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A Mannose-Specific Adherence Mechanism in *Lactobacillus plantarum* Conferring Binding to the Human Colonic Cell Line HT-29

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Two *Lactobacillus plantarum* strains of human intestinal origin, strains 299 (= DSM 6595) and 299v (= DSM 9843), have proved to be efficient colonizers of the human intestine under experimental conditions. These strains and 17 other *L. plantarum* strains were tested for the ability to adhere to cells of the human colonic cell line HT-29. *L. plantarum* 299 and 299v and nine other *L. plantarum* strains, including all six strains that belong to the same genetic subgroup as *L. plantarum* 299 and 299v, adhered to HT-29 cells in a manner that could be inhibited by methyl-α-D-mannoside. The ability to adhere to HT-29 cells correlated with an ability to agglutinate cells of *Saccharomyces cerevisiae* and erythrocytes in a mannose-sensitive manner and with adherence to D-mannose-coated agarose beads. *L. plantarum* 299 and 299v adhered to freshly isolated human colonic and ileal enterocytes, but the binding was not significantly inhibited by methyl-α-D-mannoside. Periodate treatment of HT-29 cells abolished mannose-sensitive adherence, confirming that the cell-bound receptor was of carbohydrate nature. Proteinase K treatment of the bacteria also abolished adherence, indicating that the binding involved protein structures on the bacterial cell surface. Thus, a mannose-specific adhesin has been identified in *L. plantarum*; this adhesin could be involved in the ability to colonize the intestine.

Lactobacillus plantarum is a member of the facultatively heterofermentative group of lactobacilli that are frequently isolated from plant material and various fermented foods (45). It is also one of the major *Lactobacillus* species encountered in the human intestinal tract (15, 35, 37), with mean counts of 10^7 to 10^9 bacteria per g of feces in colonized individuals (15, 35). However, the relative importance of this organism as a human intestinal inhabitant seems to be different in different study populations. *L. plantarum* was found in only 5% of a Swedish control population consisting of people who sought medical advice for gastrointestinal problems but for whom no diagnosis could be made (37). On the other hand, completely healthy Swedish adults seem to have a higher colonization rate (3), and *L. plantarum* was found in more than one-third of healthy Swedish infants who were 3 to 8 weeks old (5). Perhaps the diet and lifestyle of modern western society disfavor this species, since almost two-thirds of American Seventh-Day Adventists, who consume no or little meat, harbor *L. plantarum*, compared with around one-fourth of Americans on a western diet (15).

Little is known about the mechanisms through which lactobacilli and other members of the indigenous intestinal microflora become established and persist in the intestine. In a previous study, it was shown that two closely related strains, *L. plantarum* 299 and 299v, which were originally isolated from human intestinal tracts, became established on the intestinal mucosa of healthy volunteers and remained there for 11 days after the last dose was administered (27). One factor promoting this colonization and persistence in the intestine could be the expression of bacterial adhesins conferring binding to intestinal epithelial cells or mucins. In this study, *L. plantarum* 299 and 299v, as well as 17 other *L. plantarum* strains, were tested for the ability to adhere to cells of the human colonic carcinoma cell line HT-29.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The *L. plantarum* strains included in this study are shown in Table 1. These strains have been classified previously on the basis of the results of a restriction endonuclease analysis (REA) of their total chromosomal DNAs into different subgroups (Table 1) (28). For adhesion and agglutination experiments, the bacteria were cultured aerobically on Rogosa SL agar (42) for 24 h at 37°C. In preliminary experiments culture on MRS agar or in MRS broth (11) was found to be inferior for adhesin expression.

Six *Escherichia coli* strains expressing type 1 fimbriae with mannose-specific adhesins were used for comparison. Five wild-type strains were isolated from the rectal flora of Pakistani infants and identified as *E. coli* by biotyping (2), as described by Bettelheim and Taylor (7). These strains were cultured overnight at 37°C in static Luria broth containing 0.1% $CaCl_2$ to promote adhesin expression (13). Transformant *E. coli* 506 MS, expressing type 1 fimbriae and mannose-specific adhesins (20), was cultured on tryptic soy agar containing 25 μg of chloramphenicol per ml.

HT-29 adherence assay. The assay to determine adherence to HT-29 cells was performed as described previously (49). Human adenocarcinoma cell line HT-29 cells were cultured in Eagle's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 50 μg of gentamicin (Sigma Chemical Co., St. Louis, Mo.) per ml. A few days after the cells had reached confluence, they were detached with buffer containing 0.54 mM EDTA, washed, and suspended in Hanks' balanced salt solution (HBSS) at a concentration of 5×10^6 cells per ml. The bacteria were harvested, washed, and suspended in HBSS at a concentration of 5×10^9 cells per ml (twice the concentration that gave an optical density at 597 nm of 1.5). The HT-29 cells, bacteria, and HBSS were mixed by using a ratio of 1:1:3 and then incubated with end-over-end rotation for 30 min at 4°C. The cells were washed once with ice-cold phosphate-buffered saline (PBS) and fixed with neutral buffered formalin (Histofix; Histolab, Göteborg, Sweden). The number of bacteria attached to each of at least 40 cells was determined by using interference-contrast microscopy (magnification, ×500; Nikon Optiphot microscope equipped with interference contrast equipment; Bergströms Instruments, Göteborg, Sweden), and the mean number of bacteria per cell was calculated.

To test inhibition of adherence, the following monosaccharides were included at a final concentration of 60 mM in the adherence assay: D-glucose (U.S. Biochemicals, Cleveland, Ohio), methyl-α-D-glucoside (Sigma), D-mannose (Merck, Darmstadt, Germany), methyl-α-D-mannoside (U.S. Biochemicals), L-fucose (U.S. Biochemicals), D-galactose (Merck), *N*-acetylglucosamine (U.S. Biochemicals), *N*-acetylgalactosamine (U.S. Biochemicals), and *N*-acetylneuraminic acid (Sigma).

Yeast agglutination. Washed bacteria were suspended at a concentration of 2×10^{10} cells per ml in PBS (pH 7.2) and titrated by preparing twofold dilutions

TABLE 1. Strains of *L. plantarum* used in this study, their subgroups as determined by REA, and their sources of isolation

Strain	Subgroup[a]	Source of isolation	Reference or source
97	1a	Nigerian ogi	29
101	1a	Nigerian ogi	29
53	1a	Nigerian ogi	29
ATCC 14917^T	1b	Pickled cabbage	ATCC[b]
256	1b	Silage[c]	28
36E	1b	Silage	28
98	1b	Nigerian ogi	29
299 (= DSM6595)	1c	Human colon	37
299v (= DSM9843)	1c	Human intestine	27
107	1c	Nigerian ogi	29
105	1c	Human colon	37
79	1c	Nigerian pito	29
125	1c[d]	Nigerian ogi	29
275	1c	Human colon	37
386	1c	Human rectum	37
So5	1	Starter culture[e]	28
ATCC 8014^T	1	Maize silage	ATCC
120	2	Nigerian ogi	29
44	2	Nigerian ogi	29

[a] Genetic subgroup (cluster) as determined by REA as described by Johansson et al. (28).
[b] ATCC, American Type Culture Collection.
[c] Obtained from Sven Lindgren, Department of Microbiology, University of Agricultural Sciences, Uppsala, Sweden.
[d] Subgroup determined by a randomly amplified polymorphic DNA analysis (26).
[e] Starter culture obtained from the Swedish Sugar Company, Arlöv, Sweden.

in PBS in microtiter plates (96-well, U-shaped microtest plates; Labora, Upplands Väsby, Sweden). An equal volume of PBS or of a selected monosaccharide at a concentration of 100 mM in PBS was added, and then twice this volume of 1% (wt/vol) baker's yeast (*Saccharomyces cerevisiae*) suspended in PBS was added. The plate was shaken for 5 min and then incubated overnight at 4°C. Agglutination was determined by bright-light microscopy at a magnification of ×250. The reciprocal of the highest dilution that resulted in visible agglutination was recorded as the agglutination titer.

Hemagglutination. Washed bacteria were suspended at a concentration of 2×10^{10} cells per ml in PBS, and twofold dilutions of the bacterial suspensions were mixed on microscope slides with equal volumes of 3% erythrocyte suspensions in PBS or PBS containing 100 mM methyl-α-D-mannoside. The slides were tilted gently at regular intervals, and agglutination was determined after 15 min by visual inspection with the naked eye and by using bright-light microscopy at a magnification of ×250. The reciprocal of the highest dilution of bacterial suspension that resulted in visible agglutination within 15 min was recorded as the agglutination titer.

Binding to D-mannose immobilized on agarose beads. Tests to determine the binding of bacteria to D-mannose-coated agarose beads were performed as described by Sanchez and Jonson (43), with slight modifications. Washed bacteria that were suspended at a concentration of 10^{10} cells per ml in PBS were mixed on microscope slides with equal volumes of a 1:4 suspension in PBS of agarose beads coated with covalently linked D-mannose (Agarose-*p*-aminophenyl-α-D-mannopyranoside; Sigma) or plain agarose beads (4% beaded agarose; P-L Biochemicals, Milwaukee, Wis.). The slides were tilted for 2 min and then observed with a Nikon Optiphot interference-contrast microscope at a magnification of ×500. Tests in which bacteria adhered to mannose-coated beads but not to plain agarose beads were considered positive, and positive reactions were classified as either weak or strong.

Metaperiodate oxidation and enzymatic treatment of bacteria and HT-29 cells. Washed bacteria or HT-29 cells were suspended in 0.01 M metaperiodate (Merck) in 0.1 M citrate-phosphate buffer (pH 4.5). The bacteria were incubated at 37°C for 1 h, whereas the HT-29 cells were incubated at room temperature for 15 or 30 min. After incubation, the bacteria or cells were pelleted by centrifugation, washed twice in PBS, and resuspended in HBSS. Control incubation mixtures contained 0.01 M sodium iodate (Mallinckrodt Chemical Works, St. Louis, Mo.) in the buffer described above or buffer alone.

Washed bacteria or HT-29 cells were suspended in PBS containing 2 mg of proteinase K (15 U/mg; Sigma) per ml or in PBS alone, incubated at 37°C for 1 h, and then washed and resuspended as described above.

Adherence to human enterocytes. Colonic or ileal specimens were obtained from patients undergoing intestinal resections because of carcinoma. In each case a piece of intestine distal from the dysplastic site was selected. The mucosa was separated from the underlying muscular layer and cut into pieces that were approximately 1 by 1 cm. Bacterial adherence was tested by using a one-step method in which detachment of epithelial cells and adherence of bacteria to the cells occurred simultaneously (49). Washed bacteria were suspended at a concentration of 2×10^9 cells per ml in Weiser B solution (containing 1.5 mM EDTA and 1.5 mM dithiothreitol) (47) or in Weiser B solution containing 100 mM methyl-α-D-mannoside. A piece of mucosa was added to the suspension, and the mixture was incubated with end-over-end rotation for 30 min at 4°C. The remaining piece of mucosa was discarded, and the detached epithelial cells were pelleted by centrifugation and washed twice in PBS. After fixation with neutral buffered formalin (Histofix), the cells were examined with a Nikon Optiphot interference-contrast microscope at a magnification of ×500. For each preparation, the bacteria adhering to each of 40 enterocytes were counted, and a mean adherence value was calculated.

Statistical method. Student's *t* test for paired values was used to test for significance.

RESULTS

Adherence to HT-29 cells. Nineteen *L. plantarum* strains belonging to different subgroups, as determined by REA (28), and five *E. coli* strains with mannose-specific adhesins were tested for their ability to adhere to human colonic cell line HT-29 cells (Table 2). *L. plantarum* 299 and 299v, which previously had been shown to colonize human volunteers, adhered to HT-29 cells at a level of approximately 10 bacteria per cell. Similar results were obtained for all but one of the other *L. plantarum* strains belonging to subgroup 1c. Methyl-α-D-mannoside reduced the level of adherence of these strains by 45 to 73% (Table 2). Lower levels of adherence (two to five bacteria per cell) were observed with subgroup 1b strains, and the levels of adherence of these strains were reduced by methyl-α-D-mannoside by 33 to 58%. Other *L. plantarum* strains which did not belong to subgroup 1b or 1c adhered in low numbers, and the adherence of these organisms was not inhibited by methyl-α-D-mannoside. The appearance of *Lactobacillus* adherence is shown in Fig. 1.

D-Mannose also reduced the adherence of *L. plantarum* 299 and 299v, but to a lesser degree than methyl-α-D-mannoside (46 and 35% compared with 62 and 68% for strains 299 and 299v, respectively). None of the other monosaccharides tested (D-glucose, methyl-α-D-glucoside, L-fucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and *N*-acetylneuraminic acid) inhibited the adherence of *L. plantarum* 299 or 299v to HT-29 cells.

The *E. coli* strains with mannose-specific adhesins that were tested adhered at levels of 20 to 45 bacteria per cell (Table 2). The adherence of *E. coli* 506 MS was inhibited to the same extent (94%) by both methyl-α-D-mannoside and D-mannose.

Agglutination of yeast cells. Mannose-containing polysaccharides (mannans) are a major constituent of the cell wall of baker's yeast, *S. cerevisiae* (44). *E. coli* and other enterobacteria with adhesins specific for mannose-containing receptors agglutinate yeast cells in a mannose-sensitive manner (36, 39).

All of the *L. plantarum* strains that exhibited mannose-sensitive adherence to HT-29 cells also agglutinated *S. cerevisiae*, although at titers lower than the titers obtained with the five *E. coli* strains tested (Table 2). Methyl-α-D-mannoside inhibited the yeast agglutination induced by *L. plantarum* by at least two titer steps and the agglutination induced by *E. coli* by at least four titer steps. Two of the other monosaccharides tested, D-mannose and *N*-acetylneuraminic acid, inhibited the agglutination induced by strains 299 and 299v. *N*-Acetylneuraminic acid also inhibited the agglutination of mannose-specific *E. coli*. The nature of this inhibitory action of *N*-acetylneuraminic

TABLE 2. Adherence to HT-29 cells and agglutination of yeast (*S. cerevisiae*) cells by *L. plantarum* strains and *E. coli* strains with mannose-sensitive adhesins

Strain	Subgroup	No. of adhering bacteria/cell (mean ± SD)[a]			Yeast agglutination titer[b]	
		In HBSS	In the presence of methylmannoside	*P*	In PBS	In the presence of methylmannoside
L. plantarum strains						
97	1a	1.2 ± 0.9	1.5 ± 0.9		<1	<1
101	1a	0.5 ± 0.4	1.1 ± 0.8		<1	<1
53	1a	0.8 ± 0.4	2.0 ± 0.9		<1	<1
ATCC 14917^T	1b	5.2 ± 0.8	2.2 ± 0.7	0.070	16	1
256	1b	3.7 ± 0.3	1.8 ± 0.5	0.020	16	4
36E	1b	2.4 ± 1.8	1.6 ± 1.5	0.17	8	<1
98	1b	0.6 ± 0.6	0.4 ± 0.2		<1	<1
299 (= DSM 6595)	1c	8.1 ± 1.1	3.8 ± 0.5	0.029	32	8
299v (= DSM 9843)	1c	11.7 ± 1.4	3.4 ± 0.5	0.017	64	8
107	1c	11.9 ± 1.7	3.7 ± 0.5	0.020	64	16
105	1c	13.3 ± 5.0	3.8 ± 0.4	0.093	64	8
79	1c	12.1 ± 3.5	3.3 ± 0.4	0.056	64	16
125	1c	8.7 ± 1.6	2.9 ± 1.7	0.0036	32	8
275	1c	2.9 ± 0.3	1.6 ± 0.2	0.038	8	<1
386	1c	11.6 ± 2.8	4.1 ± 0.9	0.021	32	8
So5	1	2.2 ± 0.4	4.6 ± 2.2		<1	<1
ATCC 8014^T	1	2.7 ± 1.0	4.5 ± 1.5		<1	<1
120	2	0.9 ± 0.6	0.8 ± 0.7		<1	<1
44	2	1.4 ± 1.2	1.6 ± 0.2		<1	<1
E. coli strains						
345		18.4	0.7		256	4
253		45.4	2.7		>512	32
810		26.2	0.5		128	8
476		16.3	1.7		64	1
506 MS		34.5 ± 8.9	2.6 ± 0.9	0.020	256	1

[a] HT-29 cells were incubated with the bacteria in the presence or absence of 60 mM methyl-α-D-mannoside and washed, and the mean number of adherent bacteria per cell was calculated. Each mean is the mean of values from three experiments; single experiments were performed with *E. coli* 345, 253, 810, and 476.

[b] The titers are the reciprocals of the highest dilutions of bacteria that gave agglutination of *S. cerevisiae* in the absence or presence of 25 mM methyl-α-D-mannoside. The values are the median titers obtained from two agglutination experiments (*L. plantarum* 36E, 98, and 44) or three agglutination experiments or the titers obtained in a single experiment (*E. coli* 345, 253, 810, and 476).

acid was obscure; inhibition was observed with both *L. plantarum* and *E. coli* over a pH range of 3 to 9.

Hemagglutination patterns. The membrane-bound glycoproteins of erythrocytes contain mannose, and *E. coli* strains with mannose-specific adhesins agglutinate a wide range of erythrocytes in a mannose-sensitive manner (13). The hemagglutination patterns of the 19 *L. plantarum* strains were compared with the hemagglutinations patterns of *E. coli* strains expressing mannose-specific adhesins.

L. plantarum strains belonging to subgroup 1c agglutinated human, guinea pig, chicken, cat, dog, mouse, rat, rabbit, horse, and pig erythrocytes and, less commonly, sheep and bovine erythrocytes. With the exception of sheep and ox erythrocyte agglutination, the agglutination reactions were completely inhibited or substantially reduced by methyl-α-D-mannoside, but as observed in the adherence and yeast agglutination experiments, methyl-α-D-mannoside was often a less potent inhibitor of the hemagglutination induced by *L. plantarum* than of the hemagglutination induced by *E. coli* (Table 3). Weak mannose-sensitive hemagglutination was observed with some subgroup 1b strains, whereas strains belonging to other subgroups were negative in this test.

The pattern of the mannose-sensitive hemagglutination exhibited by *L. plantarum* resembled the pattern exhibited by *E. coli* (for instance, in the weak interaction with sheep and ox erythrocytes). However, some differences were observed (Table 3). Thus, in tests with *L. plantarum*, chicken erythrocytes gave the highest mannose-sensitive hemagglutination titers, whereas horse erythrocytes were one of the least active erythrocyte species. In tests with *E. coli*, horse erythrocytes gave the highest titers, which were slightly higher than the titers obtained with chicken erythrocytes. Guinea pig erythrocytes exhibited comparatively strong hemagglutination with both *L. plantarum* and *E. coli*, whereas human erythrocytes exhibited low levels of activity, compared with other erythrocyte species, with *E. coli* but were comparatively active in agglutination tests performed with *L. plantarum.*

Binding to D-mannose immobilized on agarose beads. Binding to D-mannose-coated agarose beads was observed with all *L. plantarum* strains which adhered to HT-29 cells and agglutinated yeast cells and erythrocytes in a mannose-sensitive manner (i.e., all strains belonging to subgroup 1c and subgroup 1b strains ATCC 14917^T [T = type strain], 256, and 36E). Most positive strains reacted strongly with the mannose-coated agarose beads; weak reactions were observed with *L. plantarum* 275 (subgroup 1c) and with subgroup 1b strains ATCC 14917^T and 256. *E. coli* 506 MS bound at approximately the same level as the strongly positive *L. plantarum* strains. Most *L. plantarum* strains which were negative for mannose-sensitive adherence were also negative in the tests performed with the mannose-

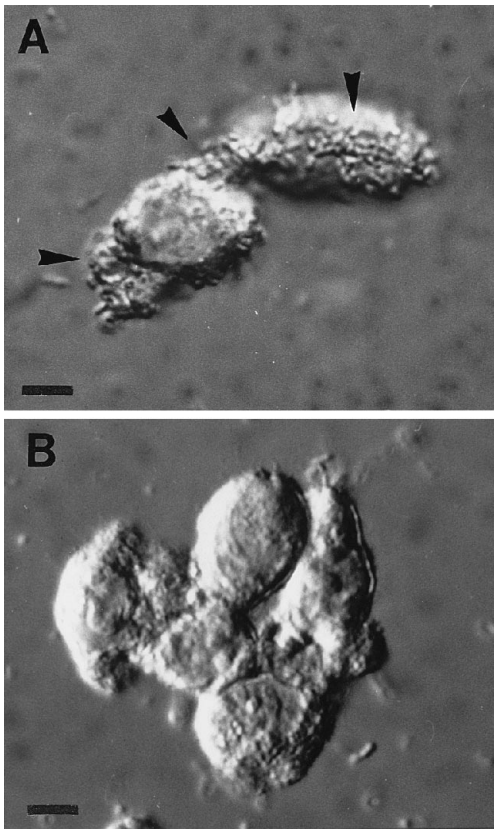

FIG. 1. (A) Adherence of *L. plantarum* 299 to human colonic carcinoma cell line HT-29 cells, as visualized by interference-contrast microscopy. Adherence occurs both directly to the cell surface and to surface-associated material. The arrowheads indicate adhering bacteria. (B) Absence of binding to HT-29 cells in the presence of 60 mM methyl-α-D-mannoside. Bar = 10 μm. Magnification, ×700.

coated beads; the only exception was subgroup 1a strain *L. plantarum* 97. However, this strain exhibited mannose-sensitive adherence in some experiments (data not shown).

Periodate oxidation and enzymatic treatment. Treatment of HT-29 cells with periodate for 15 or 30 min led to cell destruction; only 5 to 10% of the cells remained after this treatment and the subsequent adherence assay. Still, the level of mannose-sensitive adherence of *L. plantarum* to cells that had been treated for 15 min remained high ($P = 0.41$ compared with the buffer control), whereas cells that had been treated for 30 min did not bind *L. plantarum* in a mannose-sensitive manner ($P = 0.033$ compared with the buffer control; $P = 0.064$ compared with the iodate control) (Table 4). With *E. coli* 506 MS, periodate treatment of cells for 15 min reduced adherence by 74% ($P = 0.0004$ compared with the buffer control), and periodate treatment for 30 min almost abolished adherence ($P = 0.0005$ compared with the buffer control; $P = 0.0067$ compared with the iodate control). Periodate oxidation of bacterial cell surface carbohydrates did not affect binding much (Table 5).

Treatment of *L. plantarum* 299v with proteinase K completely abolished the ability of the bacteria to adhere to HT-29 cells ($P = 0.0008$) (Table 5), whereas no reduction in the adherence of *E. coli* 506 MS was observed after the bacteria were treated with this enzyme. Proteinase K treatment of HT-29 cells had no clear-cut effects on the adherence of *L. plantarum* 299v ($P = 0.36$), but tended to reduce the adherence of the type 1-fimbriated organism *E. coli* 506 MS ($P = 0.15$) (Table 4).

Adherence to human enterocytes. *L. plantarum* 299 and 299v adhered to human colonic and ileal epithelial cells obtained from surgical specimens (Table 6 and Fig. 2). The level of binding was approximately 10 bacteria per cell (i.e., 50% of the level seen with the type 1-fimbriated organism *E. coli* 506 MS). However, the presence of 100 mM methyl-α-D-mannoside did not significantly reduce the binding of *L. plantarum* 299 and 299v to colonic enterocytes ($P = 0.065$ and $P = 0.13$, respectively) or ileal enterocytes ($P = 0.49$ and $P = 0.37$, respectively). The levels of adherence of *E. coli* 506 MS to colonic and ileal enterocytes were significantly reduced by methyl-α-D-mannoside ($P = 0.0082$ and $P = 0.022$, respectively), although not as much as the levels of adherence to HT-29 cells.

L. plantarum 44 and 120, which adhered poorly to HT-29 cells and failed to agglutinate yeast cells or erythrocytes, adhered in low numbers to human enterocytes (Table 6).

DISCUSSION

In this study, we demonstrated that there is a mannose-specific adherence mechanism in certain strains of *L. plantarum*. This was manifested as mannose-sensitive adherence of the bacteria to human colonic cell line HT-29 cells, mannose-sensitive agglutination of *S. cerevisiae* and erythrocytes from different species, and direct binding of the bacteria to D-mannose immobilized on agarose beads. Metaperiodate treatment of the HT-29 cells abolished the mannose-sensitive part of bacterial adherence to HT-29 cells, which confirmed the carbohydrate nature of the cell-bound receptors.

The mannose-specific adherence mechanism was found in 12 of the 19 *L. plantarum* strains tested. One of these strains was negative in the initial experiments involving HT-29 adherence and agglutination but was positive for binding to mannose-coated agarose beads. This probably reflected variations between experiments in the expression by the bacteria of the structure(s) that mediates adherence, since this strain was also occasionally positive in adherence tests. The remaining 11 strains were positive in all tests. Eight of these strains belonged to a single *L. plantarum* subgroup, as determined by REA clustering analyses (28). All of the strains belonging to this subgroup exhibited mannose-specific adherence; this included *L. plantarum* 299 and 299v, which have previously been shown to be capable of colonizing the human intestine under experimental conditions (27), and three other strains originally isolated from human intestines (37). However, positive strains were also found in two other REA subgroups, and preliminary data indicate that members of other *Lactobacillus* species may also possess this mannose-specific adhering capacity (1).

Most likely, the mannose-specific adherence of *L. plantarum* is mediated by a proteinaceous bacterial structure(s), as treatment of the bacteria with proteinase K completely abolished the adherence capacity of the organisms. The expression of this putative adhesin was favored by aerobic culture on Rogosa agar, whereas the adhesin was weakly expressed after culture on MRS agar or in MRS broth or during culture under anaerobic conditions. Preliminary data from colonization of gnotobiotic rats indicate that adhesin expression is upregulated after in vivo colonization of rat intestines for 10 days (22), suggesting that the intestinal milieu could favor adhesin expression. Selected lactobacilli have previously been shown to adhere to human intestinal cell lines via protease-sensitive mechanisms (6, 9, 10, 19, 41), but this is one of the first reports of a defined carbohydrate specificity for the binding of *Lactobacillus* species. Previously, binding to lactosylceramide has been demon-

TABLE 3. Hemagglutination pattern of *L. plantarum* strains and *E. coli* strains expressing mannose-sensitive adhesins

Strain	Subgroup	Hemagglutination titer with the following type of erythrocytes[a]:											
		Human	Guinea pig	Chicken	Cat	Dog	Horse	Rat	Rabbit	Mouse	Pig	Sheep	Ox
L. plantarum strains													
97	1a	—	—	—	—	—	—	—	—	—	—	—	—
101	1a	—	—	—	—	—	—	—	—	—	—	—	—
53	1a	—	—	—	—	—	—	—	—	—	—	—	—
ATCC 14917^T	1b	—	1	2	ND	—	—	—	—	ND	—	—	—
256	1b	2[b]	2[b]	4[c]	1	—	—	—	—	—	—	—	—
36E	1b	—	—	1	—	—	—	—	—	—	—	—	—
98	1b	—	—	—	—	—	—	—	—	ND	—	—	—
299	1c	8	8	32	8	8	4	8	4	4	8	—	—
299v	1c	8	16	32	4[b]	4	4	4	4	2	4	(2)[d]	(2)
107	1c	16[c]	32[c]	64	8	8	8	16	16	4	8[c]	1	(4)
105	1c	16[c]	32[c]	64	16[c]	8	2	16[c]	8	8	8[c]	1	1
79	1c	16[c]	16[c]	32[c]	16[c]	16[c]	8[c]	8[c]	8[b]	4	8[b]	1	(2)
125	1c	4	4	8	4	4	2	4	4	2	2	—	—
275	1c	2[b]	2[b]	4	2	2	—	2	2[b]	ND	2[b]	(1)	(1)
386	1c	4[c]	16[c]	32[c]	4	4	2	4	4	4[c]	2	1	—
So5	1	—	—	—	—	—	—	—	—	ND	—	—	—
ATCC 1814^T	1	—	—	—	—	—	—	—	—	ND	—	—	—
120	2	—	—	—	—	—	—	—	—	—	—	—	—
44	2	—	—	—	—	—	—	—	—	ND	—	—	—
E. coli strains													
345		16[c]	256[c]	256[c]	128[c]	256[c]	256	256[c]	32[c]	16[c]	32[c]	—	—
253		128[c]	1,024[c]	1,024[c]	512	1,024	1,024	512	128	128	512	8	(8)
1121		16	256	512	256	512	1,024	512	128	64	64	—	—
810		4	64	64	32	64	128	16	16	8	16	(1)	(8)
476		—	32	64	32	32	16	32	32	4	8	—	—
506 MS		4	32	32	16	16	32	16	16	32	16	—	—

[a] The highest dilution of bacteria that gave microscopically detectable agglutination of erythrocytes was considered the hemagglutination titer. Unless indicated otherwise, the agglutination reactions were completely inhibited in the presence of 50 mM methyl-α-D-mannoside; ND, not determined. —, no hemagglutination was observed.
[b] Agglutination was inhibited one titer step by methyl-α-D-mannoside.
[c] Agglutination was inhibited at least two titer steps by methyl-α-D-mannoside.
[d] Parentheses indicate that hemagglutination was not affected by methyl-α-D-mannoside.

strated for *Lactobacillus fermentum* and *Lactobacillus acidophilus* (31).

The activity of the putative mannose-specific adhesin in *L. plantarum* was compared with the activity of the well-characterized mannose-specific adhesin of *E. coli* (13), which also binds to HT-29 cells (49) and agglutinates erythrocytes from various species (13) and baker's yeast (39). The erythrocyte membrane contains a mixture of mannose-containing oligosaccharide chains which differ in proportion in different species. Since the hemagglutination patterns of *E. coli* and *L. planta-*

TABLE 4. Effects of chemical and enzymatic treatments of HT-29 cells on the adherence of *L. plantarum* 299v and *E. coli* 506 MS

Treatment[a]	No. of adhering bacteria/cell			
	L. plantarum 299v		*E. coli* 506 MS	
	In HBSS	In the presence of methylmannoside	In HBSS	In the presence of methylmannoside
Periodate (15 min)	11.0	3.0	5.0	0.7
Periodate (30 min)	3.5	3.3	1.6	0.9
Iodate (30 min)	10.1	4.0	16.8	0.8
Buffer (15 min)	12.5	2.3	19.6	1.2
Buffer (30 min)	10.2	2.9	15.9	1.2
Proteinase K	9.2	2.2	14.0	0.5
PBS control	11.7	2.9	20.7	0.5

[a] For periodate oxidation, cells were incubated in 0.01 M periodate (citrate-phosphate buffer, pH 4.5) for 15 or 30 min at room temperature. Control incubation mixtures contained 0.01 M iodate or buffer alone. For proteinase K treatment, cells were incubated in PBS containing 2 mg of proteinase K per ml or in PBS alone at 37°C for 1 h. The cells were washed and used in the adherence assay as described in the text. Each value is the mean of values from three or four experiments.

TABLE 5. Effects of chemical and enzymatic treatments of *L. plantarum* 299v and *E. coli* 506 MS on adherence to HT-29 cells

Treatment[a]	No. of adhering bacteria/cell			
	L. plantarum 299v		*E. coli* 506 MS	
	In HBSS	In the presence of methylmannoside	In HBSS	In the presence of methylmannoside
Periodate	5.5	2.0	20.4	3.3
Buffer	7.0	2.0	23.7	0.9
Iodate	7.2	2.7	21.7	0.9
Proteinase K	0.4	0.5	22.4	3.0
PBS control	7.4	2.5	23.3	0.9

[a] For periodate oxidation, bacteria were incubated in 0.01 M periodate (citrate-phosphate buffer, pH 4.5) for 1 h at 37°C. Control incubation mixtures contained 0.01 M iodate or buffer alone. For proteinase K treatment, bacteria were incubated in PBS containing 2 mg of proteinase K per ml or in PBS alone at 37°C for 1 h. The bacteria were washed and used to test for adherence in the absence and presence of methyl-α-D-mannoside as described in the text. Each value is the mean of values from three to five experiments.

rum were not identical, there is probably a difference in preferred receptor conformation in the adhesins of these two organisms. Also, the carbohydrate receptors for *E. coli* were more sensitive to periodate treatment than the carbohydrate receptors for *L. plantarum*, which supports this hypothesis. The proposed optimal receptor structure for the mannose-specific adhesin of *E. coli* is the sequence Manα1-3Manβ1-4GlcNAc (16, 17), with the α1-3-linked mannosyl residue exposed in a terminal position (17, 38) as in short oligomannose chains. One possibility is that the putative adhesin of *L. plantarum* recognizes the Manα1-2Man sequence which occurs in abundance in the cell wall mannans of *S. cerevisiae* along with Manα1-3Man moieties (23, 44). The Manα1-2Man sequence also occurs in the least processed varieties of N-linked oligosaccharide chains and thus is present to various degrees on mammalian cells, such as erythrocytes and HT-29 cells (40). However, whether this structure is indeed part of the receptor remains to be determined. The ability of *L. plantarum* strains to associate with cells of *S. cerevisiae* is also interesting in view of the common simultaneous isolation of these species from all sorts of spontaneous lactic acid fermented products (25). Perhaps an association with yeasts has growth advantages for *L. plantarum.*

The existence of mannose-containing receptors for *L. plantarum* in the human intestine needs to be confirmed. *L. plantarum* 299 and 299v indeed bound to freshly isolated human intestinal epithelial cells, but inhibition by methyl-α-D-mannoside was not proven. However, *E. coli* strains expressing mannose-sensitive adhesins were only partially inhibited by methyl-α-D-mannoside in this system. Furthermore, two strains of *L. plantarum* that did not exhibit the mannose-sensitive adhesin adhered markedly less than *L. plantarum* 299 and 299v.

Mannose-specific adhesins are carried by a majority of *E. coli* strains that colonize the human intestine (30) and confer binding to human (13, 32, 49), rat (21), and mouse (46) intestinal epithelia. The expression of type 1 fimbriae is upregulated after colonization in vivo in the mouse intestine (33). Moreover, type 1-fimbriated *E. coli* cells adhere specifically to mannose receptors on the carbohydrate chains of secretory immunoglobulin A (48), a molecule that occurs in abundance on the intestinal mucosa, and the presence of secretory immunoglobulin A in the intestine favors the establishment of *E. coli* strains capable of expressing type 1 fimbriae (18). Mannose-specific adhesins have also been found in a variety of gram-negative bacteria, including members of the family *Enterobacteriaceae*, such as *Shigella*, *Enterobacter*, and *Klebsiella* species (14), in *Pseudomonas* strains (24), and in *Vibrio cholerae* (8). The mannose-sensitive adhesin of *Shigella* strains mediates binding to human intestinal epithelial cells (13) and the mannose-sensitive adhesin of *Salmonella* strains mediates binding to human (12), rat (34), and mouse intestinal epithelia (4). The mannose-sensitive hemagglutinin of *V. cholerae* seems to be involved in binding to rabbit small intestinal epithelium (8) and possibly also human small intestinal epithelium (50).

It seems likely that the mannose-sensitive adhesins of gram-negative intestinal bacteria are important for intestinal colonization by these bacteria. In this context, the presence of a

TABLE 6. Adherence to freshly isolated human enterocytes by *L. plantarum* and *E. coli*

Strain	No. of adhering bacteria/enterocyte (mean ± SD)[a]					
	Colonic enterocytes			Ileal enterocytes		
	No. of expts	Control	In the presence of methylmannoside	No. of expts	Control	In the presence of methylmannoside
L. plantarum 299	4	9.7 ± 4.3	7.8 ± 4.2	3	8.0 ± 3.9	7.6 ± 3.0
L. plantarum 299v	4	10.6 ± 4.5	7.8 ± 2.4	3	7.2 ± 1.9	6.1 ± 3.1
L. plantarum 44	3[b]	3.4 ± 2.0	4.3 ± 1.9	2[b]	2.4 ± 0.5	3.2
L. plantarum 120	1	2.7	3.2		ND[c]	ND
E. coli 506 MS	4	19.4 ± 6.5	7.6 ± 3.1	3	16.1 ± 5.4	5.5 ± 2.8

[a] Pieces of dissected intestinal mucosa were incubated with bacteria in Weiser B solution in the absence and presence of 100 mM methyl-α-D-mannoside. Detached epithelial cells were washed, and 40 enterocytes were examined by interference contrast microscopy for the presence of adhering bacteria.
[b] One of the experiments did not include testing in the presence of methyl-α-D-mannoside.
[c] ND, not determined.

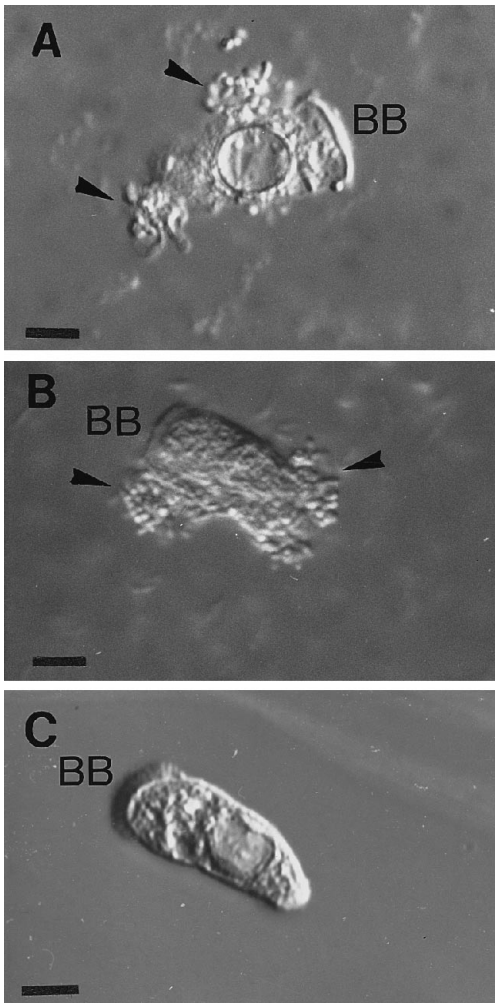

FIG. 2. Micrographs showing the adherence of *L. plantarum* 299 to a colonic enterocyte (A) and an ileal enterocyte (B) and an ileal enterocyte without adhering bacteria (C). Bars = 10 μm. Magnification, ×700. BB, brush borders. The arrowheads indicate adhering bacteria.

mannose-specific adhesin in a gram-positive bacterial species which belongs to the indigenous intestinal microflora is of great interest. Whether this adhesin contributes to the pronounced colonizing ability of *L. plantarum* 299 and 299v (27) remains to be determined.

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