British Journal of Nutrition (2002), **88**, Suppl. 1, S101–S108 DOI: 10.1079/BJN2002635

Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate

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The adhesion of *Lactobacillus rhamnosus* GG to human enterocyte-like Caco-2 cells was not inhibited by eight carbohydrates tested, namely *N*-acetyl-glucosamine, galactose, glucose, fructose, fucose, mannose, methyl-α-D-mannopyranoside and sucrose. The degree of hydrophobicity predicted the adhesion of *L. rhamnosus* GG to Caco-2 cells. *L. rhamnosus* GG, however, was able to compete with *Escherichia coli* and *Salmonella* spp. of low hydrophobicity and high adhesin–receptor interaction for adhesion to Caco-2 cells. The interference of adhesion of these gastrointestinal (GI) bacteria by *L. rhamnosus* GG was probably through steric hindrance, and the degree of inhibition was related to the distribution of the adhesin receptors and hydrophobins on the Caco-2 surface. A Carbohydrate Index for Adhesion (CIA) was used to depict the binding property of adhesins on bacteria surfaces. CIA was defined as the sum of the fraction of adhesion in the presence of carbohydrates, with reference to the adhesion measured in the absence of any carbohydrate. The degree of competition for receptor sites between *Lactobacillus casei* Shirota and GI bacteria is a function of their CIA distance. There were at least two types of adhesins on the surface of *L. casei* Shirota. The study provides a scientific basis for the screening and selection of probiotics that compete with selective groups of pathogens for adhesion to intestinal surfaces. It also provides a model for the characterisation of adhesins and adhesin–receptor interactions.

Probiotics: Gastrointestinal pathogens: Competition for adhesion

Introduction

Adhesion of probiotics to the gastrointestinal (GI) surface is considered a prerequisite for the competitive exclusion of pathogens and for the modulation of local and systemic immunological activities (Salminen *et al.* 1998; Lee *et al.* 1999). Specific adhesin–receptor interactions and non-specific hydrophobic group interactions have been suggested as the major mechanisms for the adhesion of bacteria to GI surfaces (Ofek & Doyle, 1994). The relative importance of these adhesion mechanisms however is not yet clear.

The stereo-specific adhesin–receptor interaction has been demonstrated to involve carbohydrate moieties on the intestinal surface and carbohydrate-binding adhesins on the bacterial cell surface (Ofek *et al.* 1978; Adlerberth *et al.* 1996; Yamamoto *et al.* 1996). Carbohydrates have been shown to inhibit adhesion of bacteria to the intestinal cell surface (Gusils *et al.* 1999; Neeser *et al.* 2000). One may reason that if a suitable soluble carbohydrate could mask the specific adhesin–receptor interaction, the non-specific surface interaction could be studied. Moreover, the carbohydrate inhibition profile of bacterial adhesion to the GI surface would be an indication of the binding characteristics of adhesins.

In this study, the adhesion and competition for adhesion to human intestinal cells (Caco-2 cell line) by two probiotic *Lactobacillus* species and eight strains of four GI bacteria, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella bellurup*, were studied. It was intended that this study would shed light on the mechanisms of bacterial cell adhesion to the GI surface and the competition between bacteria for adhesion.

Materials and methods

Bacterial strains

The two probiotic lactobacilli studied were *Lactobacillus casei* strain Shirota (Yakult Singapore Pty. Ltd.) and *Lactobacillus rhamnosus* strain GG (ATCC 53103). Both bacterial strains have clinically demonstrated probiotic

Abbreviations: ATCC, American Type Culture Collection; CIA, Carbohydrate Index for Adhesion; GI, gastrointestinal; NCTC, National Collection of Type Cultures; PBS, Phosphate buffer saline.

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properties (Lee *et al.* 1999). The lactobacilli were cultured in MRS broth (BBL Cockeysville, MD) at 37°C, in 5 % v/v CO_2 in air atmosphere, for 18–20 h before the study.

Escherichia coli O157, *Escherichia coli* 11775, *Salmonella choleraesuis* subsp. *choleraesuis* serotype *typhimurium* (*S. typhimurium*) 14028 and *Salmonella choleraesuis* subsp. *choleraesuis* serotype *enteritidis* (*S. enteritidis*) 13076 were obtained from American Type Culture Collection (ATCC), USA. *S. typhimurium* E10 (NCTC 8391) was obtained from), National Collection of Type Cultures (NCTC), UK. The *Escherichia coli* TG1 (Gibson, 1984) was obtained from the collection of our Department, whereas *S. typhimurium* E12 and *S. bellurup* E23 were faecal isolates provided by the National University Hospital. Bacteria were grown in Luria-Bertani broth (BBL) at 37°C for 18–20 h before use. To radiolabel the bacteria, methyl-1′,2-[^{3}H]thymidine was added to the medium at a concentration of 10 μl/ml (117 Ci/mmol). After a period of growth, the bacteria were washed twice with sterile acetate buffer (pH 5·0) and then resuspended in the same buffer.

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 ATCC. Cells were cultured in Dulbecco's modified Eagle's minimal essential medium (GIBCO-BRL), containing 25 mM-glucose, 20 % v/v heat-inactivated fetal calf serum (GIBCO-BRL), and 1 % non-essential amino acids (GIBCO-BRL). Cells were grown at 37°C, in 5 % v/v CO_2 in air. For the adhesion assay, monolayers of Caco-2 cells were prepared in 24-well tissue culture dishes (Falcon type 3047 from Becton Dickinson Labware USA) by inoculating 1×10^5 viable cells per well in 1·0 ml of culture medium. The medium was replaced every 2 days.

Carbohydrates tested

Eight carbohydrates were tested: *N*-acetyl-glucosamine, galactose, glucose, fructose, fucose, mannose, methyl-α-D-mannopyranoside and sucrose. A concentrated solution of the respective carbohydrate was added to a suspension of the bacterium in acetate buffer (pH 5·0) to give a 25 mM-carbohydrate solution. The adhesion assay was then performed in the presence of the respective carbohydrate.

Carbohydrate Index for Adhesion (CIA)

The presence of a carbohydrate alters the adhesion properties of bacteria. CIA is an indication of the receptor binding characteristics of bacterial adhesins. The adhesion value without carbohydrate is taken as 1·0 (control) and the adhesion value in the presence of a carbohydrate is taken as a fraction of the control. The CIA is the sum of the fraction of adhesion with reference to the control in the presence of the respective carbohydrate. The CIA distance between a GI bacterium and a lactobacillus is the difference between their CIA values.

Adhesion assay

Fifteen-days-postconfluent Caco-2 cell monolayers were washed once with 1 ml of sterile acetate buffer (pH 5·0) before the adhesion assay. Bacteria at concentrations between 1×10^8 and 1×10^9 CFU/ml were added to each well in 1·0 ml (total volume) of acetate buffer (pH 5·0) and incubated at 37°C, in 5 % v/v CO_2 in air, with gentle rocking. After incubation for 60 min, the monolayers were washed three times with sterile acetate buffer (pH 5·0) to remove free bacterial cells. The concentration of adhered bacterial cells was estimated from the radioactivity assayed using liquid scintillation (Ouwehand *et al.* 2001).

Hydrophobicity measurement

The hydrophobicity measurement method was a modification of Sweet *et al.* (1987). Three ml of the respective bacterial cell suspension in PBS (pH 7·0) was dispensed into 15 ml centrifuge tubes and mixed with 1 ml hexadecane by vortexing vigorously for 30 s. After phase separation (approximately 30 min), the aqueous phase was extracted and centrifuged at 3000 rpm for 10 min in a bench-top centrifuge to remove the remaining hexadecane. The optical density of the aqueous phase was determined at 600 nm. Results were reported as percentage of adherence to hexadecane.

Statistics

Differences between treatments were examined for the level of significance by the Student's *t*-test after analysis of variance. A *P*-value of $<0{\cdot}05$ was considered statistically significant.

Results

Effect of carbohydrate on adhesion of bacteria to Caco-2 cells

The percentage change in the adhesion of the two lactobacilli to Caco-2 cells are presented in Figs 1 and 2. Only *N*-acetyl-glucosamine among the eight carbohydrates tested inhibited the adhesion of *L. rhamnosus* GG to a significant degree ($P=0{\cdot}02$) compared with the control without carbohydrate (Fig. 1). Two of the carbohydrates, namely methyl-α-D mannopyranoside and sucrose, enhanced the adhesion of *L. rhamnosus* GG ($P<0{\cdot}05$). The adhesion of *L. casei* Shirota was inhibited by *N*-acetyl-glucosamine, glucose and fucose ($P<0{\cdot}05$).

The effects of carbohydrates on the adhesion of the other eight strains of GI bacteria are presented in Table 1. Each of the GI bacteria was inhibited by the various carbohydrates to different degrees.

The CIA, the sum of the fraction of adhesion with reference to the control (without carbohydrate) in the presence of the respective carbohydrate, was used to express the cumulative effect of carbohydrates tested on the adhesion of a bacterium. For example, taking adhesion for the control (without carbohydrate) as 1·0, the adhesion of *L. casei* Shirota was 0·68, 0·89, 0·69, 0·93, 0·79, 0·89, 1·0 and 0·98 in the presence of *N*-acetyl-glucosamine, galactose, glucose,

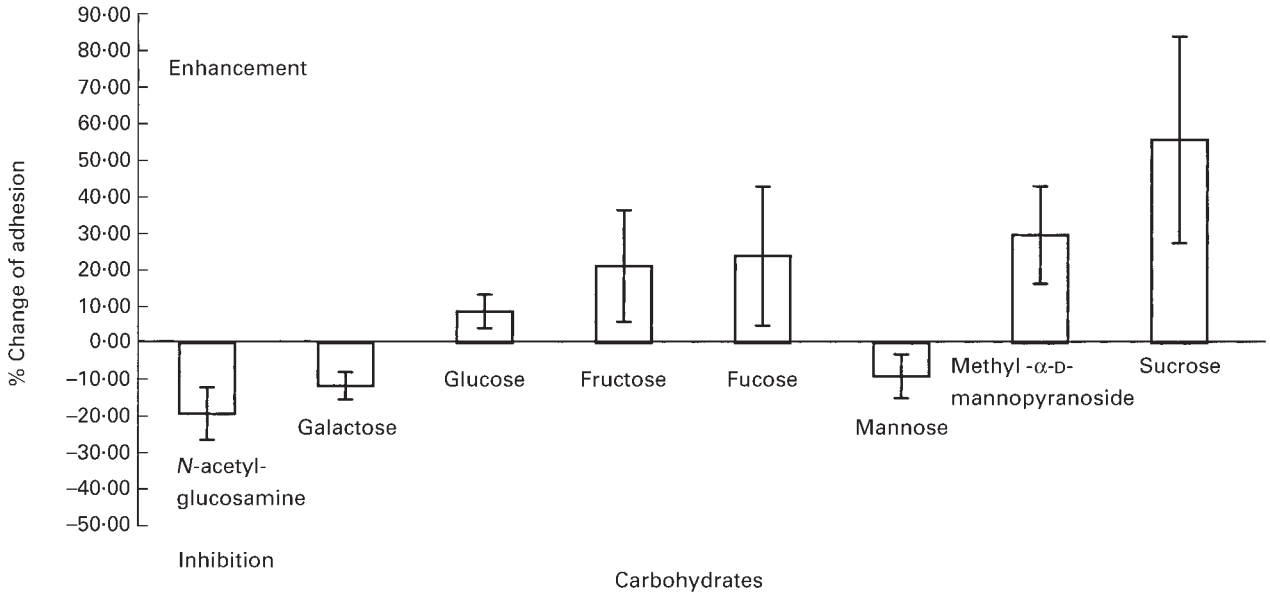

Fig. 1. The effect of carbohydrates on the adhesion of *Lactobacillus rhamnosus GG* to Caco-2 cells. The vertical bars represent the standard deviation.

fructose, fucose, mannose, methyl-α-D-mannopyranoside and sucrose, respectively. The CIA was $0{\cdot}68 + 0{\cdot}89 + 0{\cdot}69 + 0{\cdot}93 + 0{\cdot}79 + 0{\cdot}89 + 1{\cdot}00 + 0{\cdot}98 = 6{\cdot}85$. The CIA values for the ten bacterial strains tested are shown in Table 2. A CIA value close to 8, such as *L. rhamnosus* GG (8·99), indicates that the carbohydrates tested had little effect on the adhesion of the bacterium to Caco-2 cells.

Adhesion of L. casei Shirota *to Caco-2 cells in the presence of* N-*acetyl-glucosamine*

In the adhesion assay for *L. casei* Shirota, 2×10^7 to 5×10^8 cells/ml of the lactobacillus were added to a Caco-2 cell monolayer in the presence or absence of *N*-acetyl-glucosamine. The plots of the reciprocal of adhered cell concentrations versus the reciprocal of the concentration of cells added are given in Fig. 3. In both the presence and absence of the carbohydrate, a linear relationship was observed. It follows from the equation described below that the intercept on the ordinate gives the value of the reciprocal of the maximum number of bacterial cells adhered to Caco-2 cells (e_m). The intercept on the abscissa is $-1/k_x$, where k_x is the dissociation constant for the adhesion process (Lee *et al.* 2000).

$$1/e_x = 1/e_m + k_x/e_m \cdot x$$

where x is the concentration of the bacterial culture added, e_x is the concentration of the adhered bacterium.

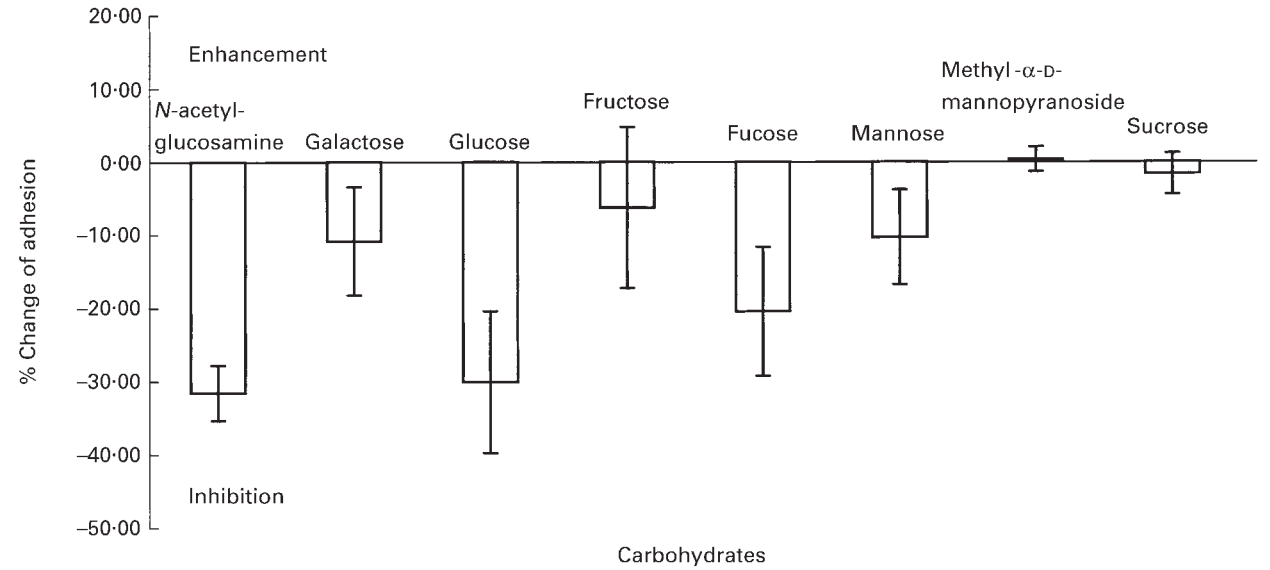

Fig. 2. The effect of carbohydrates on the adhesion of *Lactobacillus casei* Shirota to Caco-2 cells. The vertical bars represent the standard deviation.

Table 1. Adhesion of bacteria on Caco-2 cells in the presence of carbohydrates. The control study was performed in the absence of carbohydrate

Bacterium	Extent of fractional adhesion with respect to the control								
	Control	*N*-acetyl-glucosamine	Galactose	Glucose	Fructose	Fucose	Mannose	Methyl-α-D-mannopyranoside	Sucrose
E. coli 0157	1·00	0·62 ± 0·05	0·74 ± 0·06	0·74 ± 0·05	0·57 ± 0·05	0·77 ± 0·07	0·56 ± 0·04	0·71 ± 0·07	0·64 ± 0·03
E. coli 11775	1·00	0·61 ± 0·06	0·83 ± 0·07	0·74 ± 0·06	0·75 ± 0·07	0·82 ± 0·09	0·48 ± 0·08	0·49 ± 0·13	0·66 ± 0·02
E.coli TG1	1·00	0·80 ± 0·05	0·89 ± 0·14	0·92 ± 0·00	0·66 ± 0·11	0·99 ± 0·02	0·53 ± 0·03	0·50 ± 0·04	0·75 ± 0·02
S. enteritidis 13076	1·00	0·77 ± 0·09	0·90 ± 0·05	0·98 ± 0·03	0·78 ± 0·03	1·01 ± 0·06	0·71 ± 0·08	0·50 ± 0·08	0·82 ± 0·04
S. typhimurium 14028	1·00	0·85 ± 0·05	1·10 ± 0·11	1·22 ± 0·2	0·85 ± 0·06	1·10 ± 0·11	0·69 ± 0·02	0·55 ± 0·03	0·75 ± 0·10
S. typhymurium E10	1·00	0·70 ± 0·03	0·65 ± 0·08	0·64 ± 0·03	0·61 ± 0·06	0·69 ± 0·10	0·70 ± 0·09	0·87 ± 0·22	1·27 ± 0·46
S. typhimurium E12	1·00	0·87 ± 0·10	0·86 ± 0·04	0·57 ± 0·03	0·28 ± 0·06	1·06 ± 0·05	0·30 ± 0·03	0·21 ± 0·01	0·66 ± 0·07
S. bellurup E23	1·00	0·85 ± 0·10	0·91 ± 0·11	0·96 ± 0·18	1·14 ± 0·17	0·84 ± 0·03	0·95 ± 0·12	1·17 ± 0·42	0·44 ± 0·08

As calculated from the plots shown in Fig. 3 using the above equation, the maximum concentration of adhered *L. casei* Shirota to Caco-2 cells in the absence of *N*-acetyl-glucosamine was $1{\cdot}10 \times 10^7$ cells/well, whereas the dissociation constant was $6{\cdot}51 \times 10^8$ cells/well. In the presence of *N*-acetyl-glucosamine, the maximum concentration of adhered *L. casei* Shirota remained unchanged ($1{\cdot}20 \times 10^7$ cells/well), whereas the dissociation constant was about three times higher ($1{\cdot}71 \times 10^9$ cells/well). The higher dissociation constant in the presence of *N*-acetyl-glucosamine implies that *L. casei* Shirota has a lower affinity for adhesion to Caco-2 cells and adhered cells dissociate more easily in the presence of the carbohydrate.

Hydrophobicity of bacterial cells

The hydrophobicity of the bacterial cells measured by the PBS method is shown in Table 2. *L. rhamnosus* GG had the highest hydrophobicity with 73·7 % adhesion to hexadecane, which was followed by *L. casei* Shirota with 66·7 %. Among the GI bacteria, *E. coli* TG1 had the highest hydrophobicity at 63·1 %, while *S. typhimurium* 14028 had the lowest (5·1 %). The other six GI bacteria were around 20 %.

Competition for adhesion between L. rhamnosus *GG and gastrointestinal bacteria*

Equal concentrations of the respective radiolabelled GI bacterium and *L. rhamnosus* GG were incubated with Caco-2 cell monolayer for an hour. The reduction in adhesion (competitive exclusion) of the respective GI bacterium by *L. rhamnosus* GG was calculated and plotted against the hydrophobicity of the respective GI bacterium (Fig. 4). No positive correlation between the degree of competitive exclusion (% reduction of adhesion) and hydrophobicity of the GI bacteria was observed.

Competition for adhesion between L. casei Shirota *and gastrointestinal bacteria*

The reduction in the adhesion of the respective GI bacterium in the presence of an equal concentration of *L. casei* Shirota was determined and plotted against the CIA distance from *L. casei* Shirota (Fig. 5). The CIA distances were calculated from the difference between the CIA of the respective GI bacterium and that of *L. casei* Shirota. Two linear relationships were observed. A strong correlation between the degree of competitive exclusion and the CIA distance was found for *S. typhimurium* 14028, *S. bellurup* E23 and *S. enteritidis* 13076. A weaker correlation was found in the case of *E. coli* TG1, *S. typimurium* E10, *E. coli* 11775, *E. coli* 0157 and *S. typimurium* E12.

Exclusion of gastrointestinal bacteria by L. casei Shirota

In this study, *L. casei* Shirota was allowed to adhere onto Caco-2 cells and then free lactobacillus cells were washed-off. The respective radiolabelled GI bacterium was then added and its adhesion to Caco-2 cells was determined. Figure 6 shows the plot of per cent inhibition of

Table 2. The Carbohydrate Index for Adhesion of the various bacteria calculated from the data presented in Table 1

Bacterium	Carbohydrate Index for Adhesion	Hydrophobicity (% adhesion to hexadecane)
L. rhamnosus GG	9·0	73·70 ± 18·76
L. casei Shirota	6·9	66·75 ± 8·38
E. coli 0157	5·4	19·44 ± 13·28
E. coli 11775	5·3	14·23 ± 11·32
E.coli TG1	6·0	63·14 ± 11·36
S. enteritidis 13076	6·5	22·17 ± 19·95
S. typhimurium 14028	7·1	5·08 ± 19·42
S. typhimurium E10	6·1	19·68 ± 9·71
S. typhimurium E12	4·8	19·11 ± 6·70
S. bellurup E23	7·3	19·62 ± 5·03

The hydrophobicity of the various bacterial cells measured by the method of hexadecane partitioning.

adhesion of respective GI bacterium by *L. casei* Shirota (exclusion) versus the CIA distance from *L. casei* Shirota. Again, two linear relationships were observed and intercepted at *S. typhimurium* 14028. A strong correlation between the degree of exclusion and CIA distance was apparent for *S. typhimurium* 14028, *S. enteritidis* 13076 and *S. bellurup* E23. A weaker correlation was observed for *E. coli* TG1, *S. typimurium* E10, *E. coli* 11775, *E. coli* 0157 and *S. typimurium* E12.

Displacement of gastrointestinal bacteria by L. casei Shirota

In this study, the respective radiolabelled GI bacterium was allowed to adhere onto Caco-2 cells then free GI bacterial cells were washed off. *L. casei* Shirota was then added and the remaining adhered GI bacteria were determined. Figure 7 shows the plot of per cent inhibition of adhesion of respective GI bacterium in the presence of *L. casei* Shirota (displacement) versus the CIA distance from *L. casei* Shirota. Again, two linear relationships were observed. A strong correlation between the displacement and CIA distance was apparent for *S. typhimurium* 14028, *S. bellurup* E23, *E. coli* TG1, *S. enteritidis* 13076, *E. coli* 11775 and *E. coli* 0157. A weaker correlation was observed for *S. typhimurium* E10, and *S. typimurium* E12.

Discussion

It is widely suggested that carbohydrate moieties on the intestinal surface and carbohydrate-binding adhesins on the bacterial cell surface are involved in the stereospecific adhesin–receptor interaction leading to the adhesion of bacterial cells to the intestinal surface (Ofek *et al.* 1978; Adlerberth *et al.* 1996; Yamamoto *et al.* 1996). The eight carbohydrates tested in this study inhibited the adhesion of *L. casei* Shirota, the eight strains of *E. coli* and the *Salmonella* spp. to the Caco-2 intestinal cell line, but to varying degrees. Interestingly, the adhesion of *L. rhamnosus* GG to Caco-2 cells was affected by only one of the carbohydrates tested. This may suggest that the carbohydrate-mediated adhesin–receptor interaction is not the major mechanism involved in the adhesion of *L. rhamnosus* GG to Caco-2 cells. *L. rhamnosus* GG cell surface showed high hydrophobicity. The non-specific hydrophobic surface interaction may be the main adhesion mechanism for *L. rhamnosus* GG. Among the eight strains of *E. coli* and *Salmonella* spp. tested, only *E. coli* TG1 showed relatively high hydrophobicity, whereas the surface hydrophobicity of the other seven strains was low. However, *L. rhamnosus* GG was found to compete with seven strains of *E. coli* and *Salmonella* spp. and enhance the adhesion of *E. coli* 0157. As there is no obvious direct correlation between the degree of competition for adhesion

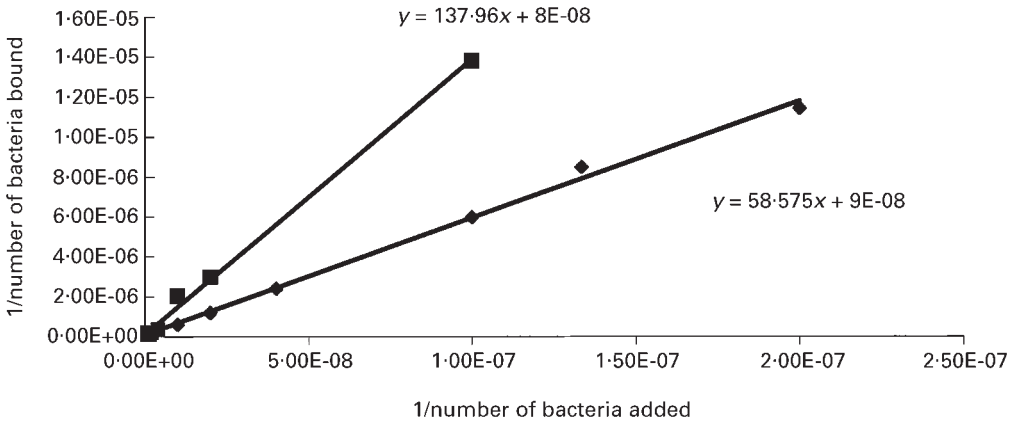

Fig. 3. The plots of 1/number of *L. casei* Shirota bound to Caco-2 cells versus 1/number of *L. casei* Shirota added to Caco-2 cultures, in the presence and absence of *N*-acetyl-glucosamine. (◆), 1/number of bacteria bound (without *N*-acetyl-glucosamine); (■), 1/number of bacteria bound (with *N*-acetyl-glycosamine).

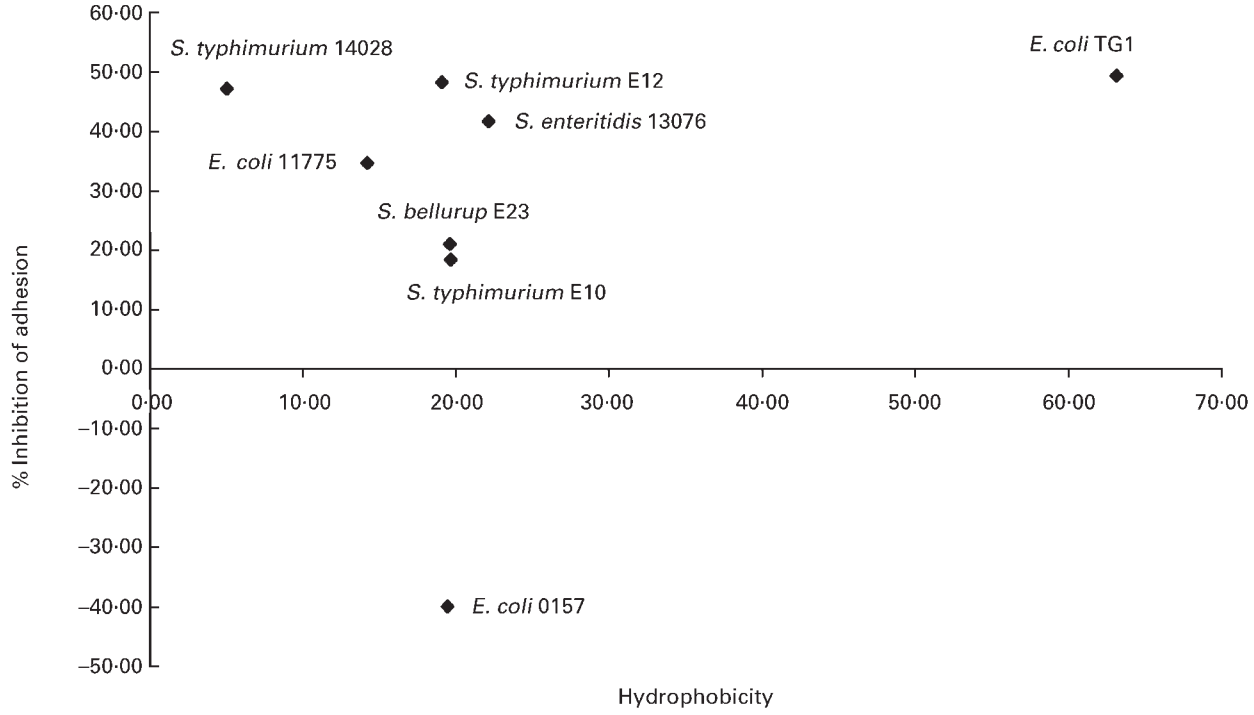

Fig. 4. Correlation between the per cent inhibition of adhesion of GI bacteria (*E. coli* and *Salmonella* spp.) to Caco-2 cells by *L. rhamnosus* GG and the hydrophobicity of the GI bacteria.

and hydrophobicity of the bacterial cells, the inhibition of adhesion of *E. coli* and *Salmonella* spp. to Caco-2 cells by *L. rhamnosus* GG was probably due to steric hindrance (Chauviere *et al.* 1992), and the degree of inhibition was related to the relative position of the hydrophobic surface and adhesin receptors. The hydrophobic surface and adhesins on the surface of *E. coli* 0157 were probably a distance apart, and the enhancement of adhesion by *L. rhamnosus* GG may be due to adhesion of *E. coli* 0157 cells on *L. rhamnosus* GG (Ouwehand *et al.* 2000). *L. rhamnosus* GG was reported to increase the adhesion of *S. typhimurium* on human intestinal glycoproteins (Tuomola *et al.* 1999).

If *N*-acetyl-glucosamine could mask the major adhesins on the surface of *L. casei* Shirota, the adhesion measured in the presence of the carbohydrate would represent the hydrophobic interaction between *L. casei* Shirota and Caco-2 cells. Interestingly, the calculated maximum concentrations of adhered *L. casei* Shirota to Caco-2 cells in the presence and absence of *N*-acetyl-glucosamine were

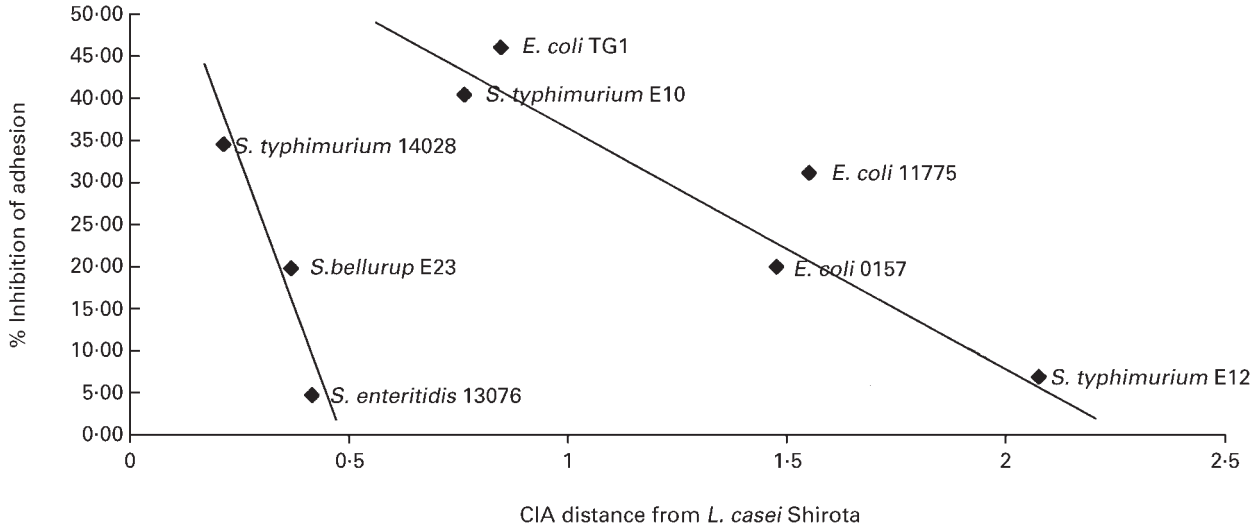

Fig. 5. Correlation between the competitive inhibition of adhesion (per cent inhibition of adhesion compared to adhesion measured in the absence of *L. casei* Shirota) of GI bacteria (*E. coli* and *Salmonella* spp.) to Caco-2 cells by *L. casei* Shirota and their Carbohydrate Index for Adhesion (CIA) distances.

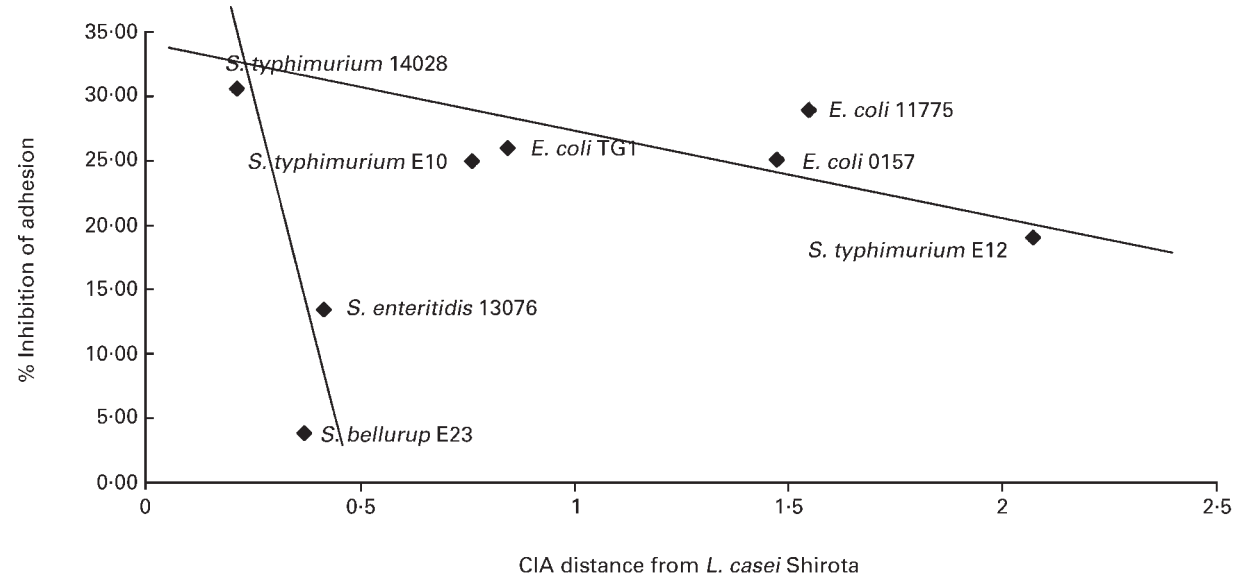

Fig. 6. Correlation between the exclusion (per cent inhibition of adhesion compared to adhesion measured in the absence of *L. casei* Shirota) of GI bacteria (*E. coli* and *Salmonella* spp.) from Caco-2 cells by *L. casei* Shirota and their Carbohydrate Index for Adhesion (CIA) distances.

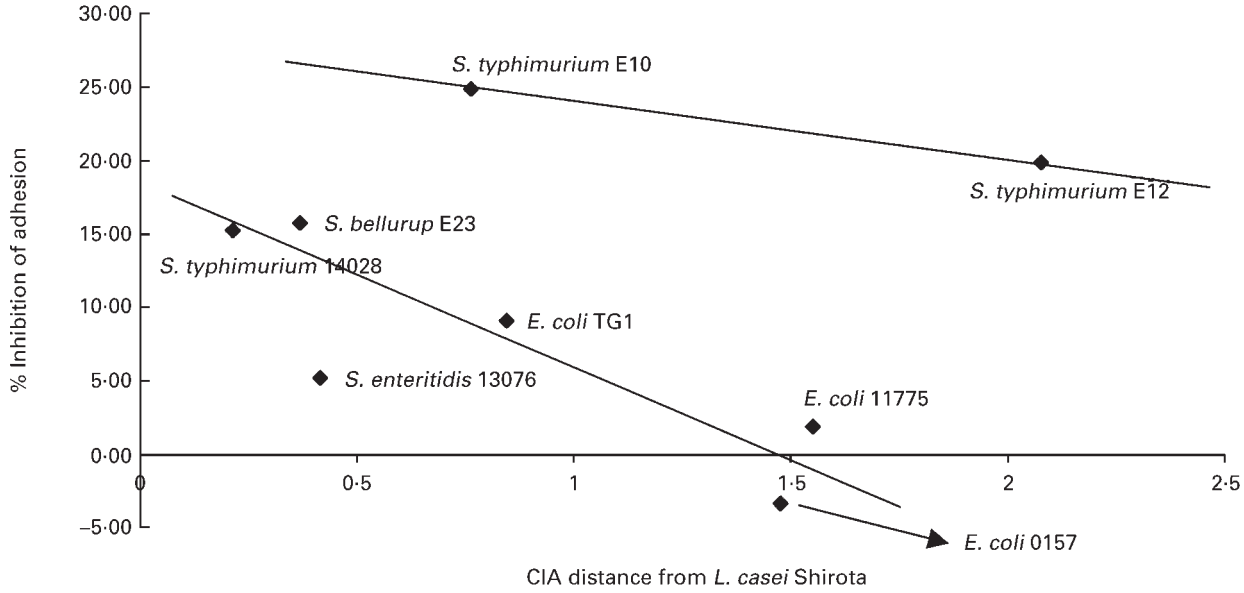

Fig. 7. Correlation between the displacement (per cent inhibition of adhesion compared to adhesion measured in the absence of *L. casei* Shirota) of GI bacteria (*E. coli* and *Salmonella* spp.) to Caco-2 cells by *L. casei* Shirota and their Carbohydrate Index for Adhesion (CIA) distance.

identical. This suggests that the adhesins and the hydrophobic surfaces on *L. casei* Shirota are in close proximity and have the same number of adhesion sites. For example, the adhesin is located at the tip of a fimbriae, whereas the stalk of the fimbriae is hydrophobic. As expected, the affinity of the adhesin for the Caco-2 receptor was about 2·6 times that of the hydrophobic surface interaction. The hydrophobic surfaces may serve to bring together the surfaces, but it is the adhesin–receptor interaction that plays the major role in the binding of *L. casei* Shirota and Caco-2 cells.

Based on the carbohydrate inhibition of adhesion profile, the adhesins on *L. casei* Shirota and the eight strains of *E. coli* and *Salmonella* spp. are probably not identical. The concept of CIA is introduced in the work to depict the receptor binding characteristic of the adhesins on bacterial surface. Two different bacteria with identical CIA would imply identical adhesins and the distance between their CIA values is zero. The greater the degree of difference between the CIA values, the greater would be the differences between the adhesins on the bacteria. In the competition study, when the degrees of inhibition of adhesion of the eight strains of *E. coli* and *Salmonella* spp. by *L. casei* Shirota were plotted against the distance between their CIA, two linear relationships were observed. This suggests that there are possibly two distinct types of adhesins on the surface of *L. casei* Shirota. One is closely related to the adhesins possessed by *S. typhimurium* 14028,

S. bellurup E23 and *S. enteritidis* 13076, while the other resembles that of *E. coli* TG1, 11775, 0157 and *S. typhimurum* E10, E12. Two types of carbohydrate-binding adhesins were found on the surface of *L. johnsonii* La1 (Neeser *et al.* 2000), one for O-linked oligomannosides and the other for the gangliotri- and gangliotetra-osylcer amides. The difference between the CIA values determined the degree of competitive exclusion of a pathogen by *L. casei* Shirota for adhesion to Caco-2 cells. This approach provides a scientific basis for the screening and characterization of probiotic lactobacilli for competitive exclusion of a specific pathogen or groups of pathogens.

The same trend was observed in the exclusion of pathogens by *L. casei* Shirota: the same two groups of *E. coli* and *Salmonella* spp. showed two distinct linear relationships between the degrees of exclusion of the pathogens by *L. casei* Shirota for adhesion of Caco-2 cells.

The displacement of the eight strains of *E. coli* and *Salmonella* spp. by *L. casei* Shirota showed two linear relationships. However, the *E coli* strains had joined the group of *S. typhimurium* 14028, *S. enteritidis* 13076 and *S. bellurup* E23, while *S. typhimurium* E10 and E12 remained separated. A second mechanism may have been involved in the displacement of *E. coli* by *L. casei* Shirota from the receptors on Caco-2 cells.

This study provides some direction for future molecular and genetic studies of adhesins on bacteria and receptors on intestinal surfaces. The study also provides a rational approach for the screening and selection of probiotics with desirable properties.

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