

The Effect of Lactose Derivatives on Intestinal Lactic Acid Bacteria

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ABSTRACT

Nine strains of lactic acid bacteria were studied for growth and fermentation end products on lactulose, lactitol, and lactobionic acid. In addition, human fecal and biopsy isolates were screened for new potential probiotic strains utilizing lactose derivatives, and one new isolate of *Lactobacillus rhamnosus* was enriched. The utilization of lactose derivatives and the effect on the fermentation end products were dependent on strain. Typical mixed-acid fermentations were observed with *Lb. rhamnosus* and *Lactococcus lactis*. Microbiota enriched from fecal and biopsy samples using modified MRS medium consisted mainly of enterococci and streptococci. The adhesion of tested strains to Caco-2 cells was not dependent on carbon source. The new *Lb. rhamnosus* strain VTT E-97800 has potential for further probiotic studies.

(**Key words:** Lactose derivatives, lactic acid bacteria, ribotyping, fermentation)

INTRODUCTION

The effects of prebiotic carbohydrates are of great interest to both consumers and food manufacturers (14). A prebiotic substance selectively enhances the growth of beneficial bacteria (probiotics) in the intestine. One source of prebiotic carbohydrates is lactose derivatives from processing whey (lactose), a main by-product of cheese processing that is traditionally used as an animal feed supplement. The lactose derivatives lactulose and lactitol are disaccharides that are not absorbed in the human small intestine and thus are substrates for colonic microbes. Lactulose and lactitol are expected to enhance the growth and metabolism of beneficial bacteria such as lactobacilli and bifidobacteria, resulting in conditions that are unfavorable to potentially harmful intestinal coliforms, clostridia, and enterococci (12, 16). Therefore, lactose derivatives have been suggested for prebiotic use (5).

Probiotics have been defined as a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (3). It has been recommended that lactic acid bacteria that potentially are probiotic be isolated from the intestine of the host. In addition, because the adhesion of intestinal bacteria to colonic cells has been shown to enhance the survival of probiotics in competition among gastrointestinal microbiota, adhesion has been regarded as an additional definition of probiotic bacteria (17).

Lactulose and lactitol have found application in the treatment of portal systemic encephalopathy, hyperammonemia, and chronic constipation (6). Moreover, lactulose is claimed to decrease the incidence of colorectal cancer, to induce a therapeutic effect in *Salmonella* spp. carriers, to have an anti-endotoxin effect, and to contribute to a decreased glycemic index in diabetics (12). Lactulose has been studied in connection with the prevention of DNA damage in colonic mucosal cells (16), and results indicate that lactulose consumption offers a degree of protection from the genotoxic effects of a known colonic carcinogen (16). However, lactulose has been found to be unpalatable and may cause nausea, vomiting, abdominal cramps, and diarrhea. These side effects are not associated with lactitol consumption (6), but the use of lactitol as a bifidogenic factor remains to be determined (15).

Generally, it is important to understand the physiological mechanisms of the metabolism of bacterial carbohydrates in order to improve the organic acid production of starters (18). This situation also applies to the probiotic field because, when probiotics are used as starters, they naturally affect the aroma of the product. Lactic acid bacteria produce acids as their main metabolic end products. Homofermentative bacteria produce mainly lactic acid, and heterofermentative bacteria produce lactic acid, CO₂, and acetic acid or ethanol. Depending on the carbohydrate and on the growth conditions (pH, nutrient density, and the number of bacterial cells in the inoculum), homofermentative lactic acid bacteria may change their metabolic pathway to produce end products of the fermentation of mixed acids (9, 19), such as

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formate, acetate, ethanol, and CO₂. These metabolic end products might have an influence on sensory properties of the products, and they may also have physiological effects, which, however, are presently largely unknown.

The objectives of this study were to define the effects of lactose derivatives on selected lactic acid bacteria and to isolate new potential strains for probiotic use from human fecal and biopsy samples. Special emphasis was placed on characterizing the effects of lactose derivatives on probiotic metabolism (homofermentative vs. mixed acid fermentation) and on adhesion to the human colon carcinoma Caco-2 cell line.

MATERIALS AND METHODS

Strains

Lactic acid bacteria were obtained from VTT Culture Collection (Espoo, Finland) and are listed in

Table 1. *Lactobacillus acidophilus* strains were used as controls for growth on lactulose and lactitol (9). For adhesion tests, *Lactobacillus rhamnosus* (*Lactobacillus* GG) was used as a positive control (10), and a nonaggregating mutant of *Lactobacillus crispatus* was used as a negative control (13).

Isolation

Biopsy and fecal isolates were enriched from samples obtained from volunteers among patients who were hospitalized for gastrointestinal disorders. The diets of the patients were not controlled. The biopsy samples (3 mm × 3 mm) were taken from the healthy part of the descending colon during the routine of diagnostic colonoscopies and transported in 10 ml of Na-thioglycollate (Difco, Detroit, MI) solution at 4°C. Biopsy samples were homogenized in 38 ml of peptone and saline using a stomacher (Stomacher 400; Seward, GWB, Finland) for 10 min. Fecal samples (1

TABLE 1. Identification and growth¹ of known lactic acid bacterial strains and colonic isolates on lactose derivatives.

Strains or isolate	Isolated or received from	Identification based on API and ribotyping	Ribo groups	Growth on lactose derivatives		Adhesion to Caco-2 cell line	
				Lactulose	Lactitol	—— (%) ——	
						\bar{X}	SE
E-96276 ^{T2}	Human isolate	<i>Lactobacillus acidophilus</i>	36-S-6	ND ³	ND	ND	
E-97976	Kneifel 145	<i>Lb. acidophilus</i>	67-S-8	+++	+++	19.5	3.5
E-97977	Kneifel A-1	<i>Lb. acidophilus</i> , <i>Lactobacillus johnsonii</i>	136-S-7	+++	+++	8.4	1.1
E-97978	Kneifel 0	<i>Lb. acidophilus</i> , unknown	192-S-1	+++	++	16.7	2.2
E-97979	Kneifel K	<i>Lb. acidophilus</i> , unknown	192-S-1	+++	++	7.8	0.8
E-96729 ⁴	Baby feces, Morelli Mu5	<i>Lactobacillus crispatus</i>	36-S-4	ND	ND	5.8	0.8
E-97851 ^T	Human blood	<i>Lb. johnsonii</i>	136-S-7	ND	ND	ND	
E-79098 ^T	Pickled cabbage	<i>Lactobacillus plantarum</i>	78-S-1	–	–	ND	
E-92142 ^T	Human intestine	<i>Lactobacillus reuteri</i>	78-S-2	–	–	ND	
E-94522 ⁵	Human feces	<i>Lactobacillus rhamnosus</i>	19-S-1	–	–	11.9	5.2
E-90414	Barley malt	<i>Lactococcus lactis</i> ssp. <i>lactis</i>	34-S-3	+	–	ND	
E-97800	Adult feces ⁶	<i>Lb. rhamnosus</i>	65-S-6	++++	++++	11.5	1.6
E-97801	Adult feces ⁶	<i>Enterococcus faecalis</i>	24-S-2	+++	+	ND	
E-97802	Adult feces ⁶	<i>Ent. faecalis</i>	24-S-3	++++	++	ND	
E-97803	Adult feces ⁶	<i>Enterococcus hirae</i>	24-S-4	+++	–	ND	
E-97843	Adult colon biopsy ⁶	<i>Streptococcus agalactiae</i>	78-S-5	++	–	ND	
E-97844	Adult colon biopsy ⁶	<i>Streptococcus gallolyticus</i>	78-S-6	+++	+	ND	
		<i>Streptococcus dysgalactiae</i> ssp. <i>equisimilis</i>	78-S-7	++	+	ND	
E-97845	Adult colon biopsy ⁶	<i>Ent. faecalis</i>	74-S-1	+++	+	ND	

¹The growth was determined as growth areas in the turbidimetric measurement: – = >50; + = 50–100; ++ = 101–150; +++ = 151–300; ++++ = >300 or counting the amount of cells in Thoma chamber using microscope (log₁₀ colony-forming units per milliliter): + = 4–5; ++ = 5–6; +++ = 6–7; ++++ = >7.

²Type strain.

³Not detected.

⁴Negative control in adhesion test.

⁵Strain *Lactobacillus* GG (positive control in adhesion test).

⁶Present study.

g) were homogenized in 99 ml of peptone and saline. The homogenates were added (10%, vol/vol, of final broth) to basic MRS broth supplemented with (2% of lactose derivatives, wt/vol of final broth) and incubated at 37°C for 24 h in anaerobic jars. The enriched cultures were plated on MRS agar (Difco 0881) and were incubated aerobically at 37°C for 72 h. The aerobic incubation was used in order to isolate more aerotolerant species for possible industrial use. Colonies were selected according to visually different morphology for further purification using MRS agar.

Media

The composition of the basic MRS broth was, per liter: 10 g of peptone from casein, 5.0 g of yeast nitrogen base without amino acids (Difco), 5.0 g of Na acetate, 2.0 g of $K_2HPO_4 \cdot 3H_2O$, 2.0 g of $(NH_4)_3C_6H_5O_7 \cdot 2 H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $MnSO_4 \cdot 4H_2O$ and 1.0 ml of Tween 80 (Fluka, Buchs, Switzerland). The basic MRS broth was supplemented with 2% (wt/vol) of the lactose derivative to be tested: lactulose [(4-O- β -D-galactopyranosyl)-D-fructose (ICN Biochemicals Inc., Aurora, OH)], lactitol [(4-O- β -D-galactopyranosyl)-D-glucitol (Xyrofin, Kotka, Finland)], or lactobionic acid [(4-O- β -D-galactopyranosyl)-D-gluconic acid (Aldrich Chemicals, Steinheim, Germany)]. Glucose (Difco), galactose (Difco), and lactose (BDH, Poole, England) were used as controls. The pH of the broth was adjusted to 5.0, 5.5, or 6.2 with HCl. The basic medium was sterilized by autoclaving at 121°C for 20 min, and the carbohydrates supplemented were sterilized using 0.22- μ m filters (Millipore S. A., Saint-Quentin, France).

Selection and Preliminary Identification

Isolates were handled in aerobic conditions and were tested for catalase activity (20), Gram (2) and cell morphology, growth at 45°C, growth with 6.5% NaCl, and growth on *Enterococcus* agar (Difco). Carbohydrate fermentation tests were carried out using the relevant API strips according to the instructions of the manufacturer (bioMérieux SA, Marcy-l'Étoile, France). Incubations were carried out at 37°C in anaerobic conditions for up to 6 d. Identifications were performed by comparing the fermentation profiles with the available databases (version 1.7.6 of ATB Plus; bioMérieux). The identifications of *Streptococcus* spp. isolates were confirmed using 16S rDNA sequence determination and traditional physiological characterization (DSMZ Identification Service; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany).

Characterization by Ribotyping

The ribotyping of the strains was carried out using the RiboPrinter™ Microbial Characterization System (Qualicon, Wilmington, DE) according to the instructions of the manufacturer. The automated system includes five stages: 1) DNA preparation and restriction by *EcoRI*, 2) separation by gel electrophoresis directly linked to a membrane transfer, 3) hybridization with a rRNA universal probe (*Escherichia coli* region encoding the rRNA 16S-23S genes) for detection, 4) extraction and visualization of the pattern (RiboPrint), and 5) characterization (RiboGroups) and, where possible, identification by computerized comparison with existing RiboPrint databases. Each strain and isolate were analyzed three times.

Growth on Lactose Derivatives

The growth in modified MRS broth on lactose derivatives was monitored through microscopic cell counting using a Thoma chamber (Assistent, Ruling, Germany) after incubation for 24 h at 37°C in an anaerobic atmosphere or a turbidometer Bioscreen C system (Labsystems, Helsinki, Finland). The turbidometer automatically plotted the bacterial growth curve and calculated the corresponding growth area. The growth curve was registered as optical density multiplied by time. The results were calculated as percentages of the growth area. Bacterial suspension (30 μ l) was added into the cuvettes of honeycomb plates (Labsystems) containing 240 μ l of the basic MRS solution plus carbohydrates (pH 6.2). The growth of the strain was compared with growth on galactose and glucose. Results are mean values of two replicates ($n = 2$ to 4 in Thoma counting); ($n = 7$ to 10 in Bio Screen Detection).

Fermentation End Products

Lactic acid, acetic acid, and formic acid were analyzed by an enzymatic method (catalogue numbers 1112821, 148261, and 979732; Boehringer-Mannheim, Indianapolis, IN) according to the instructions of the manufacturer, using an automatic sample analyzer (Cobas Mira Plus™; Roche Diagnostic Systems, Basel, Switzerland).

Ethanol, acetaldehyde, diacetyl, and CO₂ were analyzed with a Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard, Waltbronn, Germany) equipped with a Tekmar 7000 headspace autosampler (Tekmar Co., Cincinnati, OH). The compounds were detected with a thermal conductivity detector at 240°C and CO₂ at 200°C. The column was a 25-m Poraplot Q (Chrompack, i.d. 0.32 mm, layer

thickness 0.53 μm , and 0.10 μm for CO_2). The injector temperature was 150°C. The samples were run using a program in which the temperature was increased from 50 to 150°C in 3.5 min; the CO_2 samples were run isothermally at 40°C/5 min. Helium was used as carrier gas with a velocity of 31 cm/s. The split ratio was 1:30, and the injection volume was 1 ml. The quantitation were carried out using external standards. The results are mean values of two replicates ($n = 4$ to 6).

Adhesion Properties

The adhesion properties of the tested strains were studied on lactulose and glucose. The human colonic cancer Caco-2 cell line ATCC HTB 37 (American Type Culture Collection, Cockeysville, MD) was used to indicate the ability of bacteria to colonize the human gastrointestinal tract. Caco-2 cells were cultured in RPMI-HEPES medium (RPMI; Gibco BRL, Paisley, United Kingdom) supplemented with 20% fetal calf serum (PAA Laboratories GmbH, Linz, Austria), 2 mM L-glutamine (Gibco BRL), 1% nonessential amino acids (Gibco BRL), and 100 IU/ml penicillin and streptomycin solution (Gibco BRL) at 37°C in an atmosphere of 5% CO_2 and 95% air. Caco-2 cells were seeded at a concentration of 3.2×10^4 cells/ml to obtain confluence, and the cell cultures were maintained for 14 d on a Chamber Slide™ (Nunc, Naperville, IL) monolayer. The culture medium was replaced every other day. Before adhesion, the test cells were gently washed with 300 μl of PPS/L: per 13.8 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 17.9 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, and 9 g of NaCl. The 7 mM phosphate buffer (pH 7.1) was repaired and 0.14 M NaCl solution added; 300 μl of different dilutions of bacterial cell suspension (cell concentrations varying between 3.6×10^5 to 1×10^8 cfu/ml were used) in RPMI-HEPES medium without supplements. Bacterial cells were labeled using 5 μl /ml of [methyl-1,2- ^3H]thymidine (113 Ci/mmol; Amersham, Buckinghamshire, United Kingdom). After incubation for 1 h at 37°C, the Chamber Slide™ (area of one cuvette, 0.36 cm^2) was gently washed with $6 \times 300 \mu\text{l}$ of PBS and fixed with methanol for 10 min. The bacterial adhesion was visually evaluated from the nonlabeled monolayer that had been Gram stained (crystal violet and washed with Lugol solution and with ethanol) by microscopic examination (Polyvar; Reichert-Jung, Columbus, OH), and the radioactivity was measured by liquid scintillation (Wallac 1410, Liquid Scintillation Counter; Wallac, Espoo, Finland).

RESULTS AND DISCUSSION

Screening and Identification of Isolates

Of about 300 fecal or biopsy isolates, only half were Gram-positive and catalase-negative; these isolates, which were mainly cocci, were selected for further studies. Presumably, strictly anaerobic isolates died during purification and handling and, thus, did not fulfill all of the criteria for this screening purpose. From 10 isolates growing on lactose derivatives, 8 were selected for final identification on the basis of growth. The isolates obtained from fecal samples seemed to be mainly *Enterococcus* spp. and those from biopsy samples *Streptococcus* spp. (Table 1). One isolate of *Lb. rhamnosus* was present among the fecal isolates. Normal microbiota of the intestine have 10% of lactic acid bacteria (21). The colonoscopy patients used as a source of fecal and biopsy material, of course, represent a less than ideal group of subjects if the intent is to clarify the microbiology of normal gut on to isolate potential probiotic strains. However, the patients in this study did not suffer from inflammatory bowel diseases, and the biopsies were obtained from seemingly healthy mucus. Thus, the samples can be regarded as an approximation of a healthy gut.

Characterization of the Strains and Isolates by Ribotyping

The fingerprints (RiboPrints) of all of the different *Lactobacillus* spp. and the one *Lactococcus* sp. were clearly different from one another. All four probiotic *Lb. acidophilus* strains E-97976-E-97979 yielded good or excellent identification of *Lb. acidophilus* by API 50 CHL. However, the differences in their fingerprints were broad. Only one strain, E-97976, could be identified as *Lb. acidophilus* via RiboPrint databases. The similarity to the type strain E-96276 was rather low, only 88%. The strain E-97977 matched the same RiboGroup as did type strain of *Lactobacillus johnsonii* E-97851 and had a good homology (96%). The last two strains matched to the same RiboGroup and had a homology of 94%. However, the homology of this group to *Lb. johnsonii* was only 58%, and no homology to *Lb. acidophilus* was found in the current RiboPrint databases. These two strains could not be identified using the databases.

From the fecal isolates, the system was able to identify a strain, E-97800, as *Lb. rhamnosus*; this strain had 92% homology to the probiotic strain E-94522. All of the *Enterococcus faecalis* isolates (E-97801, E-97802, and E-97846) matched different RiboGroups, and the similarity between them ranged from 66 to 91%. All of the *Streptococcus* spp. isolates

belonged to different species and thus produced quite different fingerprints.

Growth on Lactose Derivatives

Bacterial growth of colonic bacteria (10^7 to 10^8 cfu/ml; anaerobic or aerobic incubation) was detected on lactulose and lactitol when the pH of the broth was 6.2 (Table 1; Figure 1). No growth was observed on lactobionic acid. An experiment using 30 isolates showed growth features to be dependent on the particular isolate. Eight selected isolates, as well as *Lactococcus lactis* and *Lb. acidophilus* strains (controls), grew well on lactulose and lactitol. Lactulose was utilized better than lactitol according to the growth results (Figure 1). The new isolate, *Lb. rhamnosus* E-97800, grew very well on lactulose (1.3×10^7 cfu/ml), but growth was weaker (2.3×10^6 to 5×10^6 cfu/ml) with the *Lb. acidophilus* strains.

Several colonic microbes have been shown to utilize lactulose and lactitol. *Bifidobacterium* spp., *Bacteroides* spp., *Clostridium* spp., and *Ent. faecalis* have been shown to utilize lactose derivatives, but somewhat contradictory results have been demonstrated with *E. coli* (7). The utilization of lactulose has previously been demonstrated for example, by, *Lb. acidophilus*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus salivarius*, *Lc. lactis*, and *Streptococcus thermophilus* (14). This finding is in accordance with our results whereby *Lb. acidophilus* strains, *Lc. lactis*, and *Lb. rhamnosus*, enterococci, and streptococci have been shown to be able to grow on lactulose and lactitol. However, *Lactobacillus* GG VTT E-94522, *Lactobacillus plantarum* VTT E-79098, and *Lactobacillus reuteri* VTT E-92142 were not able to utilize lactose derivatives in our experiment, and, thus, these strains could be candidates for new synbiotic foods [containing prebiotic and probiotic components (4)], leaving lactose derivatives intact and ensuring their intestinal effects. Thus, other LAB or bifidobacteria (1) may utilize lactose derivatives in the gut, and probiotic action of the strains could be valorized as well.

Effect of Lactose Derivatives on Fermentation End Products

All of the strains produced fermentation end products that were typical of mixed-acid fermentation (acetic acid, formic acid, CO_2 , and ethanol) on lactitol; only *Lb. rhamnosus* and *Lc. lactis* markedly changed their fermentation patterns on lactulose (Figure 2). The *Lb. rhamnosus* produced more CO_2 , and *Lc. lactis* produced less CO_2 on lactitol and lactulose than on glucose. *Lactobacillus rhamnosus* also produced ethanol on lactitol. The amounts of metabo-

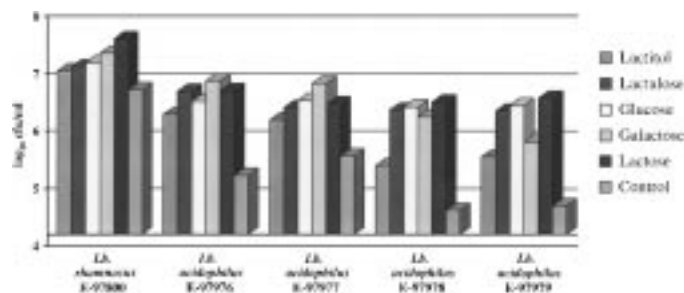


Figure 1. Growth of *Lactobacillus rhamnosus* E-97800, *Lactococcus lactis* ssp. *lactis* E-90414, *Lactobacillus acidophilus* E-97976, *Lb. acidophilus* E-97977, *Lb. acidophilus* E-97978, and *Lb. acidophilus* E-97979 on lactitol, lactulose, glucose, galactose, lactose, and broth without carbohydrate ($1 \log_{10}$ cfu/ml = 1×10^1 cfu/ml).

lites produced by all of the *Lb. acidophilus* strains were lower than those produced by the other tested strains. On glucose, the main metabolite was lactic acid (>90%) for all of the tested strains. While growing on lactose derivatives, the amount of lactic acid depended on the strain, being 10 to 85% lower on lactitol and up to 80% lower on lactulose than on glucose (Figure 2).

The end products of mixed-acid fermentation may indicate the physical condition of the bacterial cells. For example, starvation has been shown to change the metabolism from homofermentative to typical mixed-acid production (8). In this study, lactose derivatives changed the metabolic end products of lactic acid bacteria, depending on the strain, in conditions that did not involve a shortage of substrates. The comparison of growth intensities also indicates a better ability to utilize lactose derivatives by *Lb. rhamnosus* (growth intensity of 97 to 99% on lactose derivatives compared with that on glucose) than by *Lb. acidophilus* strains (growth intensity of 84 to 96%). This result suggests that strains of lactic acid bacteria are individually able to change fermentation patterns, depending on the available substrates and the metabolic pathways of the strain. Only a few studies have been done on the metabolism of lactulose or lactitol. Smart et al. (19) demonstrated that bacterial strains having either β -galactosidase or phospho- β -galactosidase activity could utilize lactulose, which indicates that lactulose metabolism is not limited to either of the known lactose utilization pathways of lactic acid bacteria (14). However, more studies should be conducted to elucidate the pathways of lactose derivative metabolism in lactobacilli.

Adhesion to the Caco-2 Cell Line

In general, all of the tested strains adhered to Caco-2 cells, regardless of the carbohydrate in the

bacterial growth medium. The adhesion profiles of the strains (grown on glucose) are presented in Table 1 and Figure 3. The most prominent adhesion was ob-

served with *Lb. acidophilus* E-97976 (20%), *Lb. acidophilus* E-97978 (18%), and the new isolate *Lb. rhamnosus* E-97800 (12%), which adhered to a simi-

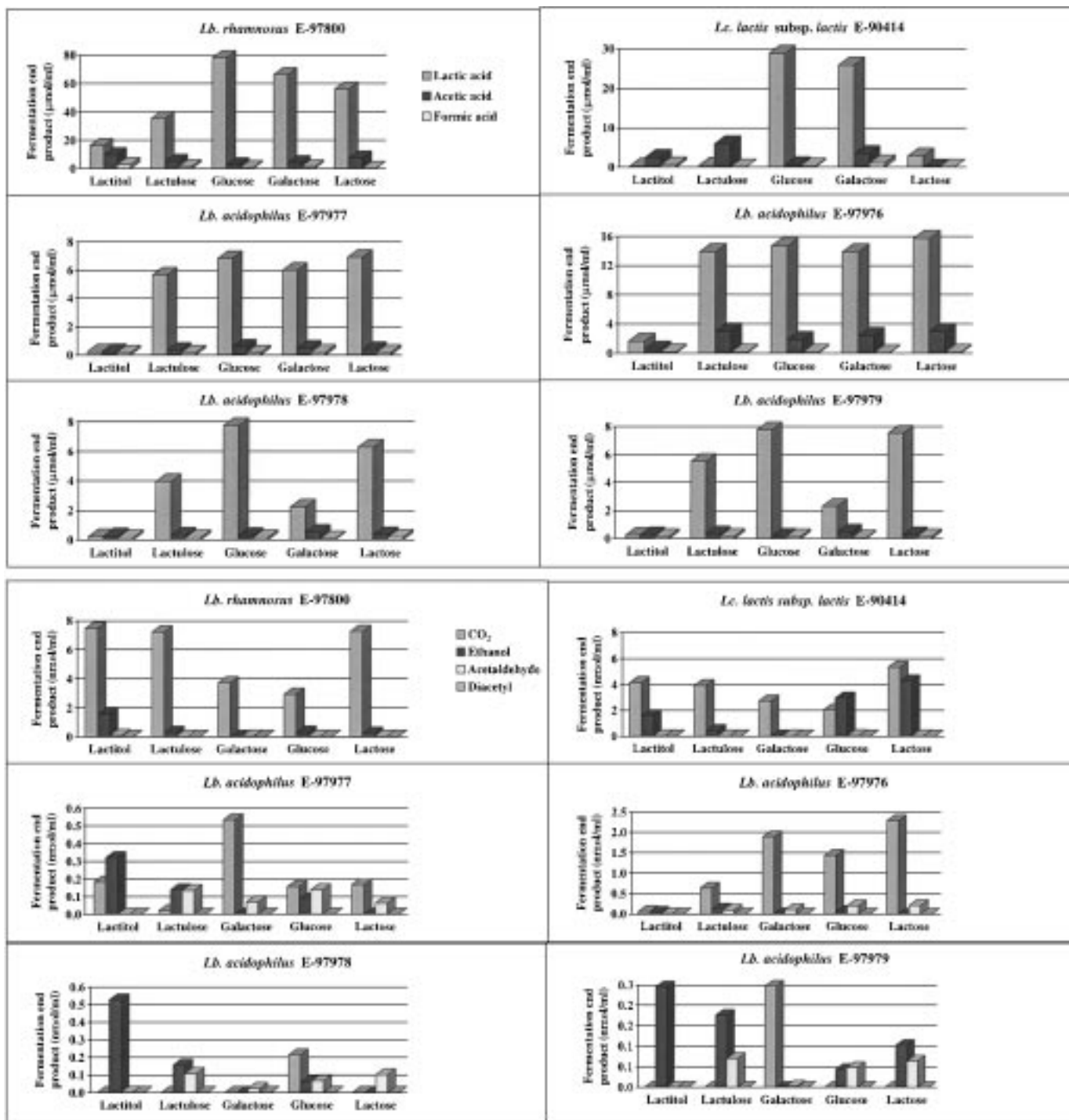


Figure 2. Fermentation end products (lactic acid, acetic acid, formic acid, CO_2 , ethanol, acetaldehyde, and diacetyl) by *Lactobacillus rhamnosus* E-97800, *Lactobacillus acidophilus* E-97976, *Lb. acidophilus* E-97977, *Lb. acidophilus* E-97978, and *Lb. acidophilus* E-97979 after 24 h of anaerobic incubation.

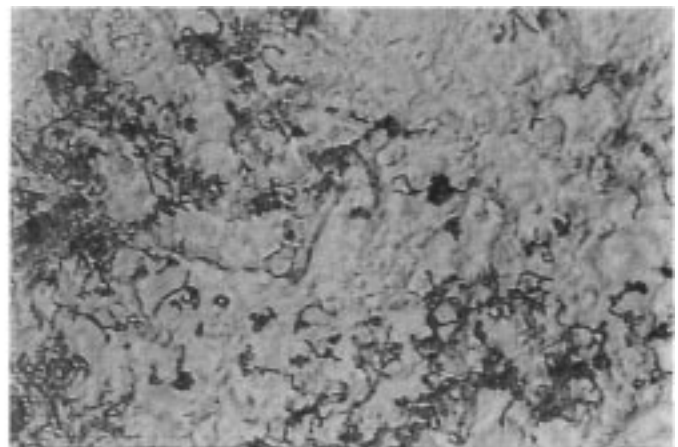
lar degree as the positive control (*Lactobacillus* GG, Table 1, and Figure 3). The adhesion of *Lb. acidophilus* varied between strains. The strains E-97976 and E-97978 seemed to adhere better than E-97977 and E-97979. The negative control *Lb. crispatus* showed only moderate adhesion (6%).

The isolated *Lb. rhamnosus* E-97800 as well as the *Lb. acidophilus* strains tested showed potential adherence properties in vitro and thus could be candidates for further probiotic studies. The adhesion depended on the amount of cells. Each strain had optimum cell density for adhesion. If cells were added beyond the optimal concentration, the cell culture

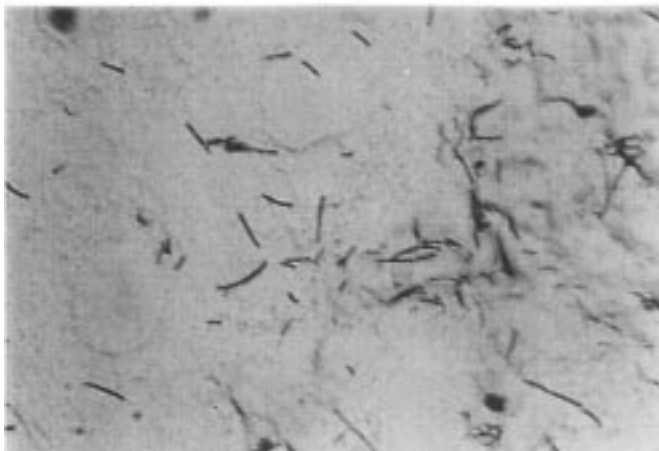
became saturated and thus gave a lower percentage of adhesion. This result may partially explain the contradictory data for in vitro adhesion that were obtained from different laboratories. Experimental conditions should be recorded more carefully in published reports in order to make the experiments more reproducible. In addition, it would be interesting to evaluate the adhesion properties of isolated enterococci and streptococci. However, it must also be remembered that some species of those bacterial groups may be human pathogens, such as *Streptococcus dysgalactiae* ssp. *equisimilis*, which was isolated in this study.



Lactobacillus rhamnosus VTT E-97800



Lactobacillus rhamnosus VTT E-94522
(*Lactobacillus* GG, positive control)



Lactobacillus acidophilus
VTT E-97979



Lactobacillus crispatus VTT E-97729
(negative control)

Figure 3. The adhesion of *Lactobacillus rhamnosus* VTT E-97800, *Lb. rhamnosus* VTT E-94522 (positive control), *Lactobacillus acidophilus* VTT E-97979, and *Lactobacillus crispatus* VTT E-97729 (negative control) to Caco-2 cells using microscopic evaluation.

The enrichment method used in this study is suitable as candidates for probiotic use that are isolated from fecal or biopsy samples. Because lactose derivatives are not selective substrates for lactic acid bacteria, it would be advisable to use a medium that is more selective than MRS in further colonic isolation experiments. The characterization of colonic isolates by ribotyping is easy to demonstrate, but the system requires improved databases to ensure identification.

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