoto, S., Tomochika, K. & Shinoda, S.

(1996). Expression of virulence-related properties by, and intestinal adhesiveness of, *Vibrio mimicus* strains isolated from aquatic environments. *Appl Environ Microbiol* **62**, 3871–3874.

Apostolou, E., Peltó, L., Kirjavainen, P. V., Isolauri, E., Salminen, S. J. & Gibson, G. R. (2001). Differences in the gut bacterial flora of healthy and milk-hypersensitive adults, as measured by fluorescence in situ hybridization. *FEMS Immunol Med Microbiol* **30**, 217–221.

Elliott, S. N., Buret, A., McKnight, W., Miller, M. J. S. & Wallace, J. L. (1998). Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. *Am J Physiol* **275**, G425–G432.

Fogh, J., Fogh, J. M. & Orfeo, T. (1977). One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J Natl Cancer Inst* **59**, 221–226.

Gibson, T. J. (1984). *Studies on the Epstein-Barr virus genome*. PhD thesis, University of Cambridge, UK.

Goldin, B. R. & Gorbach, S. L. (1984). Alterations of the intestinal microflora by diet, oral antibiotics, and *Lactobacillus*: decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. *J Natl Cancer Inst* **73**, 689–695.

Gorbach, S. L., Chang, T. W. & Goldin, B. (1987). Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet* **ii**, 1519.

Grönlund, M.-M., Arvilommi, H., Kero, P., Lehtonen, O.-P. & Isolauri, E. (2000). Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. *Arch Dis Child Fetal Neonatal Ed* **83**, F186–F192.

Isolauri, E., Kaila, M., Mykkanen, H., Ling, W. H. & Salminen, S. (1994). Oral bacteriotherapy for viral gastroenteritis. *Dig Dis Sci* **39**, 2595–2600.

Kirjavainen, P. V., Apostolou, E., Salminen, S. J. & Isolauri, E. (1999). New aspects of probiotics – a novel approach in the management of food allergy. *Allergy* **54**, 909–915.

Kutchai, H. C. (1988). The gastrointestinal system. In *Physiology*, 2nd edn, pp. 649–744. Edited by R. M. Berne & M. N. Levy. St Louis, MO: Mosby.

Lee, Y.-K. & Puong, K.-Y. (2002). Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate. *Br J Nutr* **88** (Suppl. 1), S101–S108.

Lee, Y.-K., Nomoto, K., Salminen, S. & Gorbach, S. L. (1999). *Handbook of Probiotics*. New York: Wiley.

Lee, Y.-K., Lim, C. Y., Teng, W. L., Ouwehand, A. C., Tuomola, E. M. & Salminen, S. (2000). Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. *Appl Environ Microbiol* **66**, 3692–3697.

Levine, M. M. (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *J Infect Dis* **155**, 377–389.

Ling, W. H., Hanninen, O., Mykkanen, H., Heikura, M., Salminen, S. & Von Wright, A. (1992). Colonization and fecal enzyme activities after oral *Lactobacillus* GG administration in elderly nursing home residents. *Ann Nutr Metab* **36**, 162–166.

Millar, M. R., Bacon, C., Smith, S. L., Walker, V. & Hall, M. A. (1993). Enteral feeding of premature infants with *Lactobacillus* GG. *Arch Dis Child* **69**, 483–487.

Neeser, J. R., Granato, D., Rouvet, M., Servin, A., Teneberg, S. & Karlsson, K. A. (2000). *Lactobacillus johnsonii* La1 shares carbohydrate-binding specificities with several enteropathogenic bacteria. *Glycobiology* **10**, 1193–1199.

O'Halloran, S., Feeney, M., Morrissey, D., Murphy, L., Thornton, G.,

Shanahan, F., Sullivan, G. C. & Collins, J. K. (1998). Adhesion of potential probiotic bacteria to human epithelial cell lines. *Int Dairy J* **8**, 596.

Ouwehand, A. C., Niemi, P. & Salminen, S. J. (1999). The normal faecal microflora does not affect the adhesion of probiotic bacteria in vitro. *FEMS Microbiol Lett* **177**, 35–38.

Ouwehand, A. C., Tuomola, E. M., Lee, Y. K. & Salminen, S. (2001). Microbial interactions to intestinal mucosal models. *Methods Enzymol* **337**, 200–212.

Pelto, L., Isolauri, E., Lilius, E. M., Nuutila, J. & Salminen, S. (1998). Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. *Clin Exp Allergy* **28**, 1474–1479.

Saavedra, J. M., Bauman, N. A., Oung, I., Perman, J. A. & Yolken, R. H. (1994). Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* **344**, 1046–1049.

Scaletsky, I. C. A., Fabbriotti, S. H., Aranda, K. R., Morais, M. B. & Fagundes-Neto, U. (2002). Comparison of DNA hybridization and PCR assays for detection of putative pathogenic enteroadherent *Escherichia coli*. *J Clin Microbiol* **40**, 1254–1258.

Weinstein, D. L., O'Neill, B. L., Hone, D. M. & Metcalf, E. S. (1998). Differential early interactions between *Salmonella enterica* serovar Typhi and two other pathogenic *Salmonella* serovars with intestinal epithelial cells. *Infect Immun* **66**, 2310–2318.