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Adhesion of human *Lactobacillus acidophilus* strain LB to human enterocyte-like Caco-2 cells

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Twenty-five strains of lactobacilli were tested for their ability to adhere to human enterocyte-like Caco-2 cells in culture. Seven *Lactobacillus* strains adhered well to the Caco-2 cells, of which three possessed calcium-independent adhesion properties. A high level of calcium-independent adhesion was observed with the human stool isolate *Lactobacillus acidophilus* strain LB. Scanning electron microscopy revealed that this strain adhered to the apical brush border of the cells. Adhesion increased in parallel with the morphological and functional differentiation of the Caco-2 cells. Two *Lactobacillus* components were involved in this adhesion. One was protease-resistant and bacterial-surface-associated; the other was heat-stable, extracellular and protease-sensitive.

Introduction

Lactobacilli are important in controlling undesirable microflora in the intestinal and uro-genital tracts of humans and animals. Some lactobacilli adhere to freshly isolated uro-epithelial cells (Chan *et al.*, 1984, 1985; Reid & Sobel, 1987) and living adhering lactobacilli or cell-wall fragments possess inhibiting properties against adhesion of uro-pathogenic *Escherichia coli* (Chan *et al.*, 1985; Reid *et al.*, 1987). Contradictory results have been obtained for the gastrointestinal tract (reviewed by Conway, 1988). Recently, using Caco-2 cells as an intestinal cell model, we have reported an inhibition of diarrheagenic *Escherichia coli* adhesion by the human *L. acidophilus* strain LB (Chauvière *et al.*, 1992). However, little is currently known about the mechanism of adhesion of lactobacilli to human intestinal cells (Conway, 1988; Reid *et al.*, 1990). Kleeman & Klaenhammer (1982) screened 27 human *Lactobacillus* isolates and reported that four *L. acidophilus* strains, BG2FO4, MSO1, MSO2 and MSO4 adhered specifically, in a calcium-independent manner, to human fetal intestinal epithelial cells. Conway *et al.* (1987) reported the adhesion of strain ADH to human colonic cells. Hood & Zottola (1989) observed that the cells of strain BG2FO4 closely associate with the brush border of the human intestinal cell line HITC FHs0074 (ATCC CCL 241). Recently Elo *et al.* (1991) reported that the *Lactobacillus casei* strain GG adheres to Caco-2 cells.

To gain further understanding of the mechanisms by which lactobacilli adhere to human intestinal cells, we studied the adhesion of a large number of *Lactobacillus* strains to enterocyte-like Caco-2 cells in culture. Bacterial attachment to the cell surfaces was characterized quantitatively by light and electron microscopic examination. Using the highly adhering strain LB, the adhesive determinants involved in adhesion were partially characterized. Moreover, since Caco-2 cells spontaneously differentiate in culture, the expression of *Lactobacillus* binding sites during the differentiation process was investigated.

Methods

Bacterial strains. The human *Lactobacillus* strains used in this study were *Lactobacillus acidophilus* strain Lacteol (human stool isolate, Lacteol Laboratories, Houdan, France), *L. acidophilus* CIP 6218 (human vaginal isolate), *L. acidophilus* NCK 88 (T. R. Klaenhammer, North Carolina University, Raleigh, USA), clinical stool isolates *L. casei* subsp. *casei* FPC 01.89, and *L. casei* subsp. *rhamnosus* FPC 02.90, 03.90 and 04.90. The *Lactobacillus* plant isolates were FPC 06.90, 07.90 and 08.90. The *Lactobacillus* animal isolates were: C2 and C7 (chicken), PA3, PA 19 and PA 47 (pig) (T. R. Klaenhammer). The dairy *Lactobacillus* strains were: *L. casei* GG (S. L. Gorbach, Tufts University, Boston, USA), *L. casei* CNRZ 383 (CNRZ, Jouy en Josas, France), *L. casei* subsp. *rhamnosus* ATCC 7469 and CNRZ 205, *L. helveticus* CNRZ 32 and CNRZ 240, *L. delbrueckii* subsp. *lactis* CNRZ 239 and CNRZ 1006, *L. delbrueckii* subsp. *bulgaricus* CNRZ 394 and 36. The bacteria were stored in MRS broth (De Man *et al.*, 1960)

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(Biokar, Pantin, France) containing 10% glycerol (v/v) at −80 °C. For the adherence assay, all strains were grown in MRS broth for 18 h at 37 °C.

Cell culture. The Caco-2 cell line was used between the 65th and 90th passage. Cells were routinely grown in Dulbecco-modified Eagle's minimal essential medium (25 mM-glucose) (Eurobio), supplemented with 20% (v/v) inactivated (30 min, 56 °C) fetal bovine serum (Boehringer) and 1% (w/v) non-essential amino acids (Eurobio). For assays, monolayers of Caco-2 cells were prepared on glass coverslips which were placed in six-well Corning tissue culture plates. Cells were seeded at a concentration of 4×10^4 cells cm^{-2}. All experiments and maintenance of cells were carried out at 37 °C in a 10% CO_2/90% air atmosphere. The culture medium was changed daily. Unless otherwise stated, cultures were used at post-confluence after 15 d of culture. To determine the number of Caco-2 cells in a monolayer, cells were trypsinized for 10 min at 22 °C and counted using a haemocytometer.

Adhesion assay. The adhesion of *Lactobacillus* strains to Caco-2 cells was examined using an assay previously described for ETEC adhesion (Darfeuille-Michaud *et al.*, 1990). Briefly, the Caco-2 monolayers were washed twice with phosphate-buffered saline (PBS) (138 mM-NaCl, 3 mM-KCl, 6 mM-Na_2HPO_4, 1·5 mM-KH_2PO_4). For each adhesion assay, 0·5 ml *Lactobacillus* suspension (bacteria with spent broth culture supernatant) was mixed with cell-line culture medium (0·5 ml), and added to each well of the tissue culture plate, which was then incubated at 37 °C in 10% CO_2/90% air. The final concentration of bacteria was 2 or 4×10^8 bacteria ml^{-1} as indicated. After incubation (times indicated in each experiment), the monolayers were washed five times with sterile PBS, fixed with methanol, Gram-stained, and examined microscopically under oil immersion. Each adhesion assay was conducted in duplicate with cells from three successive passages. For each glass coverslip monolayer, the number of adherent bacteria was counted in 20 random microscopic areas. Adhesion of *Lactobacillus* was expressed as the number of bacteria adhering to 100 Caco-2 cells.

Treatments of bacteria and spent culture supernatant. To characterize the bacterial determinants involved in *Lactobacillus* adhesion, bacteria with spent broth culture supernatant, bacteria alone or spent broth culture supernatant alone were subjected to various treatments. Bacterial cells and spent broth culture supernatant were separated by centrifugation. Trypsin, pronase and chemical products used were obtained from Sigma.

The bacterial suspension was incubated with Caco-2 cells in the presence of EGTA (20 mM). Where indicated, after incubation of monolayers with *Lactobacillus*, cells were washed five times with EGTA (20 mM) in PBS.

Bacterial cells alone or spent broth culture supernatant alone were heated to 60 °C or 100 °C in a water bath for 30 min and cooled by immersion in an ice-bath. Bacterial cells alone or spent broth culture supernatant alone were incubated with trypsin (2·5 mg m^{-1}) or pronase (2·5 mg ml^{-1}) for 60 min at 37 °C; trypsin and pronase were inactivated by adding inactivated (30 min, 56 °C) fetal bovine serum (Boehringer). The bacteria were preincubated with 50 mM-metaperiodate for 30 minutes, centrifuged, washed in PBS and resuspended in spent culture supernatant at 4×10^8 bacteria ml^{-1}. Bacterial viability was determined after each treatment. Only heating to 100 °C killed the lactobacilli. Adhesion inhibition by simple sugars was determined by adding D-mannose, L-fucose or D-galactose to the assay (100 mM, final concentration).

Scanning electron microscopy. Cells for scanning electron microscopy were grown on glass coverslips. After the bacterial adhesion assay, the cells were fixed with 2·5% (v/v) glutaraldehyde in 0·1 M-sodium phosphate buffer (pH 7·4) for 1 h at room temperature. After washing with phosphate buffer, cells were post-fixed for 30 min with 2% (w/v) OsO_4 in the same buffer, washed three times with the same buffer, dehydrated in a graded series (30% to 100%) of ethanol and passaged in a graded series (50% to 100%) of amyl acetate. Cells were dried in a critical-point dryer (Balzers CPD030), and coated with gold. The specimens were then examined with a Jeol JSM 25S scanning electron microscope.

Indirect immunofluorescence. Unpermeabilized Caco-2 cell monolayers on glass coverslips were used for the indirect immunofluorescence study. Cells were fixed for 10 min at room temperature in 3·5% (w/v) paraformaldehyde in PBS. Immunoreactivity of sucrase-isomaltase, a brush-border-associated hydrolase characteristic of differentiated intestinal cells (Semenza, 1986), was detected with polyclonal rabbit antibodies against sucrase-isomaltase purified from Caco-2 cells (Trugnan *et al.*, 1987) obtained from Dr Alain Zweibaum (INSERM U 178, Villejuif, France) and anti-rabbit fluorescein-coupled sheep antiglobulins (Institut Pasteur Production, Paris, France).

Results

Adhesion of lactobacilli to Caco-2 cells

Twenty-five *Lactobacillus* strains were examined for their ability to adhere to cultured Caco-2 cells (Table 1). The ability of the *Lactobacilli* to adhere to the Caco-2 cells *in vitro* varied considerably between strains. Low attachment to the differentiated Caco-2 cells was observed with the human stool clinical isolates, the *L. acidophilus* NCK 88, the human vaginal strain CIP 6218, the *Lactobacillus* plant isolates, the *Lactobacillus* animal isolates C2, PA3, PA47, and the dairy *Lactobacillus* ATCC 7469, CNRZ 36, 205, 383, 394, and 1006. High attachment was observed with the human stool isolate *L. acidophilus* strain LB and the strains CNRZ 32, 239 and 240. Moderate attachment was observed with *L. casei* strain GG and *L. acidophilus* strains C7 and PA19. However, to discriminate between calcium-dependent and calcium-independent adhesion of strains LB, GG, C2, C7, PA3, PA19, PA47, CNRZ 32, 239 and 240, we studied the attachment of strains after washing with EGTA. The rinses with EGTA had no significant effect on the adherence of strains LB, GG, C2, PA3 and PA19 and it decreased significantly the attachment of strains CNRZ 32, 239, 240, PA47 and C7. This result showed that among the adhering lactobacilli tested here, only the human *L. acidophilus* strain LB, the chicken isolate strain C2, the pig isolates strains PA3 and PA19 and the *L. casei* strain GG strain had a high calcium-independent binding capacity to differentiated Caco-2 cells in culture.

Characteristics of Lactobacillus adhesion

To characterize the bacterial components involved in *Lactobacillus* adhesion, we did experiments using the highly adhering strain *L. acidophilus* LB. The LB strain showed diffuse adhesion to Caco-2 cells (Fig. 1*a*). As observed in ultrathin sections of the post-confluent monolayer the adhesion appeared to occur at the brush

Table 1. *Adhesion of lactobacilli to Caco-2 cells in culture. Determination of calcium-dependent and independent binding*

Adhesion assay of lactobacilli strains (final concentration 4×10^8 bacteria ml^{-1}, with spent culture broth) to Caco-2 cells was monitored after 1 h at 37 °C in 10% CO_2/90% air. Mean number of Lactobacilli adhering to the cell monolayer per 100 cells in two identical adhesion assays run in parallel. Twenty randomized microscopic fields per cover slip were counted. Each adherence assay was conducted in duplicate with cells from three successive passages (70–80th cell passages: variation in reproductibility of adhesion less than 5% from each three cell passages of Caco-2 cells). Numbers in parentheses indicate standard deviations. Statistical analysis was performed using Student's *t* test.

Strains	Adhering bacteria		Reduction in adhesion (%)
	Without EGTA	20 mM-EGTA	
Lactobacillus human isolates			
L. acidophilus LB	210 (15)	175 (20)*	17
L. acidophilus CIP 6218	13 (4)	ND	–
L. acidophilus NCK 88	8 (2)	ND	–
Lactobacillus clinical isolates			
L. casei subsp. *casei* FPC 01.89	19 (7)	ND	–
L. casei subsp. *rhamnosus* FPC 02.90	36 (12)	ND	–
L. casei subsp. *rhamnosus* FPC 03.90	17 (6)	ND	–
L. casei subsp. *rhamnosus* FPC 04.90	48 (11)	ND	–
Lactobacillus plant isolates			
FPC 06.90	5 (2)	ND	–
FPC 07.90	4 (2)	ND	–
FPC 08.90	7 (3)	ND	–
Lactobacillus animal isolates			
L. acidophilus C2	43 (11)	40 (10)*	7
L. acidophilus C7	150 (12)	88 (10)†	41
L. acidophilus PA3	66 (12)	63 (12)*	4
L. acidophilus PA19	92 (9)	85 (15)*	8
L. acidophilus PA47	45 (5)	15 (2)†	67
Dairy *Lactobacillus*			
L. casei GG	125 (15)	105 (10)*	16
L. casei subsp. *rhamnosus* ATCC 7469	6 (2)	ND	–
L. casei subsp. *rhamnosus* CNRZ 205	5 (2)	ND	–
L. casei CNRZ 383	3 (1)	ND	–
L. casei CNRZ 36	6 (3)	ND	–
L. helveticus CNRZ 32	200 (21)	40 (15)†	80
L. helveticus CNRZ 240	215 (27)	35 (15)†	84
L. delbrueckii subsp. *lactis* CNRZ 239	190 (20)	90 (19)†	53
L. delbrueckii subsp. *lactis* CNRZ 1006	17 (7)	ND	–
L. delbrueckii subsp. *bulgaricus* CNRZ 394	55 (7)	ND	–

* No significant difference from control.
† $P < 0{\cdot}01$.
ND, not done.

border of the cell monolayer (Fig. 1*b*). Since spent culture supernatant of *L. acidophilus* LB contains lactic acid that could alter the brush border integrity we have observed by scanning electron microscopy that bacterial attachment occurs without cell damage (Fig. 1*c*).

The kinetics of adhesion of the *L. acidophilus* strain LB to differentiated Caco-2 cells was determined using a fixed number of monolayer cells ($4{\cdot}5 \times 10^5$ cells) and bacteria (2×10^8 c.f.u. ml^{-1}). Cover slips were washed and stained after 15, 30, 45, 60, 90 and 120 min incubation. Zero time controls consisted of monolayers to which bacteria were added and then immediately removed. The results of these experiments are presented in Fig. 2. For diffuse adhesion onto Caco-2 cells, maximum adherence occurred within 90 min and remained stable until the end of the time interval examined.

In an attempt to characterize the adhesin of *L. acidophilus* LB further, we treated the bacteria or the spent broth culture supernatant with various agents (Table 2). A highly significant loss of adhesion was observed when the spent broth was discarded or when it was replaced by fresh bacterial culture medium. These results strongly suggest that a secretory component in the

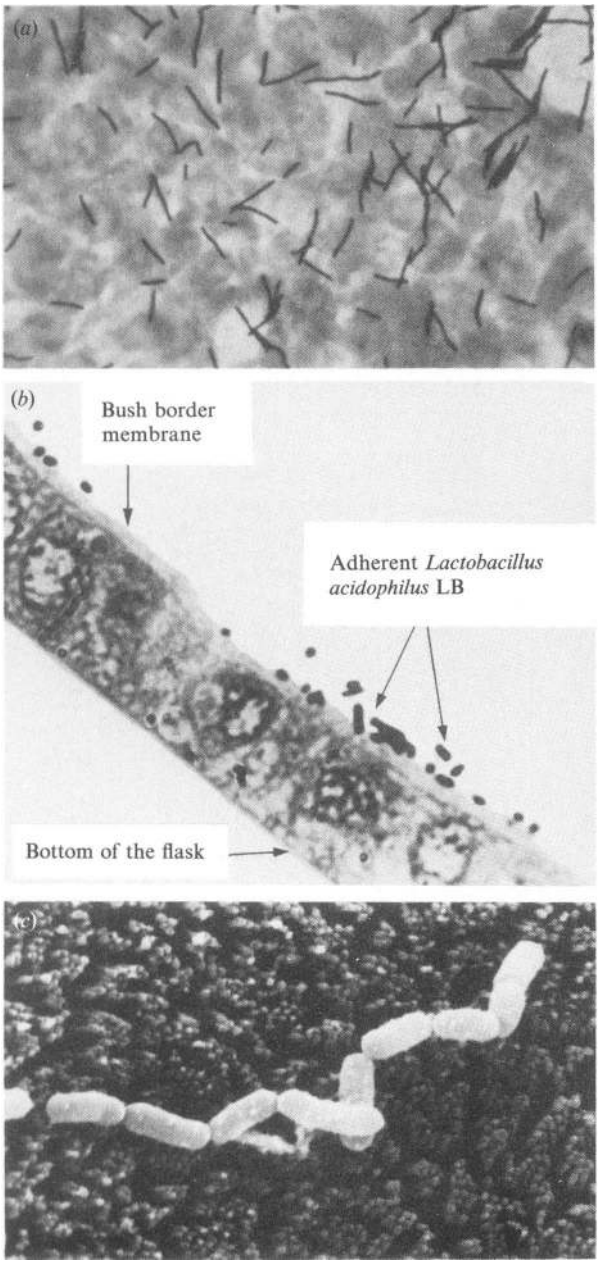

Fig. 1. Adherence of the human stool isolate *Lactobacillus acidophilus* LB to differentiated Caco-2 cells. (*a*) Light microscopy after Gram-staining; (*b*) ultrathin sections of post-confluent monolayer fixed in 3% glutaraldehyde and embedded in Epon for light microscopic examination of sections perpendicular to the bottom of the flask; (*c*) scanning electron micrograph of Caco-2 cell monolayers colonized by *Lactobacillus* LB.

spent broth was involved in adhesion of the *L. acidophilus* strain LB. Treatment of the spent broth with trypsin or pronase almost totally abolished the adhesiveness of *L. acidophilus* LB. This strongly suggests that the adhesion of strain LB is mediated by a proteinaceous component of bacterial origin in the spent culture broth. Trypsin

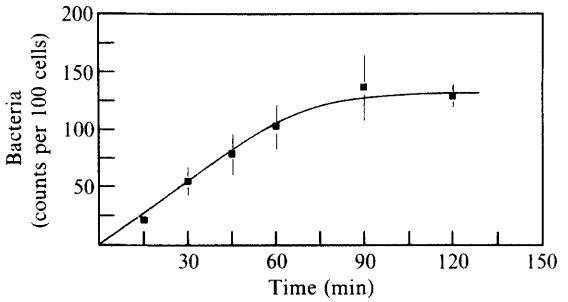

Fig. 2. Adherence time-course of the human stool isolate *Lactobacillus acidophilus* LB (final concentration 2 × 10^8 bacteria ml^{-1}) to differentiated Caco-2 cells. Each adherence assay was conducted in duplicate in three successive cell passages. Horizontal bars indicate standard deviations.

treatment of bacteria alone, without treatment of the spent culture broth, did not modify adhesion. A highly significant decrease in adhesion was observed after metaperiodate treatment of bacteria alone, indicating that carbohydrates may be involved in the interaction between the cell-surface component and the adhesion-promoting extracellular protein. The binding of *L. acidophilus* LB was not affected after heating the spent broth or the bacteria to 100 °C. Heating to 100 °C killed the bacteria. The addition of simple sugars such as D-mannose, L-fucose and D-galactose had no effect on the adhesion of strain LB (Table 2).

Expression of Lactobacillus binding sites during differentiation of Caco-2 cells

The effect of cellular differentiation was examined by measuring *L. acidophilus* LB adhesion to Caco-2 cells grown for different periods over 15 d. Adhesion was related to the age of the Caco-2 cell culture (Fig. 3). The state of the intestinal differentiation was evaluated by indirect immunofluorescence using antibody directed against the apical differentiation marker sucrase-isomaltase. After 5 d culture, the proportion of positive sucrase-isomaltase immunoreactivity on the cells was estimated to be less than 4% (Fig. 3*b*), indicating that a small proportion of the cells were differentiated. Immunoreactivity increased during subsequent days in culture (Fig. 3*c*, *d*); after 15 d, 90% of the Caco-2 cells were differentiated (Fig. 3*d*). *Lactobacillus* binding developed in parallel with the appearance of the differentiation marker (Fig. 3*a*). No adhesion of strain LB occurred on the surface of the Caco-2 cells during the first days of culture (undifferentiated cells). Bacterial binding appeared by day 9 and increased subsequently. These results indicate that *Lactobacillus* interacts with cultured human intestinal cells at cellular sites expressed in the brush border during the differentiation process of Caco-2 cells.

Table 2. *Effects of treatments of bacteria or the spent culture supernatant of strain L. acidophilus LB on adhesion to Caco-2 cells*

Adhesion assay of strain LB (final concentration 4×10^8 bacteria ml^{-1}) was monitored after 1 h at 37 °C in 10% CO_2/90% air. Mean number of lactobacilli adhering to the cell monolayer per 100 cells in two identical adhesion assays run in parallel. Twenty randomized microscopic fields per cover slip were counted. Each adhesion assay was conducted in duplicate in three successive cell passages. Number in parentheses indicate standard deviations. The control was *L. acidophilus* strain LB in presence of spent culture broth. Statistical analysis was done with the Student's *t* test.

Conditions	Pretreatment of: Bacteria	Pretreatment of: Spent culture broth	Adhering bacteria	Reduction (%)
Control	−	−	215 (16)	
Bacteria alone	−	−	37 (10)¶	83
Fresh culture medium*	−	−	31 (5)¶	86
Trypsin†	+	+	20 (3)¶	91
Pronase	+	+	33 (5)¶	85
Pronase	−	+	27 (6)¶	87
Trypsin	−	+	43 (7)¶	80
Trypsin	+	−	190 (15)‖	12
Heating 100 °C	+	+	194 (25)‖	10
Heating 100 °C	−	+	170 (14)‖	21
Heating 100 °C	+	−	145 (10)‖	32
Metaperiodate‡	+	−	86 (15)¶	60
D-Mannose§	−	−	195 (20)‖	9
L-Fucose	−	−	190 (15)‖	12
D-Galactose	−	−	200 (13)‖	7

* To determine the presence of adhesins secreted by the bacteria in the spent culture supernatant, it was replaced by a fresh culture medium before adhesion assay.

† Trypsin (2·5 mg ml^{-1}) or pronase (2·5 mg ml^{-1}) treatments for 60 min at 37 °C; trypsin and pronase were inactivated by adding inactivated (30 min, 56 °C) fetal calf serum.

‡ Bacteria were preincubated with 50 mM-metaperiodate for 30 min, centrifuged, washed in PBS and resuspended in spent culture supernatant.

§ Potential inhibitors were added separately to yield final concentrations of 100 mM.

‖ No significant difference.

¶ $P < 0{\cdot}01$.

Discussion

The mechanism by which *Lactobacilli* adhere to human intestinal cells remains unknown, but investigation of the mechanism of bacterial adhesion would be greatly facilitated by a suitable cell culture model. Ideally, isolated enterocytes could be used, but unfortunately human intestinal tissue is not readily available. Moreover, the poor viability of isolated human enterocytes and variations between different enterocyte donors have given rise to rather variable results from bacterial adhesion studies *in vitro* (Darfeuille-Michaud *et al.*, 1990). In the experiments reported here, we used the human intestinal cell line Caco-2, a well-characterized cellular model established from a human colonic adenocarcinoma by Fogh *et al.* (1977). In culture, this cell line spontaneously develops characteristics of mature enterocytes with functional brush border microvilli and apical hydrolases (Pinto *et al.*, 1983; Hauri *et al.*, 1985). Caco-2 was used extensively in studies on the organization and function of human intestinal cells (reviewed by Zweibaum *et al.*, 1991). The advantages of Caco cells are that they express morphological and functional differentiation *in vitro* and show characteristics of mature enterocytes. The cells form two clearly distinguishable domains; an apical membrane and a basolateral membrane separated by tight junctions (Simon & Fuller, 1985). With different protein and lipid compositions, these domains are strikingly different in ultrastructure. For example, the apical surface (brush border) contains peptidases and disaccharidases (Hauri *et al.*, 1985; Pinto *et al.*, 1983), the basolateral domain contains several peptide receptors involved in the control of intestinal hydroelectrolytic secretion (Laburthe & Amiranoff, 1990), and the tight junctions contain specific proteins. These cells are thought to be a convenient model for enterocytes and make an excellent model to study the cell biological processes of adhesion and bacterial invasion.

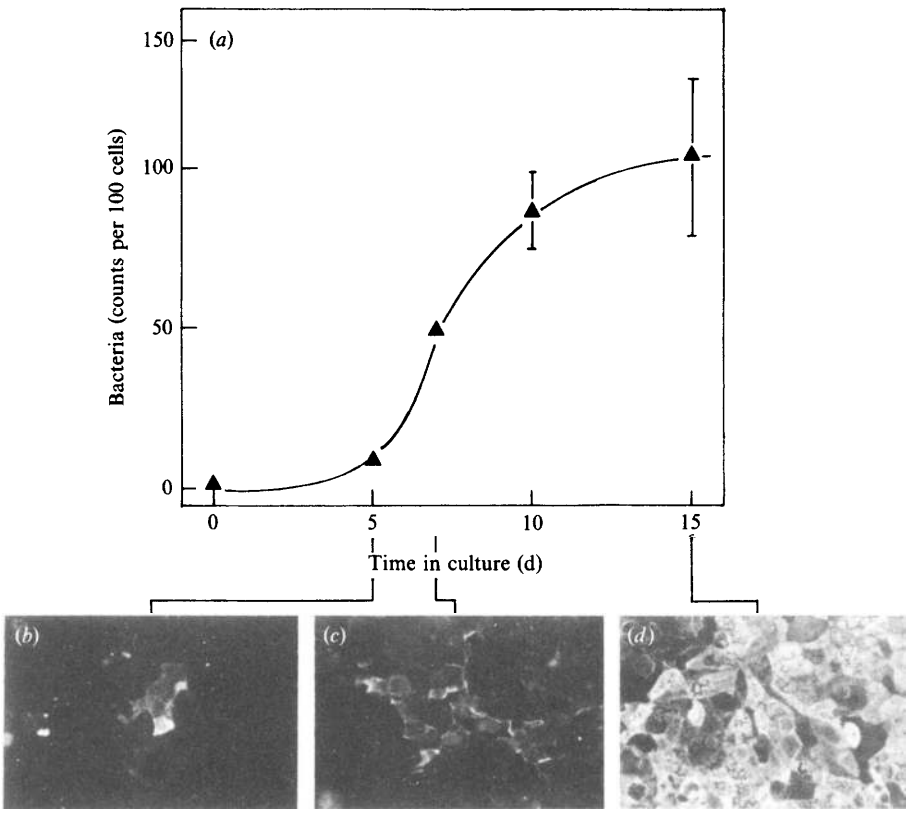

Fig. 3. Change in adhesion of *Lactobacillus acidophilus* LB to Caco-2 cells as a function of differentiation. (*a*) Day-course of *Lactobacillus* adhesion; (*b*, *c*, *d*) immunofluorescent staining with anti-human-sucrase-isomaltase antibody. Adhesion assay of strain LB (final concentration 2 × 10^8 bacteria ml^{-1}) was monitored after 1 h at 37 °C in 10% CO_2/90% air. Each adherence assay was conducted in duplicate using cells from three successive passages (70–73). Horizontal bars indicate standard deviations.

Moreover, they were recently used for studying human-specific bacteria for which animal models do not exist. Indeed, Caco-2 cells bind *Listeria monocytogenes* (Gaillard *et al.*, 1987; Mounier *et al.*, 1990), enterotoxigenic *Escherichia coli* (ETEC) producing CFA I, CFA II, CFA III, antigen 2230 adhesin factors (Darfeuille-Michaud *et al.*, 1990), diffusely adhering (DAEC) (Kerneis *et al.*, 1991) and enteropathogenic *Escherichia coli* (EPEC) (Knutton *et al.*, 1989), *Salmonella typhimurium* (Finlay & Falkow, 1990; Gahring *et al.*, 1990), *Vibrio cholerae* (Panigrahi *et al.*, 1990), *Cryptosporidium parvum* (Flanigan *et al.*, 1991) *Entamoeba histolytica* (Rigothier *et al.*, 1991), rotavirus (Svensson *et al.*, 1991) and reovirus (Ambler & Mackay, 1991). This cell line provides, post-confluence, a uniform population of differentiated cells that can be used under defined conditions in a simple reproducible assay to quantify adherent bacteria.

The ability of *Lactobacilli* to adhere to the differentiated Caco-2 cells varied considerably between strains, which showed that adhesive properties are not a universal feature of *Lactobacillus*. Particularly, the results showed that among the twenty-five *Lactobacillus* strains screened seven lactobacilli of different origin adhered well to Caco-2 cells. As indicated by Kleeman & Klaenhammer (1982), di- and trivalent cations may act by providing an ionic bridge between surfaces of bacteria and epithelial cells and play a role in many adherence systems. These authors have demonstrated that enhanced adhesion of lactobacilli to intestinal cells was promoted by the divalent cation calcium. This adhesion is distinct from the adhesion occurring in the absence of the cations. Among the adhering strains tested in the present study, three possess calcium-independent capabilities; in particular, a high level of calcium-independent binding was expressed by the human stool isolate *L. acidophilus* LB.

The adhesion of *Lactobacillus* strain LB to Caco-2 cells appeared to be mediated by two components: a cell-surface component and an extracellular factor. The cell-surface determinant of strain LB did not appear to be proteinaceous. This is of interest, since it is generally accepted that the non-proteinaceous cell-wall component lipoteichoic acid is involved in the adhesion of some Gram-positive bacteria (Araki & Ito, 1989; Chan *et al.*,

1985). The nature of the cell-surface determinant of strain LB needs to be further characterized and compared with determinants expressed on the bacterial cell surface of other intestinal adherent *Lactobacilli*, such as BG2FO4 (Kleeman & Klaenhammer, 1982; Hood & Zotola, 1989), the human-uroepithelial-adhering GR strains (Chan *et al.*, 1985; Reid & Sobel, 1987) and the strains of animal origin *L. fermentum* 737 (Conway & Kjelleberg, 1989) and *L. fermentum* 104 (Henriksson *et al.*, 1991). Trypsin and pronase treatments of the spent culture supernatant of strain LB greatly decreased the adhesion of strain LB, which suggested that the extracellular component was proteinaceous. A similar result has been reported in two comprehensive and elegant studies with the mouse *L. fermentum* strain 737 (Conway & Kjelleberg, 1989) and porcine strain 104 (Henriksson *et al.*, 1991) that adhere to mouse and porcine gastric squamous epithelia, respectively. Altogether, these results suggest that the mechanism of adhesion of Gram-positive bacteria to human intestinal cells may be quite different from that of Gram-negative bacteria, which involves fimbrial or membrane adhesins (De Graaf, 1990). Work is in progress to isolate and characterize the extracellular adhesion-promoting determinant of the human *Lactobacillus*.

In Caco-2 cells, the time-course of the differentiation process, with undifferentiated exponentially dividing cells which differentiate when the cells stop dividing (Zweibaum *et al.*, 1990), closely mimics the situation found in the small intestine. Since the differentiation of Caco-2 cells, which form tight monolayers, occurs spontaneously in culture (Pinto *et al.*, 1983), these cells represent attractive models for the study of the relationship between the state of differentiation and the *L. acidophilus* binding sites. The lack of binding to the undifferentiated Caco-2 cells during the first days of culture and the progressive increase in bacterial binding with increased duration of culture strongly suggest that the expression of specific *Lactobacillus* binding sites is related to the differentiation status of the intestinal cells. We previously noticed that the human enterocytic receptors for the diffusely adhering *E. coli* (DAEC) C1845 were also expressed during the enterocytic differentiation process of the HT-29 and Caco-2 cell lines in culture (Kerneis *et al.*, 1991). The nature of the intestinal human *Lactobacillus* binding sites remains to be determined.

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