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Adhesion of Dairy Propionibacteria to Intestinal Epithelial Tissue In Vitro and In Vivo

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ABSTRACT

Adhesion to the intestinal mucosa is a desirable property for probiotic microorganisms and has been related to many of their health benefits. In the present study, 24 dairy *Propionibacterium* strains were assessed with regard to their hydrophobic characteristics and their autoaggregation and hemagglutination abilities, since these traits have been shown to be indicative of adherence in other microorganisms. Six strains were further tested for their capacity to adhere to ileal epithelial cells in vitro and in vivo. The results of the study showed that propionibacteria were highly hydrophilic, and hemagglutination and autoaggregation were properties not commonly found among these microorganisms. No relationship was found between surface characteristics and adhesion ability, since hemagglutinating, autoaggregating, and nonautoaggregating bacteria were able to adhere to intestinal cells both in vitro and in vivo. Microscopic examination revealed that autoaggregating cells adhered in clusters, with adhesion being mediated by only a few bacteria, whereas the hemagglutinating and nonautoaggregating strains adhered individually or in small groups making contact with each epithelial cell with the entire bacterial surface. The in vitro assessment of adhesion was a good indication of the in vivo association of propionibacteria with the intestinal epithelium. Therefore, the in vitro method presented here should be valuable in screening routinely adhesive properties of propionibacteria for probiotic purposes. The adhesion ability of dairy propionibacteria would prolong their maintenance in the gut and increase the duration of their provision of beneficial effects in the host, supporting the potential of *Propionibacterium* in the development of new probiotic products.

In recent years, the use of probiotic agents to enhance a host's health has been emphasized. To date, the best studied organisms to be used as probiotic agents are *Lactobacillus* and *Bifidobacterium* strains, since they are normal inhabitants of the intestinal tracts of human beings and other vertebrate animals *(8).* Other organisms used as probiotic agents include *Streptococcus* sp., *Enterococcus* sp., *Propionibacterium* sp., and various fungi *(3).*

Attachment to the intestinal mucosa has been related to many of the beneficial health effects ascribed to probiotic bacteria, as it prolongs their survival in the gut and therefore increases the duration of their positive influence on the gastrointestinal microflora and immune system of the host *(19, 20).* Consequently, adhesion ability is regarded as an important property when probiotic microorganisms are selected.

Bacterial adhesion is initially determined by nonspecific and reversible interactions that involve physicochemical interactions (including hydrophobicity and charges) followed by specific and irreversible interactions mediated by adhesins of the bacterial surface and complementary receptors of the host cell *(1).* Properties generally associated with the initial stage are hydrophobicity of the cell surface, erythrocyte agglutination, autoaggregation, and electrical mobility *(18).* Cellular hydrophobicity is generally associated with the capacity of microbial cells of many taxonomic groups to adhere to different surfaces, including animal tissues *(7).* Hemagglutination is frequently used to monitor lectin and lectinlike substances that could act as bacterial adhesins, since the erythrocyte surface is rich in glycoconjugates and resembles the surface of tissue cells normally colonized by microorganisms *(18).* Finally, autoaggregation is thought to substantially increase the ability of lactobacilli to colonize environments with short residence times, and it was recently reported to be indicative of cell adherence in bifidobacteria *(5, 6, 22, 28).*

Dairy propionibacteria are widely used as starter bacteria for the manufacture of cheese, and research on their probiotic properties has recently begun *(9, 23, 24).* In previous studies, we demonstrated the ability of propionibacteria to survive passage through the adverse conditions of the gastrointestinal tract, and some probiotic properties were also described *(23, 24, 30).* However, little is known about the surface characteristics and adhesive properties of dairy propionibacteria. In the present study, we examined the ability of dairy *Propionibacterium* strains to adhere to intestinal mucosa in vitro and in vivo. Some surface properties were also studied to investigate a possible relationship between physicochemical properties and the ability to adhere to intestinal cells.

MATERIALS AND METHODS

Microorganisms and growth conditions. The cell surface characteristics of 24 dairy *Propionibacterium* strains were studied.

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TABLE 1. *Adhesion-related properties of dairy propionibacteria*

Strain[a]	Source	% of adhesion to[b]: p-xylene	Chloroform	Ethyl acetate	Hemagglutination	Autoaggregation
P. acidipropionici CRL 1198	Emmental	6.8 ± 1.1	0	28.0 ± 1.2	−	−
P. acidipropionici Q4[b]	Emmental	7.1 ± 1.2	12.0 ± 2.1	31.0 ± 1.8	−	−
P. freudenreichii ATCC 13673	Cheese	4.1 ± 0.9	0	23.8 ± 1.8	−	−
P. freudenreichii Ce[c]	Raw milk	5.3 ± 0.4	4.6 ± 1.0	26.2 ± 2.2	−	−
P. freudenreichii CRL 757	Emmental	9.3 ± 2.5	0	23.0 ± 2.8	−	−
P. freudenreichii E1[c]	Emmental	7.1 ± 1.5	2.2 ± 0.2	29.0 ± 0.9	−	−
P. freudenreichii E4[c]	Emmental	9.3 ± 1.1	0	31.0 ± 3.1	−	−
P. freudenreichii E6[c]	Emmental	1.1 ± 0.1	4.6 ± 0.8	24.8 ± 1.5	−	−
P. freudenreichii F1[c]	Fontina	11.4 ± 2.1	0	26.4 ± 2.8	−	+
P. freudenreichii F3[c]	Fontina	11.4 ± 3.5	0	23.8 ± 0.9	−	+
P. freudenreichii F6[c]	Fontina	7.1 ± 0.4	28.6 ± 1.2	52.0 ± 0.9	−	−
P. freudenreichii G1[c]	Gruyere	67.6 ± 2.4	62.1 ± 3.5	42.4 ± 1.2	−	+
P. freudenreichii Reβ[c]	Raw milk	3.6 ± 0.7	6.7 ± 1.3	28.2 ± 3.2	−	−
P. freudenreichii S1[c]	Gruyere	3.0 ± 0.8	5.9 ± 0.4	25.6 ± 2.5	−	−
P. freudenreichii SG1[c]	Emmental	2.6 ± 0.4	6.7 ± 0.9	11.7 ± 0.5	−	−
P. freudenreichii TL 213	Fribourg	2.6 ± 0.4	15.6 ± 1.1	25.8 ± 0.6	−	−
P. freudenreichii TL 253	Emmental	0	2.9 ± 0.8	22.7 ± 1.4	−	−
P. freudenreichii TL 26	Gruyere	9.5 ± 1.3	6.7 ± 0.3	32.3 ± 2.4	−	−
P. freudenreichii TL 495	Raw milk	9.4 ± 1.6	9.3 ± 1.4	38.8 ± 2.8	−	−
P. freudenreichii TL 502	Beaufort	9.4 ± 0.8	6.7 ± 0.9	26.7 ± 3.7	−	−
P. freudenreichii TL 503	Beaufort	4.4 ± 0.5	15.1 ± 2.8	29.0 ± 0.9	−	+
P. jensenii TL 219	Appenzell	9.3 ± 2.2	9.3 ± 1.5	33.3 ± 0.4	−	−
P. jensenii TL 246	RAS égyptien	12.8 ± 3.5	12.0 ± 1.0	36.0 ± 2.8	+	−
P. jensenii TL 494	Raw milk	4.0 ± 0.3	9.3 ± 1.2	28.0 ± 2.1	−	−

[a] CRL, Centro de Referencias para Lactobacilos Collection; ATCC, American Type Culture Collection; TL, Tecnologie laitiere, Laboratoire des Recherches en tecnologie laitiere, Institut National de la Recherche Agronomique, Rennes, France.
[b] Mean ± standard deviation.
[c] Strain isolated in our laboratory from milk or cheese.

The origins and sources of the strains are listed in Table 1. For in vitro and in vivo adhesion assays, *Propionibacterium freudenreichii* CRL 757, G1, and F3; *Propionibacterium jensenii* TL 246; and *Propionibacterium acidipropionici* CRL 1198 and Q4 were used. Prior to experimental use, organisms were subcultured at least three times every 24 h in deMan Rogosa Sharpe broth (Difco). All experiments were performed with cells in the late exponential phase (42 h). Propionibacteria were grown statically in deMan Rogosa Sharpe broth at 37°C, harvested by centrifugation (10,000 × *g*, 10 min), washed twice with 0.01 M KH_2PO_2-Na_2HPO_4 containing 0.8% NaCl and 0.2% KCl (pH 7.2; phosphate-buffered saline [PBS]), and, finally, resuspended in 0.1 M KNO_3 (pH 6.2) for the microbial adhesion to solvents test and in PBS for the other assays. The cell concentration of each culture was adjusted turbidimetrically to an optical density at 600 nm (OD_{600}) of 0.6 (~ 5×10^8 CFU ml^{-1}) with a spectrophotometer (Spectronic 20, Bausch & Lomb Spectrophotometer). The number of propionibacteria per milliliter was determined using a standard curve of absorbance at 600 nm versus CFU ml^{-1}.

Microbial adhesion to solvents. Microbial adhesion to solvents was measured by the method of Pelletier et al. *(21)* with some modifications. Two milliliters of bacterial suspension (A_0 = 0.6) was put in contact with 0.5 ml of each of the following test solvents: p-xylene (nonpolar neutral solvent), chloroform (monopolar acidic solvent), and ethyl acetate (monopolar basic solvent). After 10 min of preincubation at room temperature, the two-phase system was mixed on a vortex for 2 min. After allowing the hydrocarbon phase to rise completely, the aqueous phase was removed and the absorbance at 600 nm was determined again (A_1). The percentage of microbial adhesion to the solvent was calculated as $[(A_0 - A_1)/A_0] \times 100$.

Salt aggregation test. The salt aggregation test was basically performed according to Wadstrom et al. *(29)*. Equal amounts of serially diluted solutions of ammonium sulphate (0.2 M to 4.0 M) and bacterial suspensions (in PBS) were mixed on ordinary glass microscope slides. The ability of bacteria to form aggregates in 2 min was determined by observation under a light microscope and scored qualitatively as present (+) or absent (−).

Aggregation assay. Autoaggregation ability was measured as described by Del Re et al. *(5)*. Briefly, bacterial suspensions in PBS (OD_{600} = 0.6) were shaken and incubated at 37°C for 2 h. At 30, 60, 90, and 120 min after shaking, 1 ml of the surface of each suspension was carefully removed and transferred to another tube, and the OD_{600} was measured. The autoaggregation percentage was expressed as 1 − (OD for upper suspension/OD for total bacterial suspension) × 100.

Hemagglutination. The hemagglutination test was performed essentially as described by Mukai et al. *(17)*. The assay was carried out at room temperature with a 96-well U-shaped microtiter plate with PBS as a diluent. Twenty-five microliters of bacterial suspension (10^9 CFU ml^{-1}) was mixed with 25 μl of 2% erythrocyte suspension (type O+) pretreated with 10 mg ml^{-1} trypsin. Agglutination was read with the naked eye after 2 h of incubation. The hemagglutination titer was expressed as the reciprocal of the highest dilution showing agglutination.

Animals. Six- to eight-week-old BALB/c mice, each weighing 25 to 30 g, were used as a source of intestinal epithelial cells for in vitro and in vivo adhesion assays. The animals were obtained from the random-bred closed colony kept at our center. The mice were maintained in metal cages, were given free access to feed and water, and were fasted for 24 h before the experiments were performed. Mice were sacrificed by cervical dislocation, and their small bowels were removed for isolation of intestinal epithelial cells (IEC).

In vitro adhesion assay. A modified technique of Jin et al. *(11)* was used for the preparation of IEC and for the adhesion assay. Epithelial cells of the terminal ileum were scraped off gently with the edge of a microscope slide and suspended in PBS as previously described. The IEC suspension was washed twice with PBS (120 × *g,* 10 min) and resuspended in NCTC 135 medium (Sigma Chemical Co, St Louis, Mo.), supplemented with 2% fetal 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 for adhesion studies. Suspensions of propionibacteria (5×10^8 CFU ml^{-1} in PBS) and IEC were mixed (1:4) and incubated at 37°C for 1 h under microaerophilic conditions (5% CO_2 and 95% air). After incubation, the mixtures were kept on ice. Adhesion of propionibacteria to IEC was determined by examining 30 epithelial cells selected at random using phase-contrast microscopy *(14).* The number of IEC with adhered bacteria extrapolated to 100 IEC (adhesion percentage) and the average number of bacteria attached per IEC including only IEC with bacteria (adhesion index) were calculated. Adherence of the bacteria was also observed microscopically after Gram staining. The reaction mixtures were washed (120 × *g,* 5 min), resuspended in PBS, and filtered through an 8-μm-pore-size membrane (Millipore Corp., Bedford, Mass.). Cells with bacteria adhered that were retained in the filter were transferred to albumin-coated microscope slides, fixed with methanol, and Gram stained.

In vivo adhesion assay. Mice were randomly divided into treatment groups of five animals. For 3 days, each group received a conventional balanced diet either supplemented or not supplemented (control) with different strains of propionibacteria. Cultures of the selected *Propionibacterium* strains developed in deMan Rogosa Sharpe broth (42 h) were centrifuged (10,000 × *g,* 10 min), washed with sterile PBS, and suspended in nonfat milk (10%) to the desired concentration (10^9 CFU ml^{-1}). These bacterial suspensions were provided ad libitum instead of drinking water to the test groups of mice, whereas the control group received sterile milk. At the end of the feeding period, the animals were sacrificed and their small bowels were removed. Intestinal contents were discarded, and the gut walls were repeatedly washed with sterile PBS. Intestinal epithelial cells were exfoliated with the edge of a microscope slide and suspended in sterile PBS. The weight of the IEC was calculated by the difference between the weight of the gut walls before and after extraction of the mucosa cells. The number of viable propionibacteria adhering to the IEC was determined by a plate count method. Serial 10-fold dilutions of the IEC suspensions were carried out with sterile peptone water (0.1% peptone), and appropriate dilutions were poured into ECOTEC (Ecophysiology Technology) culture medium developed especially for propionibacteria *(2, 23).* The plates were incubated anaerobically at 35°C for 5 days in an anerobic chamber (Forma Scientifica Anaerobic System Model 1024) containing a gas mixture of 90% N_2 and 10% CO_2.

Statistical analysis. Three separate trials were conducted for each experiment, and the results are presented as means ± standard deviations. Significant differences between means were determined by Tukey's test ($P < 0.05$) after analysis of variance with the Minitab Statistic Program (release 12 for Windows).

RESULTS

Twenty-four strains of dairy propionibacteria were studied to determine their cell surface characteristics and their ability to adhere to intestinal cells. Almost all strains were highly hydrophilic, since they showed low affinity for p-xylene (Table 1). The hydrophilicity of strains was also confirmed by the salt aggregation test, since no aggregation was observed even at a high salt concentration (4 M) (data not shown), except for autoaggregating strains (*P. freudenreichii* F1, F3, G1, TL, and 503). Basic or acidic surface characteristics of propionibacteria were studied by measuring the partition of cells between the aqueous phase and chloroform or ethyl acetate, respectively. Most strains showed higher affinity for ethyl acetate, a basic solvent and an electron donor, than for chloroform, an acidic solvent and an electron acceptor (Table 1). The affinity for one solvent did not exclude simultaneous affinity for the other, suggesting a high complexity of the cell surface. However, a stronger affinity for the basic solvent indicates that propionibacteria have an acidic character. Two phenotypes were observed among the strains tested for their aggregation ability: autoaggregating cells that formed large clusters that precipitated within 2 h (≥70% autoaggregation) and nonautoaggregating cells that produced constant turbidity for long periods due to a lack of clusters (≤20% autoaggregation). Hemagglutination ability was not spread among propionibacteria, and only *P. jensenii* TL 246 was able to agglutinate erythrocytes with a titer of 32. To determine the relationship between these characteristics and adhesion ability, three nonautoaggregating strains, one hemagglutinating strain, and two autoaggregating strains were assayed for their adherence to epithelial cells both in vitro and in vivo. As shown in Table 2, the three phenotypes tested demonstrated the capacity to adhere to intestinal epithelial cells to different degrees. Autoaggregating strains did not exhibit higher adhesion levels, and no correlation was found between hydrophobicity and adhesion ability, since all strains tested (except *P. freudenreichii* G1) were highly hydrophilic. Two adhesion parameters were determined: (i) the adhesion percentage, which indicates the number of intestinal cells containing attached bacteria per 100 IEC, and (ii) the adhesion index, which gives the mean number of adhered bacteria per IEC. The adhesion percentages varied significantly for the strains tested, ranging from 28.3% for *P. freudenreichii* CRL 757 to 62.2% for *P. acidipropionici* CRL 1198. Similar levels of adherence were observed both in vitro and in vivo for *P. acidipropionici* CRL 1198 and Q4, *P. jensenii* TL 246, and *P. freudenreichii* F3 ($P > 0.05$), whereas *P. freudenreichii* CRL 757 and G1 were significantly less adherent in vitro and were also recovered in rather low numbers from the intestinal walls of mice ($P < 0,05$; Table 2).

Simultaneous analysis of both parameters revealed the mode of binding and adhesion efficiency of the strains tested, showing that *P. acidipropionici* CRL 1198 and Q4 ad-

TABLE 2. *In vitro and in vivo adhesion of dairy propionibacteria with different phenotypes to intestinal epithelial cells*[a]

Strain	Phenotype	Adhesion (%)[b]	Adhesion index[c]	In vivo adhesion (log CFU g^{-1} of cells)
P. acidipropionici CRL 1198	Nonautoaggregating	62.2 ± 2.51 A[d]	6.52 ± 1.20 A	7.46 ± 0.34 A
P. acidipropionici Q4	Nonautoaggregating	61.1 ± 7.94 A	5.45 ± 1.02 A	7.43 ± 0.62 A
P. jensenii TL 246	Hemmagglutinating	60.0 ± 3.35 A	3.83 ± 0.25 B	6.86 ± 0.36 A
P. freudenreichii F3	Autoaggregating	50.0 ± 9.40 AB	ND[e] (groups)	6.62 ± 0.50 A
P. freudenreichii G1	Autoaggregating	40.0 ± 4.00 BC	ND (groups)	2.21 ± 1.33 B
P. freudenreichii CRL 757	Nonautoaggregating	28.3 ± 2.35 C	4.80 ± 0.58 A	4.57 ± 0.53 B
Control[f]	—	0	0	3.78 ± 0.54 B

[a] Data are given as means ± standard deviations of three separate trials.
[b] Expressed as number of intestinal epithelial cells (IEC) with adhered bacteria × 100 IEC.
[c] Expressed as mean number of bacteria per epithelial cell considering only IEC with attached bacteria.
[d] Means with the same letter in the same column are not significantly different ($P > 0.05$).
[e] ND, not determined because the autoaggregating phenotype did not allow enumeration of the number of microorganisms adhered per epithelial cell.
[f] Control for in vitro assay: IEC without added bacteria; control for in vivo assay: mice fed milk without dairy propionibacteria.

hered better than *P. jensenii* TL 246 and *P. freudenreichii* CRL 757. The two *P. acidipropionici* strains bound to a larger number of IEC, with numbers of bacteria per cell similar to those of *P. freudenreichii* CRL 757 (a larger percentage of adhesion and close to the same adhesion index) and with same number of IEC but in larger numbers per cell relative to *P. jensenii* TL 246 (the same percentage of adhesion and a greater adhesion index).

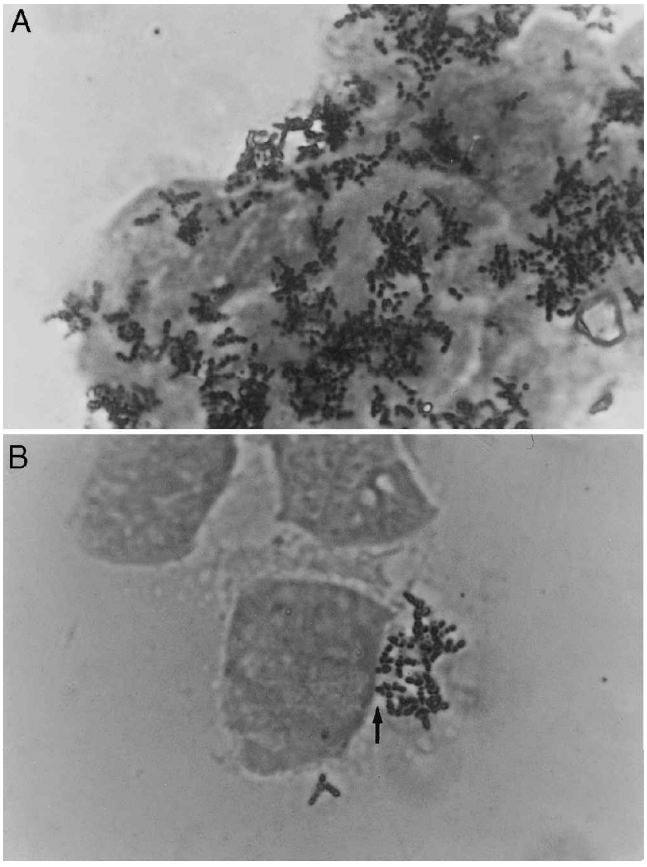

FIGURE 1. *(A) Adherence of one autoaggregating strain,* P. freudenreichii *F3, to IEC observed with light microscopy after Gram staining. (B) For all of the autoaggregating strains, the adherence of clusters to IEC was mediated by a few bacteria (arrow). Objective magnification, ×100.*

In vitro assessment of adhesion seems to provide a good indication of what may happen with propionibacteria in the intestines of living mice, since similar binding profiles were obtained in vitro and in vivo (except for *P. freudenreichii* G1). It is known that propionibacteria can be isolated from the intestines of different animals as a natural constituent of the microflora. In this study, wild strains were detected in the gut walls of BALB/c mice at approximately 5×10^3 CFU g^{-1} of IEC (3.78 log CFU g^{-1}). Ingestion of dairy propionibacteria increased the intestinal levels of propionibacteria by 0.6 ($P > 0.05$) to nearly 4 log cycles ($P < 0.05$) depending on the strain tested, indicating that in vivo adherence really occurred and demonstrating the potential for colonization of the propionibacteria tested (Table 2).

Two distinct adhesion patterns were observed with phase-contrast microscopy and Gram staining: (i) autoaggregating cells adhered in big clusters, with adhesion being mediated by only a few bacteria (Fig. 1A and 1B), and (ii) the hemagglutinating and nonautoaggregating strains adhered individually or in small groups of cells, making contact with each IEC with the entire bacterial surface (Fig. 2A and 2B).

DISCUSSION

It is thought that in order for probiotic bacteria to produce beneficial effects in vivo, they must be able to survive the adverse conditions of the gastrointestinal tract and remain in the intestine at a level that varies from 10^6 to 10^9 CFU g^{-1} of the contents, in spite of the normal washout by contractions of the gut *(25).* Therefore, microorganisms with a high growth rate and/or the ability to adhere to mucosal surfaces will have prolonged survival in the body of the host and are promising candidates for use in functional foods *(10).* In recent years, the potential of dairy propionibacteria as probiotic agents has been emphasized *(9, 23, 24).* Since they have a low growth rate, adherence is an

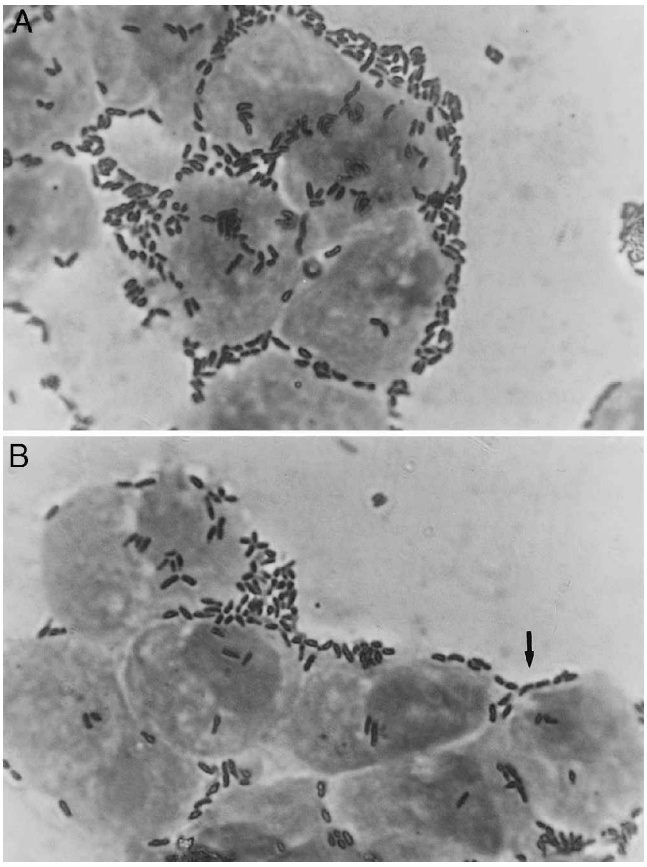

FIGURE 2. *Light micrographs illustrating the adhesion of (A) nonautoaggregating* P. acidipropionici *CRL 1198 and (B) hemagglutinating* P. jensenii *TL 246 to IEC observed after Gram staining. For these strains, individual bacteria or small groups of bacteria made contact with each IEC with their entire surface (arrow). Objective magnification, ×100.*

important property for dairy propionibacteria, and therefore a test to study their adhesion ability would be a useful tool.

The adherence of dairy propionibacteria has scarcely been studied. Lehto and Salminen *(13)* and Ouwehand et al. *(19)* reported on the adhesion of *P. freudenreichii* subsp. *shermanii* JS to Caco-2 cells and mucus, respectively, and there are few reports about the surface characteristics of dairy propionibacteria *(16).*

Adhesion of bacteria to different substrates is initially determined by physicochemical properties of the cell surface, and certain characteristics, like hydrophobicity, autoaggregation, and hemagglutination abilities, have been positively associated with cell adherence. For streptococci, lactobacilli, and bifidobacteria, a positive correlation between hydrophobicity of the cell surface and cell adherence was observed *(5, 6, 29),* and for *Bifidobacterium* strains it was reported that autoaggregation and hemagglutination abilities were bacterial traits indicative of extensive adherence to enterocytelike cells *(22).* In a recent report, Del Re et al. *(6)* demonstrated that autoaggregation of human bifidobacteria was strongly related to adhesion and proposed that cell surface hydrophobicity and the ability to autoaggregate could be used to identify potentially adhering bifidobacteria.

In this study, hemagglutination and autoaggregation were shown to be uncommon properties among dairy propionibacteria. Cell surface hydrophobicity and Lewis electron donor and electron acceptor characteristics were determined by the microbial adhesion to solvents method. Almost all strains tested showed weak affinity for p-xylene, an apolar solvent, and stronger affinity for a strong basic solvent such as ethyl acetate than for an acidic solvent such as chloroform. These results indicated that propionibacteria were hydrophilic and were strong electron acceptors and weak electron donors. The hydrophilic and acidic nature of the cell surfaces of these strains could be due in part to the presence of teichoic acids, which was previously reported and related to adhesion in dairy propionibacteria *(16).*

No relationship was found between hydrophobicity, autoaggregation or hemagglutination, and adhesion ability, and a good degree of adherence was observed for all of the phenotypes, suggesting that multiple mechanisms are involved in the adhesion process. Neither Savage *(26)* nor Ouwehand et al. *(19)* found relationships between hydrophobicity of the cell surface of lactic acid bacteria and adhesion to gastric epithelium and mucus, respectively. Therefore, this surface characteristic should not be taken as indicative of adherent microorganisms.

In the present study, the adhesion of *Propionibacterium* strains was determined by using exfoliated intestinal cells as an in vitro model of intestinal tissue and then conducting an in vivo test consisting of inoculation of experimental animals and enumeration of propionibacteria adhering to intestinal walls after the sacrifice of mice. Several authors have studied the adhesion of different lactobacilli and bifidobacteria to cultured human cell lines as in vitro models of the intestinal epithelium and have observed considerable variability in adherence among the strains tested. In general, conclusions of in vitro studies cannot be directly extrapolated to in vivo situations, and adherence in vitro is no guarantee of adherence in vivo and subsequent colonization *(10).* However, there is some evidence that relates adhesion in vitro to temporal colonization of the gastrointestinal tract by bifidobacteria *(4).* Results obtained in our study confirmed the validity of the in vitro adhesion test for predicting the in vivo association of propionibacteria with the intestinal epithelium when the strains are resistant to acid, bile salts, and proteolytic enzymes of the gastrointestinal tract *(30).* Thus, the in vitro method presented here could be used for preliminary selection of potentially adherent propionibacteria for dietary use.

Strains of dairy origin are generally used as negative controls in studies of adhesion. However, recent studies have revealed that dairy strains like *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* can adhere well to Caco-2 cells and mucus *(12, 19, 27).* Our results also demonstrate good adherence of dairy propionibacteria to intestinal cells and suggest that host specificity of bacterial strains is not strictly necessary for adhesion to occur.

Microscopic examination of IEC with adhered bacteria by phase-contrast illumination and after Gram staining allowed us to identify two different adhesion patterns in propionibacteria. Autoaggregating bacteria adhered to IEC in clusters, with adhesion being mediated by only a few bac-

teria, whereas nonautoaggregating and hemagglutinating bacteria merely adhered as individual cells or small groups of cells. For probiotic purposes, it seems better to choose an adherent autoaggregating strain, since such a strain would ensure the persistence of a greater number of bacteria in the gut. However, a whole cluster adhering to the epithelium by only a few bacteria could be washed out by the rapid flow of the intestinal contents and the peristaltic movements of the gut, resulting in a significant loss of bacteria. On the other hand, a nonautoaggregating strain that adheres to a large number of epithelial cells with many microorganisms per cell would also be effective as a probiotic agent, and removal by the normal gut-cleansing mechanisms seems less likely.

In conclusion, results obtained in the present study demonstrate the strong ability of dairy propionibacteria to adhere to intestinal cells, and these results also validate a previous report on the persistence of *P. acidipropionici* CRL 1198 in the gut *(23).* On the basis of their acid-bile tolerance *(30),* adhesion capacity, and metabolic and immune effects in a host *(15, 23, 24),* certain *Propionibacterium* strains can be considered promising bacteria for the development of new probiotic dairy products.

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REFERENCES

1. Beachey, E. H. 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. 143:325–345.
2. Bujazha, M., A. Pérez Chaia, and G. Oliver. 1998. Desarrollo de un medio de cultivo para el recuento de propionibacterias en contenido intestinal, p. 45, B 4.3. Proc. of COMBHAL 98 VI Simp. Bras. Microbiol. Aliment. Sao Paulo, Brasil.
3. Collins, J. K., G. Thornton, and G. O. Sullivan. 1998. Selection of probiotic strains for human applications. Int. Dairy J. 8:487–490.
4. Crociani, J., J.-P. Grill, M. Huppert, and J. Ballongue. 1995. Adhesion of different bifidobacteria strains to human enterocyte-like Caco-2 cells and comparison with *in vivo* study. Lett. Appl. Microbiol. 21:146–148.
5. Del Re, B., A. Busetto, G. Vignola, B. Sgorbati, and D. L. Palenzona. 1998. Autoaggregation and adhesion ability in a *Bifidobacterium suis* strain. Lett. Appl. Microbiol. 27:307–310.
6. Del Re, B., B. Sgorbati, M. Miglioli, and D. Palenzona. 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum.* Lett. Appl. Microbiol. 31:438–442.
7. Doyle, R. J., and M. Rosenberg (ed.). 1990. Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.
8. Goldin, B. R., and S. L. Gorbach. 1992. Probiotics for humans, p. 355–376. *In* R. Fuller (ed.), Probiotics, the scientific basis. Chapman and Hall, London.
9. Grant, C., and S. Salminen. 1998. The potential of propionibacterium as probiotics, p. 589–601. *In* S. Salminen and A. von Wright (ed.), Lactic acid bacteria: microbiology and functional aspects. Marcel Dekker, New York.
10. Havenaar, R., B. Ten Brink, and J. H.-J. Huis in't Veld. 1992. Selection of strains for probiotic use, p. 209–224. *In* R. Fuller (ed.), Probiotics, the scientific basis. Chapman and Hall, London.
11. Jin, L. Z., Y. W. Ho, M. A. Ali, N. Abdullah, K. B. Ong, and S. Jalaludin. 1996. Adhesion of *Lactobacillus* isolates to intestinal epithelial cells of chicken. Lett. Appl. Microbiol. 22:229–232.
12. Kimoto, H., J. Kurisaki, N. M. Tsuji, S. Ohmomo, T. Okamoto, 1999. Lactococci as probiotic strains: adhesion to human enterocyte-like Caco-2 cells and tolerance to low pH and bile. Lett. Appl. Microbiol. 29:313–316.
13. Lehto, E. M., and S. Salminen. 1997. Adhesion of two *Lactobacillus* strains, one *Lactococcus* and one *Propionibacterium* strain to cultured human intestinal Caco-2 cell line. Biosci. Microflora 16:13–17.
14. Morata de Ambrosini, V. I., S. N. González, and G. Oliver. 1999. Study of adhesion of Lactobacillus casei CRL 431 to ileal intestinal cells of mice. J. Food Prot. 62:1430–1434.
15. Morata de Ambrosini, V., S. González, G. Perdigón, A. P. de Ruiz Holgado, and G. Oliver. 1998. Immunostimulating activity of cell walls from lactic acid bacteria and related species. Food Agric. Immunol. 10:183–191.
16. Morata de Ambrosini, V., S. González, A. Pesce de Ruiz Holgado, and G. Oliver. 1998. Study of the morphology of the cell walls of some strains of lactic acid bacteria and related species. J.Food Prot. 61:557–562.
17. Mukai, T., K. Arihara, and H. Itoh. 1992. Lectin-like activity of lactobacillus acidophilus JCM 1026. FEMS Microbiol. Lett. 98:71–74.
18. Ofek, I., and R. J. Doyle. 1994. Bacterial adhesion to cells and tissues. Chapman and Hall, London.
19. Ouwehand, A. C., P. V. Kirjavainen, M.-M. Grönlund, E. Isolauri, and S. J. Salminen. 1999. Adhesion of probiotic micro-organisms to intestinal mucus. Int. Dairy J. 9:623–630.
20. Ouwehand, A. C., P. V. Kirjavainen, C. Shortt, and S. J. Salminen. 1999. Probiotics: mechanisms and established effect. Int. Dairy J. 9: 43–52.
21. Pelletier, C., C. Bouley, C. Cayuela, S. Bouttier, P. Bourlioux, and M.-N. Bellon-Fontaine. 1997. Cell surface characteristics of *Lactobacillus casei* subsp. *casei, Lactobacillus paracasei* subsp. *paracasei,* and *Lactobacillus rhamnosus* strains. Appl. Environ Microbiol. 63:1725–1731.
22. Pérez, P., Y. Minaard, E. Disalvo, and G. De Antoni. 1998. Surface properties of bifidobacterial strains of human origin. Appl. Environ. Microbiol. 64:21–26.
23. Pérez Chaia, A., M. E. Nader de Macias, and G. Oliver. 1995. Propionibacteria in the gut: effect on some metabolic activities of the host. Lait 75:435–445.
24. Pérez Chaia, A., G. Zárate, and G. Oliver. 1999. The probiotic properties of propionibacteria. Lait 79:175–185.
25. Rambaud, J.-C., Y. Bouhnik, P. Marteau, and P. Pochart. 1993. Manipulation of the human gut microflora. Proc. Nutr. Soc. 52:357–366.
26. Savage, D. C. 1992. Growth phase, cellular hydrophobicity, and adhesion in vitro of *Lactobacilli* colonizing the keratinizing gastric epithelium in the mouse. Appl. Environ. Microbiol. 58:1992–1995.
27. Tuomola, E. M., and S. J. Salminen 1998. Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures. Int. J. Food Microbiol. 41:45–51.
28. Vandervoorde, L., H. Christiaens, and W. Verstraete. 1992. Prevalence of coaggregation reactions among chicken lactobacilli. J. Appl. Bacteriol. 72:214–219.
29. Wadstrom, T., K. Andersson, M. Sydow, L. Axelsson, S. Lindgren, and S. Gullmar. 1987. Surface properties of lactobacilli isolated from the small intestine of pigs. J. Appl. Bacteriol. 62:513–529.
30. Zárate, G., A. Perez Chaia, S. González, and G. Oliver. 2000. Viability and β-galactosidase activity of dairy propionibacteria subjected to digestion by artificial gastric and intestinal fluids. J. Food Prot. 6:1214–1221.