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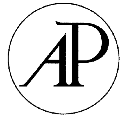

ORIGINAL ARTICLE

The isolation of lactic acid bacteria from human colonic biopsies after enrichment on lactose derivatives and rye arabinoxylo-oligosaccharides

P. Kontula*, M.-L. Suihko, T. Suortti, M. Tenkanen, T. Mattila-Sandholm and A. von Wright

Lactic acid bacteria (LAB) were isolated from human colon biopsies on LAMVAB by enrichment with different substrates such as lactose derivatives, rye arabinoxylo-oligosaccharides and rye fractions. The selected isolates were tested for their ability to adhere to Caco-2 cells. Only Lactobacillus *species were enriched under these conditions. From 161 isolates screened, 28% were identified by ribotyping as* Lactobacillus rhamnosus*, 29% as* L. salivarius*, 14% as* L. cellobiosus*, 13% as* L. paracasei *and the rest remained unidentified.* L. rhamnosus *was preferentially enriched by lactulose,* L. salivarius *by lactobionic acid,* L. cellobiosus *by lactitol and* L. paracasei *by arabinoxylo-oligosaccharides. The biopsy-derived strains* L. rhamnosus *E-97948 and* L. paracasei *E-97949 have potential for further evaluations in their probiotic and technological properties. Lactulose may have prebiotic effects on colonic LAB by favouring their growth.*
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Introduction

Lactic acid bacteria (LAB) have been used as starters in fermented plant or animal based foods for centuries. Their fermentation end-products enhance the shelf-life of the product, mainly by decreasing the pH and by production of lactic acid and thus preventing the growth of spoilage bacteria. An additional, and in industrial field nowadays more important, reason for wide use of LAB is their ability to develop a pleasant aroma in the product. Although their health improving effects were already suggested by Metchnikoff (reviewed by Ballongue 1998) in the beginning of the century, scientific interest in the possible probiotic properties of LAB was stimulated as late as in the 1970s (Kalantzopoulos 1997). Nowadays, the probiotic health effects are investigated on a worldwide basis (Salminen et al. 1996). Specifically, interest is focused on the isolation and development of new probiotic strains using improved methodologies (Salminen et al. 1996). In addition, it is suggested that probiotic LAB should be isolated from the host's intestine, because their colonization may be improved by host-specific adherence properties (Tannock 1990). Colonization has been shown to be important for the survival of probiotic strains in competition with other intestinal microbes (Saxelin 1991).

Gastrointestinal (GI) microbiota consist of approximately 400 identified anaerobic or

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aerobic species, of which LAB constitute approximately 10% (Tannock 1990). However, in the small intestine LAB may form the main population. The diversity and quantity of GI microbiota have made it difficult to evaluate the dynamic GI microbial population using traditional methods. Recently, DNA based methods such as automated ribotyping have been developed to detect and study the ecology of intestinal LAB (McCartney and Tannock 1995, McCartney et al. 1996, Kimura et al. 1997).

It is already known that diet may affect the intestinal microecology (Tannock 1990). Thus, many commercial prebiotics (fructo-oligosaccharides, xylo-oligosaccharides) have recently been introduced to specifically improve the bifidobacterial flora in the intestine (Crittenden 1996). However, prebiotics that may improve the intestinal LAB flora have been less studied. The properties of prebiotic substrates and probiotics (synbiotics) may be combined in the functional foods (Gibson and Roberfroid 1995) which mainly consists of dairy and cereal based products in the present food markets.

The aim of this study was to isolate lactic acid bacteria from human colonic biopsies by enrichment with lactose derivatives or rye fractions as possible prebiotic substrates. Special emphasis was focused on the ability of the isolates to grow on the proposed prebiotic carbohydrates and on their adhesion properties in vitro using the Caco-2 cell line. The results of this study show that potential probiotic strains can be selected on the basis of their specific affinity to certain substrates. This information can be used in the design of new functional foods as substrates in new combinations.

Materials and Methods

Lactose derivatives and preparation of arabinoxylo-oligosaccharides and rye fractions

The lactose derivatives used were lactulose [(4-O-β-D-galacto-pyranosyl)-D-fructose] (Sigma Chemical Co., St. Louis, Missouri, USA), lactitol [(4-O-β-D-galactopyranosyl)-D-glucitol] (Xyrofin, Kotka, Finland) and lactobionic acid [(4-O-β-D-galactopyranosyl-D-gluconic acid] (ICN, Eshwege, Germany). Lactitol was obtained from Valio Ltd., Helsinki, Finland.

The arabinoxylo-oligosaccharides were prepared from rye arabinoxylan (Megazyme, Bray, Ireland). Solutions of xylan (2% w/v) dissolved in 5.0 mM ammonium acetate pH 5·0 were prepared, after which 5000 nkat g^{-1} purified xylanase (Tenkanen et al. 1992) from *Trichoderma reesei* was added to the solutions. The hydrolysis was carried out for 24 h at 40°C in a water bath after which the enzyme was inhibited by heating for 20 min at 80°C. The solution was freeze-dried for 72 h (Epsilon 2-25 DS) to obtain a mixture of dry oligosaccharides. The composition of reducing sugars in the preparation was analysed by the HPLC (Dionex, Sunnyvale, California, USA) method of Tenkanen et al. (1997). The linear xylo-oligosaccharides (Megazyme) and arabinose substituted xylo-oligosaccharides (Tenkanen et al. 1996) were used as standards.

The rye fraction (pentosans) was prepared from 1 kg rye flour, which was refluxed by 90% ethanol to inactivate endogenous enzymes and to extract lipids. The solution was dispersed in water with termamyl to hydrolyze starch and the residual precipitate, isolated by centrifugation, was extracted with $Ca(OH)_2$+KOH. The solution was neutralized to pH 5·0 and the solubilized polysaccharides were precipitated with 50% ethanol to obtain the alkali-soluble pentosan fraction. The residue was an insoluble pentosan fraction. The soluble fraction was used for isolation of LAB.

Media

The composition of basic MRS-broth was, per litre: 10 g peptone from casein (Difco, Detroit, Michigan, USA), 5·0 g yeast nitrogen base w/o amino acids (Difco), 5·0 g Na-acetate, 2·0 g $K_2HPO_4 \times 3H_2O$, 2·0 g $(NH_4)_3C_6H_5O_7 \times 2H_2O$, 0·2 g $MgSO_4 \times 7H_2O$, 0·05 g $MnSO4 \times 4H_2O$ and 1·0 ml Tween 80 (Fluka, Buchs, Switzerland). The basic MRS-broth was supplemented with 2% (w/v) of the carbohydrate to be tested. The pH of the broth was adjusted to 5·0, 5·5 or 6·2 with HCl. The basic medium was sterilized in an autoclave at 121°C for 20 min and supplemented carbohydrates were sterilized using 0·22 µm filters (Millipore SA, Saint-Quentin, France) and rye fractions by radiation.

Isolation of LAB and testing the growth

Biopsy-derived bacteria were isolated from the samples obtained from voluntary patients hospitalized for gastrointestinal disorders, which made it possible to get biopsies during the routine colonoscopy. The patients had their normal diet, which was not monitored. The biopsy samples (3×3 mm) were taken from the healthy part of the descending colon and they were transported in 12 ml Na-thioglycollate (Difco) medium at 4°C to VTT, Finland. Samples were homogenized in 38 ml thioglycollate using Stomacher 400 (Seward, GWB, Finland), at normal speed for 30 s. The homogenates were added (10% v/v of final broth) to basic-MRS broth supplemented with 2% lactose derivatives, arabinoxylo-oligosaccharides or rye fraction, and incubated at 37°C for 24 h in anaerobic jars. The enriched cultures were plated on LAMVAB agar (Hartemink et al. 1997) and incubated aerobically at 37°C for 72 h. Aerobic incubation was used in order to isolate more aerotolerant species for possible industrial use. Colonies were selected on the basis of visually different morphology for further purification using MRS agar. Identified isolates were tested for their growth (Jaskari et al. 1998) in basic-MRS broth supplemented with carbohydrates using automatic turbidometer Bioscreen C system (Labsystems, Helsinki, Finland) and by plating on MRS agar after 48 h incubation. Results are presented as mean values of two tests.

Selection and identification

All isolates were handled in ambient atmosphere and were tested for Gram reaction (EBC Analytica Microbiologica, Nürnberg, Germany), catalase activity (Smibert and Krieg 1981) and cell morphology (light microscopy). Carbohydrate fermentation tests of selected isolates were carried out using the relevant API strips according to the manufacturer's instructions (bioMérieux SA, Marcy-l'Etoile, France). Incubations were carried out at 37°C in anaerobic conditions for up to 6 days. Identifications were performed by comparing the fermentation profiles with the databases contained in version 1.7.6 of ATB plus (bioMérieux SA).

Ribotyping of the isolates was carried out using the RiboPrinter® Microbial Characterization System (Qualicon™, Wilmington, Detroit, USA) according to the manufacturer's instructions. The automated system includes five stages: (1) DNA preparation and restriction by *EcoR1*, (2) separation by gel electrophoresis directly linked to a membrane transfer, (3) hybridization with an 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 the existing RiboPrint databases. During the screening step, each isolate was analysed only once. The isolates deposited in the VTT Culture Collection (Espoo, Finland) were ribotyped three times.

Adhesion properties

The adhesion properties of the tested strains were studied after growth on glucose. The human colonic tumour cell line Caco-2 ATCC HTB 37 (American Type Culture Collection, Manassas, Virginia, USA) was used to indicate the bacterial colonization ability of the human gastrointestinal tract. Caco-2 cells were cultured in RPMI-HEPES medium (RPMI, Gibco BRL, Paisley, UK) supplemented with 20% fetal calf serum (YA Kemia, Helsinki, Finland), 2 mM L-glutamine (Sigma), 1% non-essential amino acids and 100 IU ml^{-1} penicillin-streptomycin solution (Gibco BRL) at 37°C in a 5% CO_2/95% air atmosphere of 5% CO_2 and 95% air. Caco-2 cells were seeded at a concentration of $3{\cdot}2 \times 10^4$ cells ml^{-1} to obtain confluence. The cell cultures were maintained for 14 days on a Chamber Slide™ (Nunc, Naperville, Illinois, USA) monolayer. The culture medium was replaced every second day. Before the adhesion test the cells were gently washed with 300 μl PBS (PBS, per liter: 13·8 g of $NaH_2PO_4 \times H_2O$, 17·9 g of $Na_2HPO_4 \times 2H_2O$, 9 g of NaCl. Prepare 7 mM phosphate-buffer (pH 7·1) and add 140 mM NaCl solution) and they were overlapped with 300 μl of different dilutions of bacterial cell suspension (cell concentrations varying between 5×10^5 and 1×10^8 cfu ml^{-1} were used in RPMI-HEPES

medium without supplements. Bacterial cells were labelled using 5 μl ml^{-1} [methyl-1,2-^{3}H]-thymidine (113 Ci/mmol, Amersham, Buckinghamshire, UK). After incubation for 1 h at 37°C the Chamber Slide™ (area of one cuvette: 0·36 cm^2) was gently washed with 6 × 300 μl of PBS and fixed with methanol for 10 min. The radioactivity was measured by liquid scintillation (Wallac 1410, Liquid Scintillation Counter; Wallac, Espoo, Finland). Results are mean values of two tests.

Results

Composition of arabinoxylo-oligosaccharides and rye fractions

Arabinoxylo-oligosaccharides consisted of (w/v, %) arabinose (0·1), xylose (1·5), disaccharide (1·6), trisaccharides (0·2), tetrasaccharides (0·2), pentasaccharides (0·1), arabinose-xylose-trisaccharides (*c.* 30·7), arabinose-xylose-tetrasaccharides (*c.* 53·9) and arabinose-xylose-trisaccharides (*c.* 11·7) (Fig. 1). Rye fractions consisted of 60% pentosans and 40% possible protein residues.

Identification and characterization of the isolates

The numbers of identified isolates obtained using different enrichment substrates are listed in Table 1. It can be seen that the majority of strains isolated using lactulose were *L. rhamnosus*. On lactitol, the typical isolates were *L. cellobiosus*, on lactobionic acid *L. salivarius* and on arabinoxylo-oligosaccharides *L. cellobiosus* and *L. paracasei*. Rye fractions were favoured by *L. rhamnosus*, *L. salivarius*, and to a lesser extent by *L. paracasei*, while only one isolate of *L. cellobiosus* belonged to this group.

After the preliminary screening, 161 isolates were characterized and identified (lowest similarity 85%), by API tests or by ribotyping using the commercial (DUP) and VTT databases. From the isolates, 45 were identified as *Lactobacillus rhamnosus* (28·0%), 47 as *L. salivarius* or closely related to it (29·2%), 23 as *L. cellobiosus* (14·3%), 21 as *L. paracasei* (13·0%) (Table 1) and 25 remained unidentified (15·5%). However, most of the unidentified isolates were presumably members of *L. salivarius*, because their closest similarities were to the identified

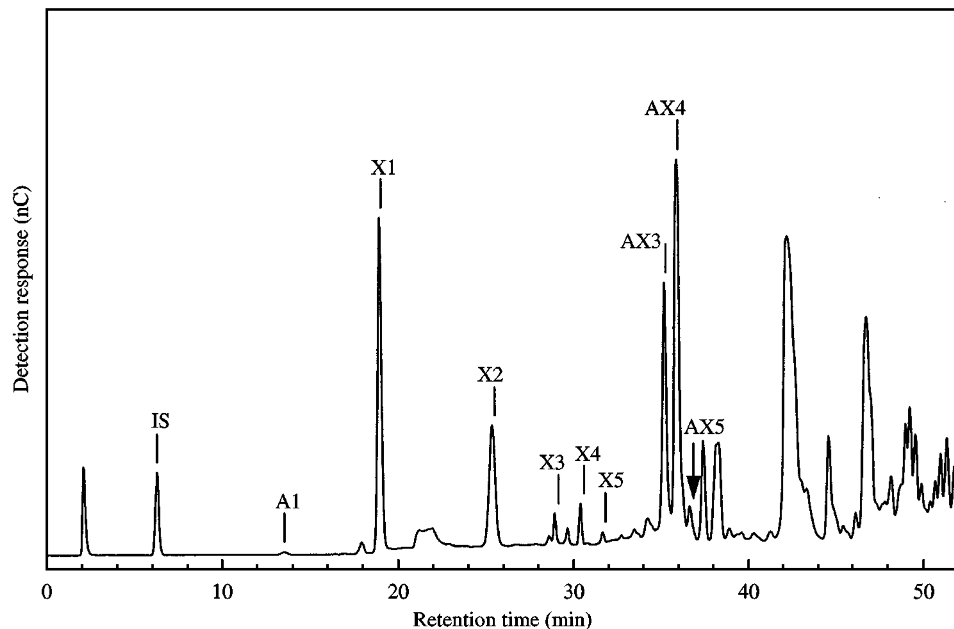

Figure 1. HPLC chromatography of arabinoxylo-oligomers. *X*, xylose; *A*, arabinose; *IS*, internal standard; figures indicate the number of monomers in the compound.

Table 1. Colonic lactic acid bacterial isolates according to their carbohydrate source in enrichment broth

	Lactulose	Lactitol	Lactobionic acid	Rye fractions	Arabinoxylo-oligosaccharides	Total
L. rhamnosus	11	—	—	34	—	45
L. salivarius	4	5	6	32	—	23
L. cellobiosus	—	18	—	1	4	47
L. paracasei	—	—	—	17	4	21
Total	15	23	6	84	8	136

L. salivarius isolates. In addition, the digestion and/or restriction with these isolates were probably only partial and at this step the analyses were carried out only once. The representatives of each group were deposited in the VTT Culture Collection (Espoo; Table 2) and they were used in further studies.

L. rhamnosus isolates were detected from the samples of the patients B1 and B2 and matched three different ribogroups with good similarity (Table 2, Fig. 2). The similarity of these ribogroups to that of the type strain E-96031 (isolation source unknown) was 71–90%. The VTT database, which includes fingerprints of

Table 2. Identification of the selected isolates deposited to the VTT Culture Collection

Strain	Patient/Enrichment		API-ID, profile % id	Ribogroup	Similarity	
					DUP	VTT
Lactobacillus rhamnosus						
E-96031[T]	—	—	*L. rhamnosus*, excellent 99·9	65-S-1	none	none
E-97951	B1	Rye	*L. rhamnosus*, doubtful 61·7	24-S-1	none	0·97
E-97948	B1	Rye	*L. rhamnosus*, doubtful 61·7	33-S-1	none	0·92
E-97959	B2	Lactulose	*L. rhamnosus*, doubtful 61·7	33-S-1	none	0·92
E-97960	B2	Lactulose	*L. rhamnosus*, doubtful 61·7	33-S-1	none	0·91
E-97962	B2	Lactulose	*L. rhamnosus*, doubtful 61·7	33-S-1	none	0·92
E-981000	B2	Rye	*L. rhamnosus*, excellent 99·9	33-S-5	none	0·97
Lactobacillus salivarius						
E-97853[T]	—	—	*L. salivarius*, excellent 99·9	125-S-3	0·96	none
E-97955	B2	Rye	*L. salivarius*, excellent 99·9	46-S-2	0·88	0·87
E-97950	B2	Rye	*L. salivarius*, excellent 99·9	46-S-2	0·87	0·86
E-98999	B4	Lactulose	*L. salivarius*, good 99·9	208-S-1	0·86	none
E-981006	B4	Lactob. acid	*L. salivarius*, doubtful 77·2	208-S-1	0·86	none
E-981007	B4	Lactob. acid	*L. salivarius*, doubtful 77·2	208-S-1	0·85	none
E-981020	B4	Lactitol	*L. salivarius*, doubtful 77·2	208-S-1	0·86	none
Lactobacillus cellobiosus						
E-8216[T]	—	—	*L. cellobiosus*, very good 99·9	210-S-1	none	none
E-97957	B2	Lactitol	*L. cellobiosus*, excellent 99·9	169-S-1	none	none
E-97958	B2	Lactitol	*L. cellobiosus*, excellent 99·9	169-S-1	none	none
E-98997	B2	Xylo-oligos.	*L. cellobiosus*, excellent 99·9	169-S-1	none	none
E-981003	B2	Xylo-oligos	*L. cellobiosus*, good 97·3	169-S-1	none	none
Lactobacillus paracasei						
E-93490[T]	—	—	*L. paracasei*, good 98·7	35-S-8	none	
E-97949	B1	Rye	*L. paracasei*, very good 99·3	146-S-1	none	0·88
E-981004	B1	Xylo-oligos	*L. paracasei*, good 97·7	146-S-1	none	0·9

[T] Type strain.

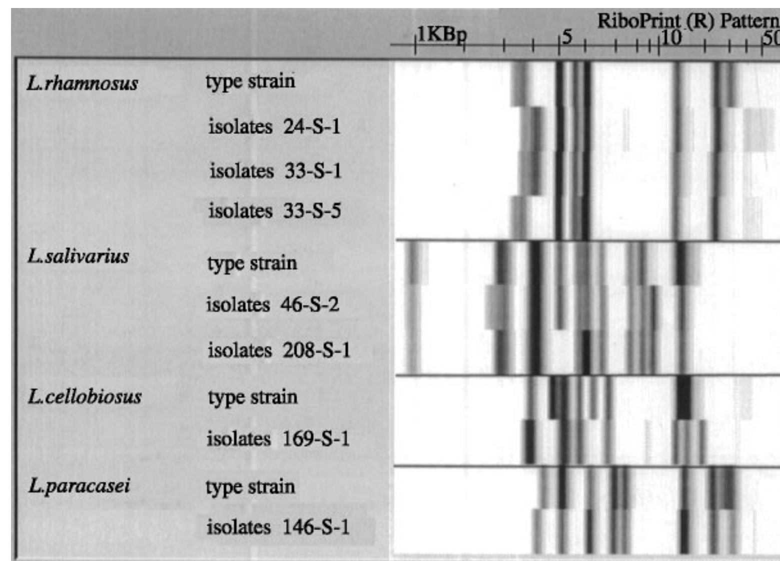

Figure 2. RiboPrinter® fingerprints of the identified biopsy isolates and of the relevant type strains.

isolates from the dairy industry, identified the isolates reliably, but the profiles of API identifications, with one exception, were only doubtful for these biopsy isolates.

L. salivarius species was detected from the samples of the patients B2 and B4. These isolates matched two ribogroups and the identification with the existing databases was poor. The similarity of these groups to that of the type strain E-97853, isolated from rumen, were 82% and 88%, respectively. The profiles of API identifications ranged from doubtful to excellent.

L. cellobiosus isolates were detected only from samples of the patient B2. All of these isolates matched the same ribogroup, which was not identified by the existing databases. The similarity of this group to that of the type strain E-82167, isolated from saliva, was only 67%. The profiles of API identifications ranged from good to excellent.

L. paracasesi isolates were detected only from the samples of the patient B1. All of these isolates matched the same ribogroup. The similarity of this group to that of the type strain E-93490y, isolated from dental caries, was 87% and it was identified by the VTT database. The profiles of API identifications were good or very good.

Growth of selected isolates

The growth of bacteria ($5{\cdot}0 \times 10^3$–$1{\cdot}1 \times 10^9$ cfu ml^{-1}) was dependent on the carbon source (Table 3). The best growth occurred with the strains *L. rhamnosus* E-97948 and E-97951, *L. paracasei* E-97949 and *L. salivarius* E-97950. In addition, lactobionic acid seemed to enhance the growth of *L. cellobiosus* E-97957 and E-97958 when compared to their growth on lactulose, lactitol or arabinoxylo-oligosaccharides. With the exception of lactulose, the best substrate for growth was not the same as used in enrichment. For example the strains from E-97948 to E-97951, which were enriched with a rye fraction, showed better growth on the other carbohydrates studied, but not on arabinoxylo-oligosaccharides, The rye fraction was not tested because of the quantity of protein residues it contained and because it was insoluble in water, which caused false results in the Bioscreen analysis. According to API carbohydrate tests *L. rhamnosus* utilized D-arabinose, but none of the strains were able to utilize

Table 3. Growth of isolates on different carbohydrates in 24 h measurement using Bioscreen

LAB	VTT code	The main carbohydrate in enrichment broth	Lactulose	Lactitol	Lactobionic acid	Lactose	Galactose	Glucose	Arabinoxylo-oligo-saccharides	No carbo-hydrate
L. rhamnosus	E-97959	Lactulose	++	+	+	+++	++	+++	+	—
L. rhamnosus	E-97960	Lactulose	++	++	+	++++	++	+++	+	—
L. rhamnosus	E-97962	Lactulose	++++	+++	+	± ± ± ± ±	+++	++++	+	—
L. rhamnosus	E-97948	Rye fraction	± ± ± ± ±	± ± ± ± ±	+++	± ± ± ± ± ±	± ± ± ± ± ±	± ± ± ± ± ±	+++	+ +
L. rhamnosus	E-97951	Rye fraction	++++	+++	+	++++	++++	++++	++	++
L. rhamnosus	E-981000	Rye fraction	+	+	+	++	++	++	+	+
L. rhamnosus	E-98952[a]	—	± ± ± ± ± ±	++++	+++	± ± ± ± ± ±	± ± ± ± ±	± ± ± ± ±	++	++
L. salivarius	E-98999	Lactulose	+	+	+	+	+	+	+	+
L. salivarius	E-981006	Lactobionic acid	± ± ± ± ± ±	++	++	+++	+	++	++	+
L. salivarius	E-981007	Lactobionic acid	± ± ± ± ± ±	+	++	++	+	++	++	+
L. salivarius	E-97950	Rye fraction	++++	++	++	+++	++	+++	++	++
L. salivarius	E-97955	Rye fraction	+++	++	++	+++	++	+++	++	++
L. cellobiosus	E-98957	Lactitol	+	+	++	+	+	++	+	+
L. cellobiosus	E-97958	Lactitol	+	+	++	+	+	++	+	+
L. cellobiosus	E-98997	Arabinoxylo-oligos.	++	+	± ± ± ± ±	+	+	± ± ± ± ± ±	+	+
L. cellobiosus	E-981003	Arabinoxylo-oligos.	+	+	++++	+	+	++++	+	+
L. paracasei	E-97949	Rye fraction	++++	++++	+++	++++	± ± ± ± ± ±	++++	+++	++
L. paracasei	E-981004	Arabinoxylo-oligos.	++	+	+	+	+ +	+++	+	+

[a]Control *L. rhamnosus* L56 received from Prof Kneifel, Austria — no growth.
The areas $<$ 100 represent +; 101–200 + +; 201–300 + + +; 301–400 + + + +; 401–500 ± ± ± ± ± and $>$ 501 ± ± ± ± ± ±.

xylose. However, *L. rhamnosus* E-97948 and *L. paracasei* E-97949 showed moderate growth on arabinoxylo-oligosaccharides (Table 3). The growth of the four selected bacteria was also enumerated by plating, whereby the growth was enhanced 1–3 $\log_{10}$ cfu ml^{-1} on all supplemented carbohydrates (data not shown).

Adhesion properties of selected isolates

Over 75% adhesion was observed with *L. salivarius* E-98999. This result is, however, an artefact of the very strong cell aggregation observed with this strain. Good adhesion (>20%) was detected with the strains *L. rhamnosus* E-97948, E-97951, E-97959, E-97960, E-971000 and *L. paracasei* E-97949 and E-971004 (Fig. 3). Adhesion of 4–6% was detected with *L. salivarius* E-97955, *L. cellobiosus* E-97957 and E-98997 and *L. rhamnosus* E-97952 (negative control). *Lactobacillus* GG E-96522 and *L. rhamnosus* E-97800 (positive controls) showed 15% and 30% adhesion, respectively. The optimal amount of bacterial cells for adhesion was strain dependent and generally between 7×10^6 and 8×10^8 cfu ml^{-1}.

Discussion

LAMVAB agar used in this study had a much higher potential to select lactic acid bacteria from intestinal material than the modified MRS agar used in our earlier study when mainly enterococci and streptococci were enriched (Kontula et al. 1998). This is also in agreement with the previous study of Hartemink et al. (1997) who demonstrated the growth of intestinal isolates on LAMVAB agar. However, some lactic acid bacteria of dairy origin, e.g., *L. acidophilus*, fail to grow on it (this study, data not shown; Hartemink, pers. comm), and this may be due to the different lactic acid bacteria content of different individuals (Tannock 1997, Kimura 1997, this study, Table 2), but most likely that *L. acidophilus* are sensitive to vancomycin used in LAMVAB. Hamilton-Miller and Shah (1998) found that *L. acidophilus* strains isolated from probiotic supplements or foods were sensitive to vancomycin. However, in their study the real origin of the strains remained unclear. For the purpose of isolating LAB, an advantage of LAMVAB medium is that bifidobacteria, estimated as the fourth largest group in the intestinal microbiota (Tannock 1990), do not grow on it (Hartemink 1997, this study, data not shown).

The current starter cultures from 50 *Lactobacillus* species in commercial use are mainly strains of *L. acidophilus*, *L. delbrüeckii* subsp. *bulgaricus*, *L. casei*, *L. fermentum*, *L. helveticus*, *L. johnsonii*, *L. kefir*, *L. paracasei*, *L. plantarum*, *L. reuterii* and *L. rhamnosus* with possible antimicrobial or probiotic action (Davidson

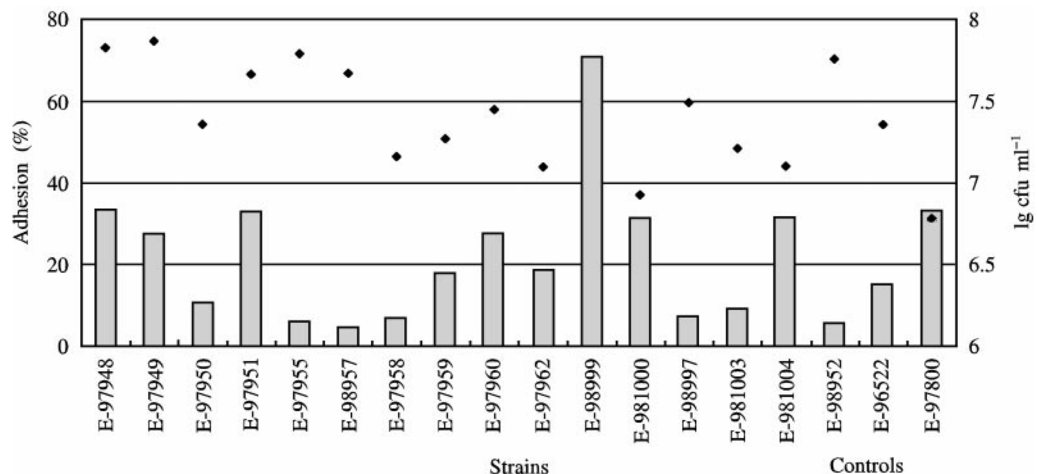

Figure 3. Adhesion of the selected isolates (%) on the Caco-2 cell line. Adhesion (%) (▢); lg cfu ml^{-1}(◆).

and Hoover 1993, Salminen et al. 1993). Traditional dairy starters are *L. bulgaricus* (yogurt) and *L. helveticus* (cheese). In our previous study, a potential probiotic strain *L. rhamnosus* (E-97800) was isolated from a faecal sample (Kontula et al. 1998) and in this second survey the most promising strains (E-97948, E-97949) are members of *L. rhamnosus* and *L. paracasei*. These strains showed good *in vitro* adhesion and growth on lactulose and rye fraction.

The advantages of the automated system are that it has a high discrimination power, and that it is rapid, standardized, labour-saving, easy and versatile. The ability to characterize below the species level can be applied in tracing the source of isolates. The disadvantages of the system are that, at present, it is suitable only for bacteria, it is expensive to buy and the running costs are high. Before the method can be used efficiently, many fingerprints have to be collected for different organisms and the customer's own databases for RiboGroups and identification created.

The results indicate that different carbohydrates favor the enrichment of different bacterial species from intestinal samples and it is possible to isolate strains with substrates specificities. However, in most cases there was no correlation between the carbohydrate used for enrichment and the ability to utilize that substrate later for growth. The growth of all isolates on arabinoxylans was weak, and on the other hand, lactulose was utilized most efficiently. Interestingly, the growth of *L. cellobiosus* isolated from arabinoxylo-oligosaccharides enrichment broth was markedly enhanced by lactobionic acid. Tested strains showed utilization of lactose and D-arabinose, but not xylose.

The adhesion of strains tested by radioactive labelling was highly dependent on the concentration of the cells. Therefore, we recommend the use of several concentrations in testing the strains to avoid saturation of the Caco-2 cell culture.

Using vancomycin containing LAMVAB medium, this study showed that lactose derivatives and rye fraction mixture select *L. rhamnosus*, *L. paracasei*, *L. cellobiosus* and *L. salivarius* species from colonic biopsy material. The adhesion to Caco-2 cells depended on the isolate. Many of the strains isolated with the carbohydrates used showed even better adhesion than the positive control probiotic *Lactobacillus* GG. This might indicate that this kind of human colonic material shows good promise as a source of potential probiotic isolates. However, results should be verified with *in vivo* experiments.

Acknowledgements

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