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ORIGINAL ARTICLE

Dairy bacteria remove *in vitro* dietary lectins with toxic effects on colonic cells

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Abstract

Aims: To assess *in vitro* the ability of some dairy bacteria to bind concanavalin A (Con A), peanut agglutinin (PNA) and jacalin (AIL), preventing their toxicity on mouse intestinal epithelial cells (IEC).
Methods and Results: Con A and AIL reduced significantly IEC viability *in vitro*, as determined by Trypan Blue dye exclusion or by propidium iodide/fluorescein diacetate/Hoescht staining. Different strains of dairy bacteria were able to remove lectins from the media. Two strains were subjected to treatments used to remove S-layer, cell wall proteins, polysaccharides and lectin-like adhesins. They were then assayed for the ability to bind dietary lectins and reduce toxicity against IEC and to adhere to IEC after interaction with lectins. Con A and AIL were removed by *Propionibacterium acidipropionici* and *Propionibacterium freudenreichii* by binding with specific sugar moieties on the bacterial surface. Removal of lectins by bacteria impaired IEC protection. Adhesion of *P. acidipropionici* to IEC was reduced but not abolished after binding Con A or AIL.
Conclusions: Removal of Con A or AIL by dairy propionibacteria was effective to avoid the toxic effect against colonic cells *in vitro*.
Significance and Impact of the Study: Consumption of foods containing these bacteria would be a tool to protect the intestinal epithelia.

Introduction

Plant lectins are proteins or glycoproteins of nonimmune origin possessing at least one noncatalytic domain, which binds reversibly to a specific mono- or oligosaccharide through hydrogen bonds and van der Waals interactions (Peumans and Van Damme 1995; Lis and Sharon 1998). They are found in all tissues of plants, such as roots, leaves, flowers, bulbs and rhizomes, but in higher amount in seeds (Broekaert *et al.* 1987; Ratanapo *et al.* 1998; Van Damme *et al.* 1998).

Lectins participate in the plant defence mechanisms against phytopathogenic micro-organisms, insects and nematodes (Peumans and Van Damme 1994; Oka *et al.* 1997; Ripoll *et al.* 2003). Ingested by plant-eating animals, these glycoproteins, with high stability in a large range of pH and resistance to proteolytic enzymes, pass over the gastrointestinal tract where they interact with carbohydrates placed on the surface of the epithelial cells. The different deleterious effects that exhibit each plant lectins depend on their resistance to degradation within the intestine and their specificity to membrane carbohydrates moieties expressed by the epithelial cells. The binding of lectins cause morphological and physiological changes in the intestinal epithelium (Pusztai 1991). Those changes include inhibition of digestive enzymes, shedding of brush border membranes and shortened of microvilli that conduce to reduction of the absorptive function and nutrient utilization, and increases on cellular proliferation and turnover. Moreover, much of the plant lectins are mitogenic and would act as tumour promoters by stimulating cell proliferation (Kiss *et al.* 1997; Ryder *et al.* 1998). Some studies have shown that peanut lectin acts as a mitogen for colorectal cancer cell line and increases crypt cell proliferation rates in normal colonic epithelium *in vitro* (Ryder *et al.* 1992).

As plant lectins are commonly consumed by humans in foods such as vegetables, fruits, cereals, beans and

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mushrooms (Nachbar and Oppenheim 1980), it is likely that the colonic epithelium is exposed to their deleterious effects.

It is also known that plant lectins have the ability to interact with bacterial surfaces and precipitate polysaccharides from bacterial origin. Concanavalin A (Con A), the lectin from Jack bean (*Canavalia ensiformis*), binds an arabinogalactan of *Mycobacterial* cell walls and produces its agglutination (Pistole 1981). It also agglutinates *Actinomyces* cells and precipitates polyglycosylglycerol phosphate teichoic acid from *Bacillus subtilis*. The lectin from snail (*Helix pomatia*) precipitates lypopolisaccharides of *Salmonella* and polysaccharides from group C streptococci. Wheat germ lectin interacts with intact bacterial cell surfaces or complex molecules extracted from bacterial cell walls that contain *N*-acetyl-D-glucosamine residues (Lotan *et al.* 1975). Due to the ability to interact directly with bacteria, lectins can be used for typing bacterial strains (Kellens *et al.* 1995).

In the intestinal ecosystem, where a complex microbiota inhabits free within the lumen or adhered to the mucosal surface, lectins can interact with the bacterial cells depending on the carbohydrates expressed on their surface. This lectin-bacteria interaction may interfere with the epithelial cell-lectin interaction preventing toxic effects. With this concept in mind, some bacteria with appropriated cell wall carbohydrates moieties could be consumed as a part of human or animal diets to interfere with the recognition process between lectins and intestinal epithelial cells (IEC).

The aim of our investigations was to assess *in vitro* the ability of some species of dairy bacteria to bind Con A, peanut agglutinin (PNA) and jacalin and protect colonic cells against the cytotoxic effect of lectins. The relation between bacterial adhesion to epithelial cell and protection against lectins was also investigated.

Materials and methods

Lectins

The lectins used in this study were concanavalin A (Con A, from *C. ensiformis*) which binds glucose and mannose; peanut lectin (PNA, from *Arachis hypogaea*) and jacalin (AIL, from *Artocarpus integrifolia*) which bind galactose-β-1,3-*N*-acetylgalactosamine. All chemicals were provided by Sigma Chemical Co, St Louis, MO, USA.

Micro-organisms and culture conditions

The strains used in the present study, *Propionibacterium acidipropionici* CRL 1198 and Q4, *Propionibacterium freudenreichii* S1 and *Bifidobacterium longum* ML1, were obtained from Laboratorio de Ecofisiología Tecnológica, CERELA, Argentina (CRL, Centro de Referencia para Lactobacilos, Tucumán, Argentina). The strain *Propionibacterium jensenii* TL 494 belongs to the collection of INRA, Rennes, France. Cells were grown on Laptg broth (Raibaud *et al.* 1973) for 24 and 12 h for propionibacteria and bifidobacteria respectively, harvested by centrifugation (5000 ***g***, 10 min, 4°C) washed twice with 0·01 mol l^{-1} KH_2PO_2-Na_2HPO_4 buffer, containing 0·8% NaCl and 0·2% KCl, pH 7·2 (PBS) and suspended in the same buffer to 1×10^8 bacteria ml^{-1}.

Intestinal epithelial cells

Six week-old Balb/c mice, each weighing 25–30 g, were used as the source of IEC. The animals were obtained from the random-bred closed colony kept at our laboratory. The mice were maintained in metal cages, with free access to feed and water and fasted for 24 h before the experiments were performed. Mice were killed by cervical dislocation and the large bowels were removed for isolation of IEC according to Jin *et al.* (1996) with some modifications. Colonocytes were scraped off gently with the edge of a microscope slide and washed twice with PBS (120 ***g***, 10 min). Finally, they were suspended in NCTC 135 medium, pH 7·4 (Sigma Chemicals Co) supplemented with 2% foetal bovine serum and adjusted to a concentration of 5×10^5 cells ml^{-1}. The IEC suspension was maintained on ice and used within 2 h.

Lectin-bacteria and lectin-IEC interactions

A screening of the ability of lectins to interact with some dairy bacteria was carried out by agglutination assays carried out on microscope slides. Twenty-five microliters of bacterial suspensions (10^8 bacteria ml^{-1}) in PBS were mixed with 25 μl of each lectin solution with final concentrations of 1–500 μg ml^{-1} in PBS. After 60 min at 25°C, bacterial agglutination was determined by observation of slides under optical microscopy at 40× magnification.

The interaction between lectins and colonic cells was studied by agglutination assay of mouse IEC on microscope slides or in 96-well U-shaped polystyrene microtitre plates. The mixtures were prepared as indicated for the interaction lectins-bacteria but incubated under microaerophilic conditions (5% CO_2 : 95% air atmosphere) during 120 min at 37°C. Agglutination was determined by observation of slides under optical microscopy at 40× magnification or at naked eye in microtitre plates. Observation of a granular mesh in a shadowed background indicated a positive result and a compact disc in

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the bottom of the wells of microtitre plates, negative result.

Cytotoxic effects of lectins on mouse colonic cells

The effect of lectins on IEC was determined by Trypan Blue dye exclusion after incubation of IEC with lectins in NCTC 135 medium in a final concentration of 100 μg ml^{-1}. Mixtures were incubated during 120 min at 37°C under microaerophilic conditions and then equal volume of the colon cell suspensions treated with lectin and 0·4% Trypan Blue in 0·85% NaCl were mixed. Stained and nonstained cells were counted in a Neubauer cell chamber at 40× magnification in a conventional light microscope (Zeiss–Axiolab; Cool Zeiss, Jena, Germany) and cells that exclude Trypan Blue (viable cells) were reported as the percentage of total cells counted.

In some experiments, effects were evaluated by propidium iodide/fluorescein diacetate/Hoescht staining. Freshly made combinations of 2·5 μg propidium iodide, 7·5 μg fluorescein diacetate and 1·0 μg Hoescht-33342 in 10 μl of PBS were added to 100 μl of colon cells suspensions after lectin interaction and incubated on ice for 10 min in the darkness. The cells were washed once in PBS and counted immediately by fluorescence microscopy in a conventional fluorescence microscope (Leica DM LS2; Leica Microsystems, Wetzlar, Germany) by using the appropriated filters. Cells with green cytoplasm and normal nuclei (viable), condensed blue–green nuclei (early apoptotic cells) and bright red nuclei (necrotic cells) were reported as a percentage of the total number of cells.

Lectins removal assays

Two strains, *P. acidipropionici* CRL 1198 and *P. freudenreichii* S1, and two lectins, Con A and AIL, were selected for this study.

In order to determine the maximum binding ability of the strains, equal volume of bacterial suspensions and lectins, in final concentrations of 400 μg ml^{-1} in PBS, were incubated as above described. After centrifugation of the mixtures, remnant lectins concentrations were quantified in the interaction supernatants by Bradford (1976) procedure. The assays were also carried out by including solutions of 50 mmol l^{-1} D-glucose, D-galactose, D-mannose or *N*-acetyl-D-galactosamine in the reaction mixtures.

Chemical and enzymatic treatments of bacteria

Both bacterial strains were subjected for 60 min to different treatments used to remove S-layer (5 mol l^{-1} LiCl); cell wall proteins (2 mg ml^{-1} pronase E en PBS, pH 7·2); polysaccharides (10 mg ml^{-1} sodium periodate in 0·1 mol l^{-1} acetate buffer pH 4·5) and lectin-like adhesins (0·03 mmol l^{-1} phenylmethylsulfonylfluoride in PBS at 0°C) as previously published (Zárate *et al.* 2002b). After treatments, cells were washed and suspended in PBS to the desired concentration for further studies.

Effects of remnant lectins on the colonic cells viability

Treated and nontreated bacteria were incubated with Con A in a final concentration of 100 μg ml^{-1} during 60 min at 25°C. The mixtures were then centrifuged (10 000 ***g***, 10 min, 4°C), and supernatants and bacterial cells were collected for further use. IEC were incubated in 100 μl of each supernatant under microaerophilic conditions during 120 min at 37°C. After incubation, treated IEC were stained with Trypan Blue and observed as previously described for cytotoxic effect. Results were compared with trials carried out without bacteria.

Bacterial cells obtained from each sample were stored at 4°C to study the influence of lectins on the ability of bacteria to adhere to colonic cells.

In vitro adhesion assay

Adhesion of micro-organisms to IEC was also determined in treated and nontreated bacteria from the different trials as was previously published (Zárate *et al.* 2002a). Briefly, suspensions of 10^8 bacteria ml^{-1} and 5×10^5 IEC ml^{-1} were mixed (1 : 4) and incubated 1 h in 5% CO_2 : 95% air atmosphere. After incubation, the mixtures were kept on ice. Adhesion was determined by examining 30 epithelial cells selected at random using phase contrast microscopy. Results were reported as number of IEC with attached bacteria extrapoled to 100 IEC (adhesion percentage).

Results

Agglutination of colonic and bacterial cells by interaction with lectins

All the assayed strains showed agglutination in the presence of different concentrations of Con A (Table 1). Cells agglutination was detected between 1–500 μg ml^{-1} of lectin when both strains of *P. acidipropionici* and *P. jensenii* were evaluated. *Propionibacterium freudenreichii* and *B. longum* showed agglutination with 10 and 50–500 μg ml^{-1} of Con A respectively. Only *P. jensenii* TL 494 showed microscopically a weak interaction with PNA and AIL at the highest lectins concentration assayed. Higher concentrations of Con A and AIL were needed in order to agglutinate colonic cells whereas PNA did not shown macro or microscopic interaction with IEC.

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Table 1 Agglutination of propionibacteria and bifidobacteria in the presence of different concentrations of dietary lectins

		Concentration (μg ml^{-1})					
Cells	Lectins	1	10	50	100	250	500
Propionibacterium acidipropionici CRL1198	Con A	+*	+	+	+	+	+
	PNA	–	–	–	–	–	–
	AIL	–	–	–	–	–	–
Propionibacterium acidipropionici Q4	Con A	+	+	+	+	+	+
	PNA	–	–	–	–	–	–
	AIL	–	–	–	–	–	–
Propionibacterium jenseni TL 494	Con A	+	+	+	+	+	+
	PNA	–	–	–	–	–	+d
	AIL	–	–	–	–	–	+d
Propionibacterium freudenreichii S1	Con A	–	+	+	+	+	+
	PNA	–	–	–	–	–	–
	AIL	–	–	–	–	–	–
Bifidobacterium longum	Con A	–	–	+	+	+	+
	PNA	–	–	–	–	–	–
	AIL	–	–	–	–	–	–
Colonic cells	Con A	–	–	–	+	+	+
	PNA	–	–	–	–	–	–
	AIL	–	–	–	–	–	+

Con A, concanavalin A; PNA, peanut agglutinin; AIL, jacalin.
*+: granular mesh in a shadowed background (positive result), –: compact disc in the bottom of the wells of the microtitre plates (negative result), +d: weak and variable reaction.

Effect of lectins on the colonic cells viability

The cytotoxic effect of lectins on IEC was evaluated after incubation of colonic cells with 100 μg ml^{-1} lectins and further determination of viable cells that exclude Trypan Blue. As it is shown in Table 2, the three lectins induced cells death in a different extent. The effect was remarkable only with Con A and AIL as they reduced the percentage of viable cells from 88 ± 12% to 63 ± 10% and 64 ± 12% respectively after 120 min of contact. Lower damage was produced by PNA as the viable cells percentage was reduced to 70 ± 3·5%. These results were close related to the agglutination assays (Table 1) that showed a visible interaction between IEC and Con A (100–500 μg ml^{-1}) and AIL (500 μg ml^{-1}) but not PNA.

Table 2 Cytotoxic effect of dietary lectins on epithelial cells from mouse colon*

	Viable cells (%)†
None	88 ± 7
Con A	63 ± 10
AIL	64 ± 12
PNA	70 ± 3·5

Con A, concanavalin A; PNA, peanut agglutinin; AIL, jacalin.
*The effect of lectins on IEC was determined by Trypan Blue dye exclusion after incubation of IEC with 100 μg ml^{-1} lectins (see Materials and methods).
†Cells that exclude Trypan Blue (viable cells) were reported as the percentage of total cells counted.

Lectin removal by bacteria

Con A and AIL were selected for further studies as there was a remarkable toxic effect of these two lectins on colonic cells. Two bacterial strains, with and without the property of adhesion to IEC, were studied for their ability to remove the selected lectins from the reaction mixture. High concentration of lectins (80 μg of protein in a final volume of 200 μl) was assayed in order to determine the maximum binding ability of the bacterial suspensions, and remnant lectins were determined in the interaction supernatants. Both strains of propionibacteria were able to remove 60–70% of lectins as determined by the free protein detection in supernatants (Fig. 1).

Carbohydrate residues involved in the lectin-bacteria interactions were the expected according to the lectins specificity. However, the inhibition of lectin-bacteria interaction by free sugar solutions was different depending on

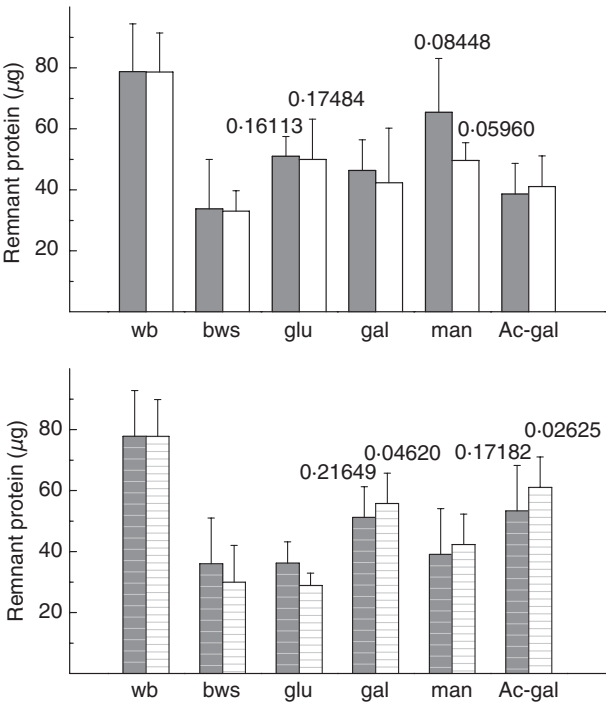

Figure 1 Lectins removal by dairy propionibacteria measured as protein remnant in the interaction supernatants lectins-bacteria in the absence/presence of different carbohydrates. Upper panel: Con A removal by *Propionibacterium acidipropionici* CRL 1198 (■) and *Propionibacterium freudenreichii* S1 (□). Lower panel: AIL removal by *P. acidipropionici* CRL 1198 (▤) and *P. freudenreichii* S1 (▥). wb, without bacteria, bws, bacteria without sugar; glu, 50 mmol l^{-1} D-glucose; gal, D-galactose; man, D-mannose; Ac-gal, *N*-acetyl-D-galactosamine.

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the bacterial strain and lectin studied. Lower removal of Con A was observed by *P. acidipropionici* and *P. freudenreichii* when they were incubated in the presence of 50 mmol l^{-1} D-mannose ($P < 0{\cdot}1$), and in lesser extent, D-glucose ($P = 0{\cdot}16113$ and $0{\cdot}17484$ respectively). The interaction of both strains with AIL was inhibited by D-galactose and *N*-acetyl-D-galactosamine but the inhibition was more significant for *P. freudenreichii* ($P < 0{\cdot}05$) than for *P. acidipropionici* (Fig. 1).

Protection of colonic cells by lectin removal

Bacteria were allowed to interact with lectins during 60 min and supernatants of the incubation mixtures where assayed for their toxic effect against colonic cells. Results of viable and nonviable cells stain are represented in Fig. 2.

Control cells incubated with PBS instead interactions supernatants showed, as expected, a high viability percentage of about 85%. Low number of necrotic cells (10%) or in an early stage of apoptosis (5%) was detected. In contrary, incubation of IEC with Con A or AIL produced a significant cellular necrosis.

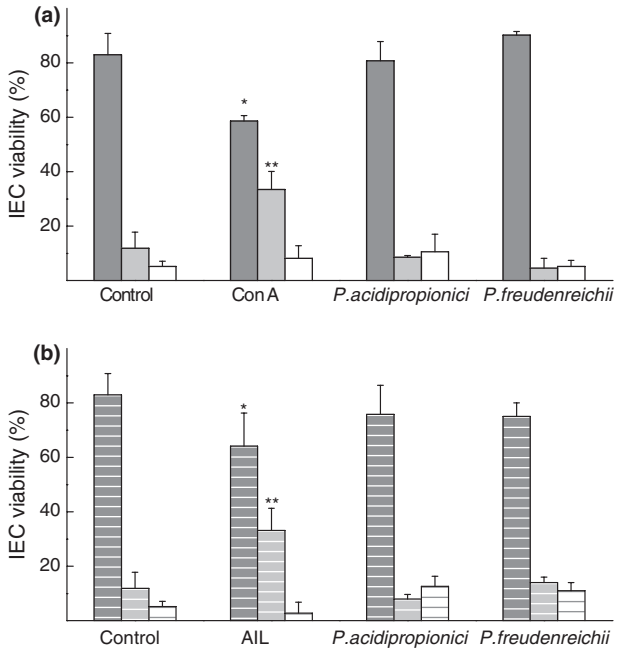

Figure 2 Cytotoxic effects of lectins and protection of colonic cells by lectin removal by propionibacteria. Panel A: Viability of IEC exposed to PBS (Control); Con A and to the interaction supernatants Con A-propionibacteria. (▩) Viable cells; (▨) necrotic cells; (□) apoptotic cells. Panel B: Viability of IEC exposed to PBS (control); AIL and to the interaction supernatants AIL-propionibacteria. (▩) Viable cells; (▨) necrotic cells; (□) apoptotic cells.

When Con A solutions were incubated with *P. acidipropionici* or *P. freudenreichii* before the incubation with IEC, there was a reduction on the necrotic cells percentage from 33% to 9% and 5% respectively. No significant difference was observed between strains, lectin types or apoptotic cells percentage.

Influence of the bacterial surface composition on lectins removal and adhesion property

Bacteria were subjected to chemical and enzymatic treatments before Con A-bacteria interactions in order to determine whether there are bacterial cell surface components other than carbohydrates involved in the lectin removal. The reduction in the Con A content from interaction supernatants was biologically determined by toxicity assays on mouse intestinal cells. Data presented in Fig. 3 suggested that different components are involved in

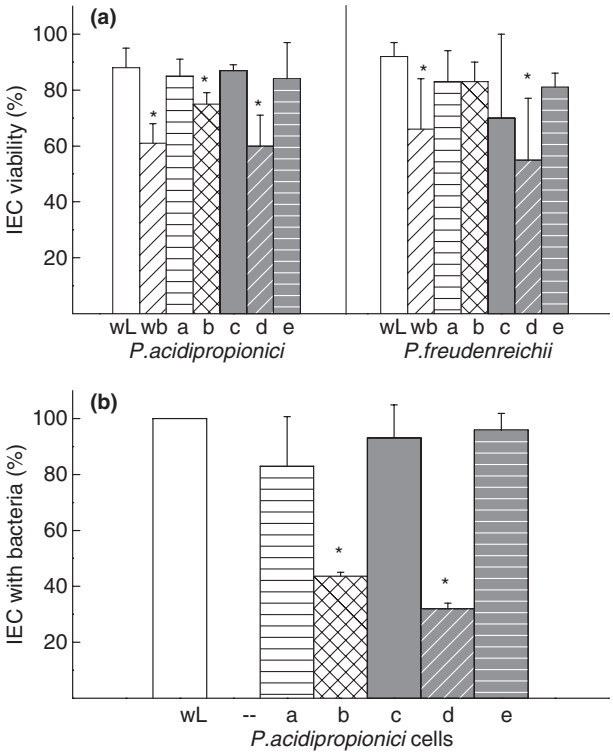

Figure 3 Influence of bacterial surface components on lectins removal (a) and adhesion property (b). (a) Viability of IEC exposed to the interaction supernatants of Con A and propionibacteria treated with chemical agents in order to remove cell surface structures. (b) Adhesion ability (%) of treated propionibacteria after incubation with Con A. wL, propionibacteria without lectin interaction, wb, lectin without bacteria; a, Nontreated bacteria; b, protease treatment (cell wall proteins remotion); c, LiCl treatment (S-layer); d, periodate treatment (polysaccharides); e, phenylmethylsulfonylfluoride treatment (lectin-like adhesins).

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the Con A-bacteria interaction depending on the strain studied. Preincubation of Con A with nontreated cells of *P. acidipropionici* allowed to restore the IEC viability from 61 ± 7 to 85 ± 6% (Fig. $3a_1$). This protective effect was not observed when polysaccharides located on the bacterial surface were removed, while a significantly reduced protection was achieved by elimination of cell wall proteins. In contrast, the lectin removal by a nonadherent strain of *P. freudenreichii* only depended on cell wall carbohydrates as periodate treatment of bacterial cells was the only responsible for the loss of protective effect of this strain on IEC (Fig. $3a_2$).

The sites for attachment of lectin on the bacterial surface and the adhesion determinants seems to be related in *P. acidipropionici*, as loss of the property to adhere to IEC and of the ability of the strain to remove Con A were both observed after treatments with periodate and pronase E (Fig. $3a_1$ and 3b). However, Con A linked to *P. acidipropionici*, prevented in low extent the adhesion of this strain to IEC. The relative percentage of IEC with adhered bacteria was reduced to a value of 83 ± 18% after the interaction of this bacteria with Con A (Fig. 3b), suggesting that carbohydrates other than glucose and mannose on the bacterial surface are also involved in the bacteria-IEC interaction.

Discussion

It is well known that mono and oligosaccharides have the ability to interfere with carbohydrate–protein interaction and therefore inhibit the cell-cell recognition and adhesion processes, which play an important role in cancer growth and progression (Nangia-Makker *et al.* 2002). It has been reported that the galactose-*N*-acetylgalactosamine-binding dietary lectins, such as PNA, have the potential to act as tumour promoters and that a high dietary intake of galactose-containing carbohydrates (such as galactose-containing vegetable fibre) would offer protection from these effects by binding free lectin in the colonic lumen (Ryder *et al.* 1994; Evans *et al.* 2002). The same role could be played by bacteria with galactose-containing receptors. In a global view, suitable sugar moieties on the bacterial surface would inhibit the interaction between dietary lectins and colonic cells by competing for the sites where these molecules bind. That should avoid the toxicity of some lectins on IEC and the consequent antinutritional effect for humans and animals.

In this study, three different lectins were assayed for their ability to bind to the surface of five dairy bacteria. All the bacteria assayed were able to interact with Con A and showed agglutination at concentrations lower than 500 μg ml^{-1} of lectin. The interactions of surface carbohydrates from different bacteria with PNA or AIL were less evident as they did not resulted in bacterial agglutination, with the single exception of *P. jenseni* TL 494 which showed a variable and weak reaction with the higher concentration of PNA and AIL assayed.

Studies on carbohydrates contents of the cell wall of dairy propionibacteria indicated that *P. acidipropionici* and *P. jensenii* have glucose as the main cell wall sugar, mannose is in minor quantities and galactose appeared to be present in little amount or absent (Allsop and Work 1963) *P. freudenreichii* have larger amounts of galactose and, in lower extent, mannose in its cell wall. Galactose, glucose and rhamnose are important sugars in the cell wall of *Bifidobacterium* (Poupard *et al.* 1973). Therefore, interactions of Con A with all the strains used in this study was possible, as Con A have specificity for both D-mannose present in the cell wall of *P. freudenreichii* and D-glucose present in significant amounts in the surface of the other strains. High concentrations of PNA or AIL showed a weak agglutination of *P. jensenii* TL 494 and no agglutination of the other strains even when the lectins have specificity to galactose that is always present in *Bifidobacterium* and *P. freudenreichii*. That indicated that PNA and AIL would interact with carbohydrates of the bacterial surface even when they were not able to agglutinate the strains studied (Table 1). Two strains with an expected different pattern of surface carbohydrates and different surface properties related to IEC adhesion (Zárate *et al.* 2002a) were selected for further studies. Con A and AIL were the lectins subjected to more studies as they have different sugar specificity and showed opposite response in the agglutination assays of bacteria. The inhibition of lectin-bacteria interaction by sugars was a useful tool to elucidate which carbohydrates moieties were involved in the interaction. Mannose was the most important carbohydrate moiety involved in the interaction of both strains with Con A, but higher amount of molecules seems to be linked through this carbohydrate in *P. acidipropionici*. It is known that mannose containing receptors with Manα1-3Manβ1-4GlcNAc and the putatitive sequence Manα1-2Man occurs in *N*-linked oligosaccharides chains that are present to various degrees on micro-organisms and mammalian cells and could act as the optimal receptor structure for mannose-binding lectins (Adlerberth *et al.* 1996). Such oligosaccharides would be expressed on the cell wall of the strains of our study. *N*-acetylgalactosamine and galactose were also expressed on the cell wall of both strains of propionibacteria, and were responsible for the higher AIL removal from the interaction mixture by *P. freudenreichii*.

The maximum amount of lectin bound to the bacterial surfaces under the studied conditions was 40–45 μg in interaction mixtures of both strains and lectins when

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400 μg ml^{-1} Con A or AIL were used. Therefore, a lower concentration of lectin in the reaction mixtures was expected to be fully removed. A significant loss of viability of colonic cells was observed when they were incubated with 100 μg ml^{-1} lectins in the reaction mixtures, but the cellular damage was almost completely abolished when lectin solutions were preincubated with bacteria. Therefore, it was evident that micro-organisms remove these compounds from the media avoiding their deleterious effects on cells.

Although the Jack bean is not a regular component of human diets, Con A is a good model to study the behaviour of members of the mannose binding lectins family, which include, among others, lectins found in lentils and kidney beans. In contrast, Jack bean is considered an interesting alternative in birds diets. Even when the Jack Bean seeds have a high protein content of almost 24%, its incorporation in broiler chicken diets is possible only after heat treatment as it has been demonstrated that 10% of raw seeds in the diet, that is equivalent to approximately 0·3% pure Con A, is enough to produce depressed feed intake and stunted growth of animals (Mendez *et al.* 1998). Among the different antinutritional factors known to be present in that bean, Con A seems to be the most deleterious as it induce a wide array of physiological and biochemical changes in the cells. Mendez *et al.* (1998) have reported that after continuous feeding a diet containing Jack bean for 6-weeks, there is Con A endocytosis and transferal to the general circulation in chickens. Histological changes on the mucosa, villi showing vascular degeneration and inflammatory reaction in the lamina propia were also observed. By contrast, Nakata and Kimura (1985) reported that rats receiving a unique dosis of 25 mg Con A in the diet eliminate almost all the lectin during the following 5 days, but reduction in the sucrose activity from the intestinal epithelium was detected after the first 24 h. An intestinal bacteria fragment (IB) obtained from 10 g of faeces and dried at room temperature, was *in vitro* assayed for its ability to adsorb 3 mg of Con A. Arbitrary amounts of this fragment of 10, 25 or 50 mg bound 35, 50 and 70% Con A respectively. The *in vivo* treatment used by the authors in a rat experimental model was not really effective to avoid deleterious effects of Con A as the binding of the lectin to IB was abolished at the stomach pH. In our experiments, 10^7–10^8 propionibacteria were able to remove *in vitro* 40–45 μg Con A suggesting that almost 4 mg should be adsorbed by 10^9–10^{10} bacteria, amount that is possible to find in the caecal content of mice fed with diets containing some strains of this genus (Perez Chaia and Zárate 2005).

The presence of active lectins in fresh or incompletely cooked foods has led to depressed growth in animals and a number of outbreaks of food poisoning in humans (Miyake *et al.* 2007) due to the lack of enough information about the effects of lectins on the gut cells and appropriated tools to counteract them. We conclude that the consumption of foods with suitable viable bacteria, that ensure its development and maintenance within the intestine, may be an effective tool to avoid lectins-epithelia interactions and its undesirable effects both in animals and humans.

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