Survival of Lactic Acid Bacteria in the Human Stomach and Adhesion to Intestinal Cells

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ABSTRACT

The survival of four strains of lactic acid bacteria in human gastric juice, in vivo and in vitro, and in buffered saline, pH 1 to 5, has been investigated. The strains studied include two *Lactobacillus acidophilus* strains, *Lactobacillus bulgaricus,* and *Streptococcus thermophilus.* In addition, the adhesion of these strains to freshly collected human and pig small intestinal cells and to pig large intestinal cells has been studied and the effect of milk on both survival and adhesion tested. As a result of these investigations, an in vitro test system for screening potential cultures for use as human dietary adjuncts can be developed. The ability to survive in gastric juice and to adhere varied significantly for the strains tested; *L. acidophilus* ADH survived and adhered better than the others while *S. thermophilus* survived and adhered poorly. For all strains, both survival and adhesion was enhanced by milk. As all strains adhered to some extent to both human and pig intestinal cells, the adhesion mechanism is probably a nonspecific attachment as opposed to other reported specific *Lactobacillus* adhesion to gastric tissue. From the survival and adhesion data it seems feasible to obtain elevated levels of viable *Lactobacillus* sp. in human intestine by careful selection of the bacterial strains ingested. Furthermore, the in vitro methods used here should be valuable to screen potential strains. The data presented here can then be correlated with human in vivo studies monitoring the beneficial effect of ingestion of these *Lactobacillus.*

INTRODUCTION

The ingestion of lactic acid bacteria, which was initially proposed by Metchnikoff as a means to reduce intestinal putrification and prolong life (20), has been extensively investigated as a beneficial dietary adjunct for gastrointestinal disorders in humans and animals (16). Some workers (1, 18, 25) have suggested the use of *Lactobacillus* to prevent and treat diarrhea induced by *E. coli, Salmonella,* or *Shigella.* This work has paralleled in vitro studies demonstrating bacteriocin production by *Lactobacillus* strains (3, 19, 24). Other workers (2) have shown a correlation between *L. acidophilus* consumption and a decreased need for laxatives in constipated elderly people; Gilliland et al. (8) have demonstrated assimilation of cholesterol by *L. acidophilus.* Many antitumor properties of *Lactobacilli* have been reported (7). Goldin and coworkers have shown that oral *L. acidophilus* supplements given to rats (9) and humans (11) lowered the fecal activity of the enzymes β-glucuronidase, nitroreductase, and azoreductase. Using an animal chemical carcinogenesis model, occurrence of tumours was decreased significantly when *L. acidophilus* was administered orally (10).

The strains of lactic acid bacteria and the form of bacterial preparation has varied enormously for the various studies (26). These

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include freeze-dried commercial preparations of *L. acidophilus* and *L. bulgaricus* (23); defined laboratory strains of *L. acidophilus* administered with skim milk (10); dairy cultures used routinely to produce commercial fermented milk products including various *Streptococcus* sp. (15, 21, 22). More recently, emphasis has been placed on the selection and preparation of *Lactobacillus* strains (12, 13, 17). Kleeman and Klaenhammer (13) reviewed the need to select strains that can survive and establish within an environment as hostile as the gastrointestinal tract and have tested the ability of various strains to adhere to a human fetal intestinal cell line. For successful implantation of ingested *Lactobacillus,* bacteria must be viable within the gastrointestinal tract and also have adhesive properties to avoid the transient passage as in an in vivo human study (23).

The aim of this work was to test, both in vivo and in vitro, survival of several lactic acid bacteria when exposed to human gastric juice and the effects of an additive, such as milk, on survival. In addition, adhesion of various strains to freshly collected human and pig intestinal cells has been studied. Correlation between in vivo and in vitro data demonstrated the reliability of the in vitro methodology.

MATERIALS AND METHODS

Bacterial Cultures

The following bacterial cultures were a gift from T. R. Klaenhammer at the North Carolina State University, Department of Food Science. All strains were supplied and maintained in skimmed milk in 1-ml vials in liquid nitrogen. They were thawed immediately before use and not refrozen. *Lactobacillus acidophilus* ADH was a human isolate reported as strain MSO2 (13); *L. acidophilus* N_2, originally given to us by M. L. Speck, was the strain used by Goldin and Gorbach (10); *L. bulgaricus* and *S. thermophilus* strain were dairy culture strains. The frozen vials contained the following viable cells: *L. acidophilus* ADH 1.2×10^{10}/ml; *L. acidophilus* N_2 3.8×10^{10}/ml; *L. bulgaricus* 1.7×10^{9}/ml; *S. thermophilus* 1.7×10^{9}/ml.

Bacteriology for Survival Studies

Lactobacillus cultures were grown anaerobically in MRS broth (Difco) at 37°C overnight and transferred to fresh MRS broth for a further 24 h. *Streptococcus thermophilus* was similarly subcultured using brain-heart infusion broth (BHI) (Difco). Cultures were centrifuged at 3000 × *g*/10 min/4°C, washed once in sterile phosphate-buffered saline [(PBS) NaCl, .8%; .1 *M*, pH 7.2], and resuspended to one-tenth of the culture volume. These suspensions were used for the in vitro survival studies. The number of viable *Lactobacillus* cells was determined by serial 10-fold dilution in PBS and .1-ml aliquots were spread evenly on MRS agar. Plates were incubated anaerobically at 37°C for 72 h and the colony forming units estimated. Verification of the identity of the colonies was by Gram stain and the catalase reaction. The *Streptococcus thermophilus* PBS serial 10-fold dilutions were enumerated on blood agar plates (BHI plus 5% blood) incubated anaerobically at 37°C for 72 h. Colonies were verified by Gram stain morphology.

Effect of pH on Survival

The survival of each of the four bacterial suspensions was studied by the addition of .1 ml of the suspension into a series of 2-ml volumes of sterile PBS at pH 1, 3, and 5 (adjusted using NaOH). The incubation mix was maintained at 37°C and the viable organisms enumerated at 0, .5, 1.0, 1.5, 2.0, 3.0, and 4.0 h.

In Vitro Survival in Gastric Juice

Gastric juice was obtained by aspiration through a nasogastric tube from patients after at least 4 h fasting. For each of the four strains, a .1 ml volume of the bacterial suspension was added to 1.0 ml of gastric juice. To study the effect of the presence of milk, parallel tubes were prepared; .1 ml PBS was added to one series and .1 ml skim milk was added to the other series. The pH was measured at 0 and 4 h. Viable bacterial cells were enumerated at 0, .5, 1.0, 1.5, 2.0, 3, and 4 h.

Stability of Aspirated Gastric Juice pH

The pH of gastric juice was measured 30 min after aspiration and again at 1, 2, 5, and 20 d after storage at −20°C. The pH of 1-ml aliquots of the gastric juice also was studied at 0 and 3 h at 37°C after the addition of the following compounds: skim milk (10%); NaOH to take

pH initially to 6; mylanta (3 and 6%) (Parke Davis Pty. Ltd.).

Survival in Gastric Juice In Vivo

Survival of the three *Lactobacillus* cultures, in the absence and presence of skim milk, was studied in vivo using healthy human volunteers aged 21 to 31 (except subject D, 42 yr) with conventional American-style eating habits. *Streptococcus thermophilus* was not tested due to poor survival in the in vitro studies. All subjects completed medical and dietary history forms. A standard light low fat breakfast was completed 4 h before commencement of the study. The breakfast consisted of 125 ml Special K cereal (Kelloggs), one slice of white bread with jelly, 250 ml skim milk, and black tea or coffee (with sugar if desired). The bacterial cultures used were the 1-ml stock vials maintained at −70°C and thawed just prior to use. A nasogastric tube was inserted and 10 ml of gastric juice was aspirated. The *Lactobacillus* suspension (1.0 ml) was introduced into the stomach via the tube. An additional 20 ml of sterile saline was added to ensure all of the culture reached the stomach (tube volume = 20 ml). At 0, 20, 40, 60, 90, 120, and 140 min after addition of the culture, a 25-ml aspirate was collected. After the 140-min aspirate was collected, subjects were allowed to rest with the tube remaining in place until 240 min. The procedure was then repeated with the addition of skim milk prior to the introduction of the bacterial culture. An additional 25 ml of aspirate was taken and then sterile skim milk (250 ml) was added via the tube. Another aspirate was immediately taken and then a further 1 ml of the appropriate bacterial culture was added. Aspirates of 25 ml were collected at 0, 20, 40, 60, 90, and 180 min after addition of the culture. The pH and number of viable cells were determined for all aspirates immediately after collection. A Radiometer pH meter was used and the number of viable *Lactobacillus* enumerated by serial 10-fold dilution of 1 ml samples into PBS; then aliquots were spread on MRS agar as for the in vitro studies. Three subjects received *L. acidophilus* N_2 and two subjects each received *L. acidophilus* ADH and *L. bulgaricus*.

Cell Wall Stability

Overnight 5-ml MRS broth cultures of *L. acidophilus* ADH and N_2 were centrifuged at 3000 × *g*/10 min/4°C, washed in 4 ml TE buffer [50 m*M* Tris base plus 50 m*M* ethylenediaminetetraacetate (EDTA) pH 8.5], and after centrifuging again, were resuspended in .2 ml of a sucrose solution (25% in TE buffer). The lysozyme solution (.05 ml of 10 mg/ml solution in .25 *M* Tris) was added and the mixture incubated at 37°C for 1 h. Tubes were observed for visible lysis during this incubation. Ethylenediaminetetraacetate (.08 ml of .25 *M* pH 8.0) was added (final concentration 1%) and the tubes were held at room temperature for 10 min. To lyse the cells further, .3 ml of Triton X-100 (.2% in 50 m*M* Tris-HCl and 50 m*M* Na EDTA at pH 8.5) was added and the tubes held at 65°C for 15 min. The tubes were observed for visible lysis.

Preparation of Bacterial Cells for Adhesion Study

Lactobacilli cultures were grown in MRS broths overnight at 37°C in an anaerobic chamber and cultures were then transferred to fresh MRS broth containing 25 μCi each of [^{3}H] alanine and [^{3}H] leucine (New England Nuclear Corp., Boston, MA) and then incubated in an anaerobic chamber overnight at 37°C. The *Streptococcus thermophilus* culture was grown in BHI broth overnight at 37°C and subcultured into fresh BHI broth containing the 25 μCi each of [^{3}H] alanine and [^{3}H] leucine and incubated at anaerobically 37°C overnight. All bacterial suspensions were centrifuged at 3000 × *g*/10 min/4°C, washed once in cold PBS, and resuspended in cold PBS to give an optical density of .35 using a Coleman model 44 spectrophotometer. Bacterial suspensions were used immediately in the binding study. The loss of the tritiated label from the bacterial cells into the fluid phase of the bacterial suspension was monitored at .5-h intervals for 3 h and found not to increase.

Binding Assay

The binding of all four bacterial suspensions to either human or pig ileal cells and pig cecum or colon cells was carried out according to the method of Deneke et al. (6). A 1-ml sample of

the radioactively labelled bacterial suspension, filtered through 5 μm filter, was incubated with 1 ml of the intestinal cell suspension or 1 ml of buffer in glass test tubes in a 37°C water bath for 2 min; then three samples of .5 ml from each were filtered through a polycarbonate filter (pore size, 2 μm; Nucleopore); each filter was washed with 5 ml PBS. Duplicate .5-ml samples of the bacterial suspension were similarly filtered and washed. The radioactivity (counts per min) on each filter was determined in Aquasol II scintillation fluid (New England Nuclear) using a Beckman LS-3133P liquid scintillation counter.

The number of bacteria bound per filter, and subsequently per intestinal cell, was estimated from the bacterial specific activity, based on the radioactivity of the bacterial suspension filters and the corresponding optical density measurement; the number of bacteria was estimated from a standard graph prepared for the optical density and the colony forming units per milliliter. The colony forming units per milliliter were determined by serial dilution and quantitation on agar plates as for viable counts.

Each fresh supply of intestinal cells was initially tested using a known positive and negative binding *E. coli* strain, B2C and B44, respectively (5). Only cell preparations producing binding patterns consistent with those of Deneke et al. (5, 6) with human and pig ileal cell preparations were used.

Preparation of Intestinal Suspension

All intestinal cell suspensions, collected as detailed, were maintained at 4°C in tissue culture medium NCTC 135 (Gibco Laboratories, Grand Island, NY) containing penicillin (100 μg/ml), streptomycin (100 μg/ml), and gentamycin (100 μg/ml) diluted to 25% with buffer 2 (5) and used in the binding assay as described by Deneke et al. (5, 6).

Ileal Cell Suspension

The human and pig ileal cell suspensions were collected as described by Deneke et al. (5, 6). A concentration of 10 ileal cells/ml was used in the binding assay.

Pig Cecum and Colon Cell Suspension

The cecum and colon were individually removed from the freshly sacrificed suckling pig (4 to 5 weeks old). Buffer 2 with the mannose omitted was used throughout. As gentle lavaging failed to release epithelial cells (observed by direct microscopy), the cecum and colon were opened longitudinally. The inner surfaces were washed gently with 2 × 20 ml cold buffer and the washing discarded; then inner surfaces were gently scraped with the end of a glass slide at an angle of 45° and epithelial cells collected in cold buffer. The dish containing the open cecum or colon was held on ice during the harvesting. A concentration of 10 cells/ml was used in the binding assay.

Effect of Mannose on Binding to Pig Ileal Cells

Pig and human ileal cells were routinely harvested and collected in buffer 2 (which contained 1% mannose). As mannose may influence adhesion, 1-m sections of pig small intestine were lavaged and the ileal cells harvested, maintained, and used in buffer 2 without any mannose. The binding assay was conducted for the four bacterial strains with the ileal cells either in buffer 2 without mannose or with 1% mannose included with the ileal cells for 5 min before the addition of the bacteria.

Effect of Milk on Binding to Human Ileal Cells

The lactobacilli cultures were transferred twice in MRS broth and the *Streptococcus thermophilus* were transferred twice in BHI broth before inoculation into the radioactively labelled broths. This additional subculture was carried out to reduce the presence of milk from the maintenance fluid in the bacterial suspension used in the binding assay. Skim milk (1%) was added to a second series of radioactively labelled broths prior to inoculation and the binding compared with milk free cultures.

RESULTS

Survival Studies

Effect of Physiological Saline pH. The viable count of all four bacterial strains decreased rapidly at pH 1.0 and more slowly at pH 3 and 5 (Table 1). The *L. acidophilus* ADH survived better than all the other strains, *L. acidophilus* N_2 better than *L. bulgaricus*, which in turn was better than *S. thermophilus*.

TABLE 1. Survival of bacterial strains in phosphate-buffered saline at various pH, as determined by viable counts.

Time	pH	Viable counts						
		0	.5	1.0	1.5	2.0	3.0	4.0
(h)		($\log_{10}$ cfu/ml)						
Lactobacillus acidophilus ADH	1	7.3	4.0	<1	<1	<1	<1	<1
	3	7.5	5.8	5.0	4.9	4.5	3.3	2.0
	5	7.8	7.7	5.0	4.1	4.3	4.5	4.5
Lactobacillus acidophilus N_2	1	7.0	3.7	<1	<1	<1	<1	<1
	3	7.6	5.8	5.5	5.9	6.2	4.4	3.8
	5	7.9	7.8	6.1	5.6	5.0	5.4	5.2
Lactobacillus bulgaricus	1	8.3	2.0	<2	<1	<1	<1	<1
	3	8.5	6.3	3.5	<2	<1	<1	<1
	5	9.8	9.8	8.1	8.2	7.7	7.6	7.5
Streptococcus thermophilus	1	4.9	<2	<1	<1	<1	<1	<1
	3	5.9	<3	3.0	<2	<1	<1	<1
	5	5.9	5.7	4.2	4.0	3.9	3.6	4.3

Stability of pH of Aspirated Gastric Juice. The pH of freshly aspirated gastric juice varied from 1 to 2.5 and remained unchanged when stored at −20°C for the 20 d studied. When gastric juice of pH 1 was treated with 1) skim milk (10%); 2) NaOH; 3) mylanta 3%; 4) mylanta 6%; pH of 3, 6, 2.5, and 4.5, respectively, were recorded. When these gastric juice samples were incubated at 37°C for 3 h, no shift in pH was recorded, which demonstrated the stability of the aspirated gastric juice samples.

Effect of Gastric Juice In Vitro. Survival patterns similar to those observed using physiological saline pH 1 and 3, were noted for the four bacterial strains when exposed to gastric juice in vitro (Table 2). A significant decrease in the viable cell count was noted between .5 and 1 h with *L. acidophilus* ADH surviving better than *L. acidophilus* N, which was better than *L. bulgaricus* and *S. thermophilus.* The presence of skim milk raised the pH and increased the survival of all strains.

TABLE 2. In vitro survival of bacteria in human gastric juice from donor A (pH 1) and donor B (pH 2.5) as determined by viable counts. The addition of skim milk (10%) to juice from donor A raised the pH to 3.

Time	pH	Skim milk	Viable counts					
			0	.5	1.0	2.0	3.0	4.0
(h)			($\log_{10}$ cfu/ml)					
Lactobacillus acidophilus ADH	1	−	7.0	5.9	<3	<3	<3	<3
	2.5	−	9.6	9.5	9.1	7.5	7.3	7.0
	3	+	7.8	7.8	7.1	6.3	5.0	2.6
Lactobacillus acidophilus N_2	1	−	6.2	<3	<3	<3	<2	<2
	2.5	−	9.5	8.8	6.8	<4	<4	<4
	3	+	6.6	5.9	6.1	2.9	3.3	2.0
Lactobacillus bulgaricus	1	−	9.1	5.8	3.5	<1	<1	<1
	2.5	−	9.9	8.3	<5	<5	<5	<5
	3	+	9.1	6.5	6.4	3.6	3.4	<2
Streptococcus thermophilus	1	−	5.3	5.6	<3	<1	<1	<1
	2.5	−	10.7	9.8	5.6	<5	<5	<5
	3	+	5.3	5.6	3.3	<1	<1	<1

Effect of Gastric Juice In Vivo. The in vivo data reflected a pattern similar to the observations in vitro. Survival time was directly related to pH (Table 3) and could be increased by the addition of milk (Table 4). The *L. acidophilus* ADH strain survived between 40 and 60 min in the stomach without the addition of milk and for 2 h when milk was included. *Lactobacillus acidophilus* N_2 survived for at least 40 min. Subject C, administered *L. acidophilus* N_2, maintained significant numbers of lactobacilli up to 240 min in the aspirated sample. However, these may represent indigenous lactobacilli continually passing from the mouth region, because the baseline value of lactobacilli before the addition of the culture was significant for this subject (from 2.3 to 3.3 $\log_{10}$ cfu/ml). A similar situation can be noted for Subject E. The decrease in viable lactobacilli in the stomach aspirate was consistent with recorded pH up to 90 min, after which time the subject was irritated by the tube and produced and swallowed excessive quantities of saliva. This is reflected in the increased pH and detectable lactobacilli, which may have originated from the mouth or oesophageal region, as this subject also had significant numbers of lactobacilli (3.2 $\log_{10}$ cfu/ml) before the addition of *L. acidophilus* N_2. Survivability of *L. acidophilus* N_2 was extended up to 1 h in Subject E by the addition of skim milk; however, this effect may have been reduced in Subjects C and D due to a sharp decrease in pH after 20 min. As both subjects admitted consciously longing for food after being shown the dinner menu, it is possible that this increased the acid production in the stomach, which could explain a sharp drop in pH. *Lactobacillus bulgaricus* survived very poorly in Subjects F and G. Addition of milk did extend *L. bulgaricus* survival to 40 min.

Cell Wall Stability. Lactobacillus acidophilus ADH could not be completely lysed by treat-

TABLE 3. In vivo survival of bacteria in human gastric juice as determined by viable counts in stomach aspirates.

Time (min)	*Lactobacillus acidophilus* ADH				*Lactobacillus acidophilus* N_2			
	Subject A		Subject B		Subject C		Subject D	
	pH	(cfu/ml)	pH	(cfu/ml)	pH	(cfu/ml)	pH	(cfu/ml)
BL[1]	2.8	0	1.3	0	2.9	2.3	3.6	1.0
0	4.5	6.9	2.0	5.4	1.9	6.6	2.3	7.1
20	3.2	6.2	1.5	<4	2.2	4.4	1.8	1.7
40	3.0	4.2	2.0	<3	2.4	4.0	1.7	<1
60	2.2	2.0	1.8	<2	1.8	3.5	1.6	<1
90	1.5	<1	2.0	<1	1.7	3.4	1.6	<1
120	1.5	<1	2.0	<1	1.5	2.7	1.6	<1
240	1.0	<1	1.0	<1	1.6	3.3	1.6	<1

Time (min)	*Lactobacillus acidophilus* N_2		*Lactobacillus bulgaricus*			
	Subject E		Subject F		Subject G	
	pH	(cfu/ml)	pH	(cfu/ml)	pH	(cfu/ml)
BL[1]	2.5	3.2	2.6	1.5	6.3[2]	2.1
0	3.0	6.9	2.3	2.0	3.2	8.8
20	2.2	5.3	2.4	<1	1.8	<1
40	1.7	3.2	2.2	<1	1.7	<1
60	1.7	<1	1.7	<1	1.5	<1
90	2.2	<1	1.7	<1	1.6	<1
120	3.7[3]	4.0[3]	1.5	<1	1.8	<1
240	7.0	3.2	1.6	<1	2.1	<1

[1] Baseline value before the addition of culture.

[2] Large volume of sterile water used to insert tube.

[3] Subject experienced extreme saliva production due to irritation by tube.

TABLE 4. In vivo survival of the three *Lactobacillus* sp. in human gastric juice. Viable counts expressed as $\log_{10}$ colony forming units per milliliter of stomach aspirate. Sterile skim milk (250 ml) added via nasogastric tube before addition of the culture.

Time (min)	*Lactobacillus acidophilus* ADH				*Lactobacillus acidophilus* N_2			
	Subject A		Subject B		Subject C		Subject D	
	pH	(cfu/ml)	pH	(cfu/ml)	pH	(cfu/ml)	pH	(cfu/ml)
BBM[1]	1.0	<1	1.0	<1	1.6	3.3	1.6	<1
BAM[2]	5.0	2.5	6.0	<1	6.2	3.6	6.3	7.0
0	5.0	8.7	6.0	8.7	6.2	7.7	6.3	5.9
20	4.0	7.9	5.5	8.8	2.4[3]	6.6	2.4[3]	<3
40	4.0	7.8	4.5	7.5	1.5	<3	1.5	<1
60	2.5	7.6	3.0	7.2	1.4	1.3	1.4	<1
90	2.0	7.1	2.0	5.5	1.4	3.2	1.6	<1
120	1.5	4.3	2.0	1.8	1.6	2.8	1.9	<1
180	1.0	1.0	2.0	<1	1.6	3.4	2.3	<1

Time (min)	*Lactobacillus acidophilus* N_2		*Lactobacillus bulgaricus*			
	Subject E		Subject F		Subject G	
	pH	(cfu/ml)	pH	(cfu/ml)	pH	(cfu/ml)
BBM	7.0[4]	4.0[4]	1.6	<1	2.1	<1
BAM	6.5	3.2	6.2	<1	6.0	<1
0	6.1	7.7	6.1	7.1	5.9	7.6
20	5.8	5.1	4.0	5.6	5.3	6.4
40	4.0	2.7	2.0	4.0	2.3	<1
60	2.2	1.6	2.0	<1	2.0	<1
90	2.0	<1	1.8	<1	1.7	<1
120	1.8	<1	1.6	<1	1.7	<1
180	1.9	<1	1.7	<1	1.7	<1

[1] BBM = Baseline value before addition of milk or culture.

[2] BAM = Baseline value after addition of milk and culture.

[3] Subjects showed menu for meal at completion of study.

[4] Subject experienced excessive saliva production which ceased with repositioning of the tube.

ment with lysozyme and then Trition X-100, but the *L. acidophilus* N_2 culture showed complete lysis after incubation with the lysozyme for 10 min at 37°C. The difficulty in lysing the *L. acidophilus* ADH suggests the presence of a much more resistant cell wall.

Adhesion Studies

Adhesion to Ileal Cells. Lactobacillus acidophilus ADH bound significantly better to human ileal cells than did *L. acidophilus* N_2 or *L. bulgaricus,* whereas *S. thermophilus* bound very poorly (Table 5). The data are expressed as numbers of bacteria bound per ileal cell, assuming 10^5 ileal cells were used. Although this is an approximation, the same ileal cell preparation was used within each experiment and all four bacterial strains were studied at the same time. A similar binding pattern was obtained for the four bacterial strains using pig ileal cells (Table 6). A direct comparison of human and pig ileal cells may be made using Experiment A (Table 5) and dilution 1 of Experiment B (Table 6). These experiments were carried out at the same time using the same bacterial cell suspensions. All bacterial strains bound in higher numbers to pig ileal cells but in the same pattern as for human cells. Components of buffer 2 may enhance adhesion of the bacterial strains as Experiment B and Experiment C (Table 6) were carried out simultaneously using the same bacterial suspensions. Binding was significantly less when the pig ileal cells were suspended in PBS (Experiment C) rather than buffer 2 (Experiment B). Although these

TABLE 5. Adhesion of four bacterial strains to human ileal cell suspensions.

Strain	Experiment[1] A	B	C	$\bar{X}$	SD
Lactobacillus acidophilus ADH[2]	195	120	205	173	46
Lactobacillus acidophilus N_2	51	69	55	58	9
Lactobacillus bulgaricus	31	66	101	66	35
Streptococcus thermophilus	14	3	10	9	5.5

[1] Each run in triplicate. Bacterial cells bound per ileal cell.

[2] *Lactobacillus acidophilus* ADH was significantly higher than *Lactobacillus acidophilus* N_2 ($P<.01$); *Lactobacillus bulgaricus* ($P<.05$); *Streptococcus thermophilus* ($P<.01$).

experiments were performed on the day of collection of the pig ileal cells, observed decreased binding to ileal cells in PBS also may reflect alterations of the ileal cells in the absence of stabilizing components in buffer 2.

Effect of Mannose and Milk on Adhesion. Although there is a consistent slight decrease, no significant reduction of binding of the four bacterial strains was observed when mannose was added to the ileal cell suspension before the addition of the bacterial cells (Table 7). The number of bacterial cells bound per ileal cell was higher when cultures used in the adhesion assay were grown in the presence of 1% skim milk (Table 8).

Adhesion to Cecum and Colon Cells. The *Lactobacillus* sp. all adhered well to pig cecum or colon epithelial cells whereas *S. thermophilus* adhered in very low numbers (Table 9).

DISCUSSION

A major consideration in the choice of lactic acid bacteria used as dietary adjuncts must be to chose a strain that cannot only survive stomach acidity but also establish within the digestive tract. The results presented here

TABLE 6. Adhesion of four bacterial strains to pig ileal cell suspensions in buffer 2 (Experiments A and B) or in phosphate-buffered saline (Experiment C). Bacterial suspensions were diluted twofold and adhesion tested for the same ileal cells within each experiment.

Strain	Dilution of bacteria	Bacterial cells bound per ileal cell in Experiment[1] A $\bar{X}$	A SD	B $\bar{X}$	B SD	C $\bar{X}$	C SD
Lactobacillus acidophilus ADH	1	170	53	375	67	191	170
	2	86	8	208	21	86	63
Lactobacillus acidophilus N_2	1	115	7	226	111	111	8
	2	20	4	68	21	8	3
Lactobacillus bulgaricus	1	56	47	114	20	39	9
	2	20[2]		35	6	20	7
Streptococcus thermophilus	1	10	9	37	17	14	4
	2	2	1	9	4	9	4

[1] Each run in triplicate.

[2] Standard deviation not estimated as value obtained by summation rather than mean of individual values.

TABLE 7. Effect of mannose on adhesion of bacterial cells to pig ileal cells maintained in buffer 2 without added mannose. The mannose (.5%) was added to ileal cells 5 min before addition of bacterial cells.

Strain	Experiment	Bacterial cells bound per ileal cells[1]			
		0% Mannose		.5% Mannose	
		$\bar{X}$	SD	$\bar{X}$	SD
Lactobacillus acidophilus ADH	1	92	34	80	21
	2	14	12	11	8
Lactobacillus acidophilus N_2	1	31	20	10	9
	2	NB[2]		NB	
Lactobacillus bulgaricus	1	131	40	118	67
	2	63	8	70	14
Streptococcus thermophilus	1	5	7	2	
	2	7	2	4	2

[1] Each run in triplicate.

[2] NB = No binding.

illustrate the range of survival of two *L. acidophilus* strains and two other common dairy culture strains *L. bulgaricus* and *S. thermophilus* when exposed to human gastric juice. These data highlight the need for such measurements to be made on potential dietary adjunct cultures. A comparison of Tables 1 and 2 illustrates that a key factor influencing survival of bacteria in gastric juice is the pH. The viable count of *L. acidophilus* ADH decreased in .5 h by: 1 × 1 $\log_{10}$ cfu/ml in gastric juice at pH 1 and by 3.3 $\log_{10}$ cfu/ml in PBS at pH 1; by 0 $\log_{10}$ in gastric plus skim milk at pH 3 but by 1.6 $\log_{10}$ in PBS at pH 3. The same slight reduction in survivability in PBS at the same pH as gastric juice is evident for all strains tested and suggests components in the gastric juice may confer some protective effect on the bacterial cell. Because survivability is slightly less when PBS is used rather than gastric juice, it seems reasonable to use PBS at the desired pH, as used by Mäyrä-Mäkinen et al. (17), to screen strains for their ability to maintain viability when exposed to gastric juice. Addition of 10% skim milk significantly raised pH and enhanced survival. This is consistent with the in vitro data of Pettersson et al. (21), which demonstrated better survival of *L. acidophilus* when the pH was raised from 3.8 to 6.8 using milk and that *L. acidophilus* survived better than other strains to produce fermented milk products. The better survival of *L. acidophilus* over *L. bulgari-*

TABLE 8. Influence of skim milk (1%) on binding of bacterial cells to human ileal cells. Bacterial cells used in the adhesion assay were grown in broth with or without skim milk.

Strain	Bacterial cells bound per ileal cell[1]			
	No Milk		Milk	
	$\bar{X}$	SD	$\bar{X}$	SD
Lactobacillus acidophilus ADH	120	38	422	89
Lactobacillus acidophilus N_2	54	25	55	59
Lactobacillus bulgaricus	67	10	201	77
Streptococcus thermophilus	5	1	20	15

[1] Each run in triplicate.

TABLE 9. Adhesion of bacteria to epithelial cells from the small intestine and the large intestine (cecum and colon) of the pig.

Strain	Experiment[1]	Bacterial cells bound per intestinal cell[2]					
		Ileum		Cecum		Colon	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Lactobacillus acidophilus ADH	1	92	34	1752	273	1264	223
	2	14	12	336	160	287	112
Lactobacillus acidophilus N_2	1	31	20	570		1192	361
	2	NB[3]		55	27	30[4]	
Lactobacillus bulgaricus	1	131	40	1800	300	400[4]	
	2	63	8	631	321	529	371
Streptococcus thermophilus	1	5	7	48[4]		111[4]	
	2	7	2	70	12	81	26

[1] Experiment 1 and 2 used same epithelial cells and twofold different concentration of bacteria.

[2] Each run in triplicate.

[3] NB = No binding recorded.

[4] Standard deviation not estimated as value obtained by summation rather than mean of individual values.

cus is consistent with the in vitro and in vivo studies of Lindwall and Fonden (15). The better survival of *L. acidophilus* ADH over *L. acidophilus* N_2 may be related to the more resistant cell wall of *L. acidophilus* ADH demonstrated here by exposure to lysozyme.

Comparison of data in Tables 2, 3, and 4 provides evidence of the justification for extrapolation from the in vitro to in vivo. Although there are large variations among subjects, these correlate directly with an alteration in pH, as is clearly illustrated in Table 4 for Subjects A and B. At zero time, for Subject A the pH was 4.5 and the viable count 6.9 $\log_{10}$ cfu/ml and for Subject B a pH of 2.0 and a viable count of 5.4 $\log_{10}$ cfu/ml was recorded. After 20 min, for Subject A a pH of 3.2 and a viable count 6.2 $\log_{10}$ cfu/ml are higher than for Subject B with a pH of 1.5 and <4 $\log_{10}$ cfu/ml. Such variation of pH among human subjects exposed to the same conditions could help explain inconsistencies of early lactobacilli studies using limited numbers of subjects (13, 23). High viable counts of Subjects C and E before the addition of a *Lactobacillus* culture suggests that in these subjects, a high concentration of lactobacilli colonize the oesophagus or mouth region and are continually seeding the stomach. It would be surprising to find commensal organisms in the stomach that grow at such low pH.

The addition of skim milk (250 ml) into the human stomach raised the pH by 4 to 5 units in all subjects and subsequently prolonged the survival time of all strains as seen in Table 4. As *L. acidophilus* ADH maintained a viable count of 4.3 $\log_{10}$ cfu/ml after 2 h residence time in the stomach in the presence of milk, and because stomach emptying times varies from 0 to 3 h, it seems plausible that viable lactobacillus could enter the small intestine. This is consistent with studies that demonstrated viable *L. acidophilus* in the human ileum up to 4.5 h after ingestion of acidophilus milk (22) and increased lactobacillus in feces (15).

As demonstrated by other studies (22, 23), bacterial strains that can remain viable after passage through the human stomach may only remain in the small intestine for a several hours. It seems feasible that if a strain can adhere to the intestinal wall, residence time could be extended to allow the bacterial cell sufficient time to multiply and, if possible, to colonize. Elevated *L. acidophilus* have been maintained for one human subject 1 wk after cessation of *L. acidophilus* ingestion (15). Numerous reports describe the specificity of adhesion of lactic acid bacteria to gastrointestinal cells of animals (4, 14, 17), but practical limitations have restricted data available for humans. Kleeman

and Klaenhammer (13), using a human fetal intestinal cell line, demonstrated specific and calcium-dependent nonspecific adhesion using 32 isolates.

One of the goals of the data presented here was to test a strain of Kleeman and Klaenhammer (13) that adhered specifically to the fetal intestine cell line plus the other cultures tested in this study for the capacity to adhere to freshly collected human intestinal cells. The intestinal cells were viable, as determined by the trypan blue exclusion test, and surrounded by an extracellular carbohydrate layer, as demonstrated by periodic acid-Shiff test (5). Adhesion to the human ileal cells has been demonstrated for all strains to various degrees (Table 4). The *L. acidophilus* ADH strain, which adhered well to the fetal cell line (13), also adhered well in this system. As a similar binding pattern for the four bacterial strains was achieved using the pig ileal cells (Table 6), it seems feasible that pig intestinal cells or the fetal cell line may be used to screen routinely adhesive properties of strains for human use. This overlapping of adhesive properties also has been demonstrated by Mäyrä-Mäkinen et al. (17), because *Lactobacillus fermentum* strains isolated from and adhered to the calf intestine could also adhere to pig epithelial cell. But the overlapping of adhesive properties conflicts with the results of Barrow et al. (4). The data in Tables 5 and 6 illustrate that the lactic acid bacteria used for the commercial production of cultured dairy products can adhere to human and pig ileal cells to various degrees. Although this is the converse of the finding of Mäyrä-Mäkinen et al. (17), Barrow et al. (4) used different commercial strains. As all strains showed some binding to both human and pig intestinal cells, the binding may be nonspecific as compared with that reported for *Lactobacillus* sp. in the murine, (19) chicken, and pig systems (4).

Because adhesion was unaffected by mannose (Table 7), inclusion of mannose in the ileal cell suspension according to Deneke et al. (5) to inhibit mannose sensitive type 1 fimbriae did not alter the adhesion patterns of the cultures. The promotion of adhesion by the inclusion of milk (Table 7) is consistent with the enhancement of binding in the presence of calcium (13) and could be valuable to increase adhesion in the intestine. The demonstration of adhesion to both pig small and large intestinal cells strengthens the suggestion that implantation may be possible and need not be restricted to the small intestine.

It has been shown that survival of lactic acid bacteria within the human stomach is closely related to pH, which can vary largely among individuals. Some lactic acid bacteria survive much longer in human gastric juice over the pH range 1 to 5, and it is encouraging that the strain showing best survival in gastric juice, *L. acidophilus* ADH, also adhered best to human and pig ileal cells. As the addition of milk both extended survival times of bacteria exposed to gastric juice and enhanced adhesion, the administration of *Lactobacillus* in milk or as cultured milk products should optimize *Lactobacillus* levels within the digestive tract. The in vitro system presented here for studying survival and adhesion would be valuable for screening potential lactic acid bacteria for dietary use. Once such conditions are defined it is possible to correlate information on strains with effects produced in vivo which can be monitored quantitatively. Studies continue to determine the effect of *L. acidophilus* ADH on fecal enzyme activity.

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