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Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract

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ABSTRACT

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Aims: The study of two human strains of *Lactobacillus* to be used as probiotics in the gastrointestinal tract.

Methods and Results: The *Lactobacillus acidophilus* UO 001 and *Lact. gasseri* UO 002, were resistant to the gastrointestinal conditions (pH 2 and 3, presence of pepsin, pancreatin or bile salts), the resistance was enhanced in the presence of skimmed milk. Additionally, adhered to Caco-2 cells through glycoproteins in *Lact. gasseri* and carbohydrates in the case of *Lact. acidophilus*. These strains are able to inhibit the growth of certain enteropathogens: *Salmonella*, *Listeria* and *Campylobacter* without interfering with the normal microbiota of the gastrointestinal tract, as stated by using the mixed culture and the spot agar test. Finally, strongly adherent *Lact. gasseri* were found to inhibit the attachment of *Escherichia coli* O111 to intestinal Caco-2 cells under the condition of exclusion.

Conclusions: These results indicate that the two strains of *Lactobacillus* from human origin present important properties for survival in, and colonization of, the gastrointestinal tract, that give them potential probiotic.

Significance and Impact of the Study: Two strains of *Lactobacillus* isolated from human vagina of healthy premenopausal women could be promising candidates to be used in the preparation of probiotic products and for their use as health-promoting bacteria.

Keywords: adherence, gastrointestinal tract, inhibition, *Lactobacillus*, pathogens, probiotic.

INTRODUCTION

Probiotics have been used in animal production for the last two decades, their efficiency on animal performance having been widely discussed. However, the mode of action of probiotics still remains unclear. It has been proposed that probiotics could maintain the healthy intestinal microbiota through competitive exclusion and antagonistic action against pathogenic bacteria in the animal intestine (Fuller 1989).

The ability of lactic acid bacteria to inhibit the growth of various Gram-positive or Gram-negative bacteria is well known. This inhibition may be due to the production of organic acids such as lactic and acetic acid (Gilliland and Speck 1977), hydrogen peroxide, bacteriocins, bacteriocin-like substances and possibly biosurfactants (Velraeds *et al.* 1996), which are active against certain pathogens and may be produced by different species of *Lactobacillus*. On the other hand, several studies have suggested that adhesive probiotic bacteria could prevent the attachment of pathogens and stimulate their removal from the infected intestinal tract (Lee *et al.* 2000).

These antagonistic properties could be very useful in probiotic products. Apart from this, successful probiotic bacteria should be able to survive gastric conditions and colonize the intestine, at least temporarily, by adhering to the intestinal epithelium (Lee and Salminen 1995). Such probiotic microorganisms appear to be promising candidates for the treatment of intestinal disorders produced by abnormal gut microflora and altered gut mucosal barrier functions (Salminen *et al.* 1996a, 1996b; Álvarez-Olmos and Oberhelman 2001). The most studied probiotics are the lactic acid bacteria, particularly *Lactobacillus* and *Bifidobacterium*.

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This paper reports a study of two human strains of *Lactobacillus* described in a previous report (Boris *et al.* 1998) that presents properties that may allow their use as biotherapeutic agents in the genitourinary tract. Other characteristics such as acid and bile tolerance, adherence to intestinal epithelial cells and the antagonistic effect *in vitro* against certain enteropathogenic bacteria, three important properties for survival in, and colonization of, the gastrointestinal tract are described.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Lactobacillus acidophilus UO 001 and *Lact. gasseri* UO 002, two previously characterized (Boris *et al.* 1997; Boris *et al.* 1998) vaginal isolates, were employed in this study. The other bacterial strains used as enteropathogens were clinical specimens: *Escherichia coli* O111, *Salmonella choleraesuis* serotype Enteritidis, *Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni, Clostridium difficile* and *Cl. perfringens*. The following were used as members of normal microbiota: *E. coli, Enterococcus faecalis, Bacteroides fragilis* and *Bifidobacterium bifidum* CECT 870. All strains except *Bif. bifidum* were obtained at the Hospital Monte Naranco and the Hospital Central de Asturias (Oviedo).

Lactobacilli were incubated on LAPTg broth or agar (Raibaud *et al.* 1961). Other media such as eosin–methylene-blue (Oxoid) were used for enterobacterias; KF agar (Scharlau, Barcelona, Spain) for *Ent. faecalis; Campylobacter* medium (Oxoid) for *Camp. jejuni*, BHI (Biokar, Beauvais, France) for *Listeria*; TGY (Tryptone 30 g l^{-1}, yeast extract 20 g l^{-1}, glucose 5 g l^{-1} and thioglycolic acid sodium salt 1 g l^{-1}) for *Cl. perfringens* and *Cl. difficile*; TPY (Adsa, Barcelona, Spain) for *Bifidobacterium* and TPY supplemented with haemine (5 μg ml^{-1}) for *Bacteroides* and Chapman (Pronadisa, Madrid, Spain) for *Staph. aureus*.

The incubation temperature was 37°C. The strains were incubated in aerobic conditions, except for *Bacteroides, Bifidobacterium* and *Clostridium,* which were propagated under anaerobiosis.

Resistance to artificial gastric and intestinal fluids

Simulated gastric digestion was tested essentially as described in (Zárate *et al.* 2000). Briefly, 50 ml of LAPTg medium were inoculated at 2% (v/v) with lactobacilli strains and incubated at 37°C for 24 h. After washing in sterile saline solution (NaCl, 0·9%) and centrifugation, the cell suspensions were added to 50 ml of artificial gastric juice with the following composition: NaCl, 125 mmol l^{-1}; KCl 7 mmol l^{-1}; $NaHCO_3$, 45 mmol l^{-1} and pepsin, 3 g l^{-1}. The final pH was adjusted with HCl to pH 2 and 3 and with NaOH to pH 7. The bacterial suspensions were incubated with agitation (200 rev min^{-1}) to simulated peristalsis. Aliquots were taken for the enumeration of viable at 0, 90 and 180 min. The effect of gastric digestion was also determined by suspending the cells in skimmed milk instead of saline solution before the inoculation of gastric juice at pH 2.

As described in Zárate *et al.* (2000), simulated intestinal fluid was prepared by suspending the cells (after 180 min of gastric digestion) in 0·1% (wt/v) pancreatin (Sigma) and 0·15% (w/v) Oxgall bile salts (Sigma) in water and adjusting it to pH 8·0 with 5 mol l^{-1} NaOH. The suspensions were incubated as above and samples for total viable counts were taken at 0, 90 and 180 min.

Adherence assays

The adherence of lactobacilli to Caco-2 cells was examined as previously described (Coconnier *et al.* 1992). Briefly, the Caco-2 monolayers were washed twice with phosphate-buffered saline (PBS), pH 7·3. For each adhesion assay, 1 ml of *Lactobacillus* suspension (10^8 bacteria per ml in PBS) was added to each well of the tissue culture plate, which was incubated at 37°C in 5% CO_2. After 90 min of incubation, the monolayer was washed five times with sterile PBS, fixed with methanol, stained with Gram stain and examined microscopically. Each adherence assay was conducted in duplicate over three successive passages of intestinal cells. For each monolayer on a glass coverslip, the number of adherent bacteria was counted in 20 random microscopic areas. Adhesion of lactobacilli was expressed as the number of bacteria adhering to 100 Caco-2 cells. *Lact. delbrueckii* subsp. *lactis* UO 004 (an intestinal isolate) and *Lact. delbrueckii* subsp. *bulgaricus* (dairy origin) were used as positive and negative controls, respectively, for adherence.

The bacteria were subjected to various treatments in order to characterize the bacterial determinants involved in lactobacilli adhesion. The bacterial suspension was heated to 100°C in a water bath for 10 min and cooled by immersion in an ice bath. Treatments with trypsin (2·5 mg ml^{-1}, 37°C, 1 h), lipase from *Rhizopus arrhizus* (Sigma, Madrid, Spain) (2·5 mg ml^{-1}, 37°C, 1 h), sodium metaperiodate (10 mg ml^{-1}, 1 h, room temperature) and ethylenediaminetetraacetic acid (EDTA) (20 mmol l^{-1}) in PBS at pH 7·02 were performed as described previously (Barrow *et al.* 1980; Chauviére *et al.* 1992). The adherence experiment was performed as indicated above.

At the same time, in order to confirm the results obtained above, a radiolabelled method was used (Greene and Kleanhammer 1994). In our case LAPTg replaced MRS as culture medium.

In vitro interaction between lactobacilli and some enteropathogens, and with some members of the normal microbiota

Lactobacilli were tested for inhibition of representative gastrointestinal tract pathogens using two methods: the mixed culture (Bathia *et al.* 1989) and the agar spot test described in Jacobsen *et al.* (1999). In the former, lactobacilli and the enteropathogens were incubated separately in LAPTg broth under aerobiosis until $O.D._{600} = 0{\cdot}6$. Aliquots of each *Lactobacillus* culture were mixed with equal volumes of each of the enteropathogen cultures and incubation was resumed. Samples from the mixed cultures were plated at 4-h intervals for 24 h on the appropriated media. The plates were incubated at 37°C for 48 h and colony-forming units (CFU) counted (Bathia *et al.* 1989). The experiment was performed twice. The method described by Jacobsen *et al.* (1999) was used for the agar spot test. Briefly, aliquots of 2 μl of test cultures were seeded onto LAPTg agar plates and incubated for 24 h. Thereafter, 100 μl of the overnight cultures of the indicator bacteria were mixed with 7 ml of soft agar (7 g l^{-1}) using the aforementioned medium for each strain. The plates were then incubated at 37°C for 48 h in aerobiosis, anaerobiosis or in a 5% CO_2 atmosphere, depending on the tested strain, and inhibition zones were observed. When clear zones reached more than 1 mm, these were scored as positive. Each test was performed twice. This method was used for *Listeria*, *Campylobacter* and *Clostridium* sp.

Similar methods were used in the case of some strains of the normal microbiota, the mixed culture for *Ent. faecalis* and *E. coli* and the second one for *Bact. fragilis* and *Bif. bifidum*.

Identification of the inhibitor

To further determine the properties of the inhibitor, Supernatants of *Lact. acidophilus* cultures (24 h) were subjected to different treatments: (i) sensitivity to proteases; trypsin and proteinase K were added to a final concentration of 1 mg ml^{-1}, incubated at 37°C for 1 h, and the samples were adjusted to pH 4 with 1 M HCl; (ii) heat; 100°C for 30 min; (iii) ammonium sulphate precipitation (85%); the pellet was resuspended in distilled water and the supernatant was dialysed using a dialysis membrane with a pore exclusion unit of 1200 Da; (iv) chloroform extraction; the chloroform was combined with an equal volume of filtered supernatant, shaken and allowed to separate, the organic phase was evaporated and resuspended in saline solution at pH 3; (v) the 24-h culture supernatant of *Lact. acidophilus* was incubated with 250 μg l^{-1} L-lactic dehydrogenase (Sigma Chemical Co., St Louis, MD, USA) for 2 h at 37°C.

All the samples were tested using the well diffusion assay (Schilinger and Lucke 1989), the plates being prepared by adding 100 μl from an overnight culture of *E. coli* O 111 to 10 ml of LAPTg containing 1·2% (w/v) agar. After solidification, wells (5-mm diameter) were made with a sterile cork-borer and filled with 15 μl of each sample.

Interference assays

Interference experiments were performed with *E. coli* O111 and *Lact. gasseri*, as they showed a significant capacity to adhere to Caco-2 cells. The procedures described in (Spencer and Chesson 1990) were used. Briefly, for the exclusion tests, *Lact. gasseri* (10^8 CFU ml^{-1}) were added to Caco-2 monolayers and incubated together for 45 min; radiolabelled *E. coli* (10^8 CFU ml^{-1}) was subsequently added, and incubation was continued for a further 45 min. For competition tests, *Lactobacillus* and the radiolabelled pathogen were added to wells and incubated for 90 min. For displacement tests, the radiolabelled *E. coli* was added to Caco-2 monolayers and incubated together for 45 min, *Lact. gasseri* was then added, and incubation was continued for a further 45 min.

Subsequently, the monolayers were washed and solubilized as described in the adherence assays. Samples harvested were added to 10 ml of scintillation liquid and counted in a scintillation model Rack Beta 1211 LKB counter (Turku, Finland).

The controls used were the following: C1, a suspension of radiolabelled *E. coli* in PBS pH 7·3; C2, the Caco-2 cells incubated with 1 ml of PBS pH 7·3; and C3, the Caco-2 cells incubated with 500 μl of radiolabelled *E. coli* and 500 μl of PBS pH 7·3.

The percentage of the total added radioactivity associated with the washed monolayers was calculated by the equation:

$$\frac{\text{Test}_{\text{cpm}} - \text{C1}_{\text{cpm}}}{\text{C1}_{\text{cpm}}} \times 100$$

Each assay was conducted in triplicate twice.

Statistical analysis

Two-factor analysis of variance (ANOVA) was used to evaluate the statistical significance of the experimental results. Factor one represents the three conditions (i.e. exclusion, competition and displacement) and the control group. The second was a factor block with two levels, one of which represents an independent repetition of the experiment; all measurements being carried out with a minimum of duplicate samples per variable for each experiment.

Data are expressed as mean ± typical deviation and the *posteriori* comparison was analysed by the Tukey's DHS.

The statistical significance for treatments and *posteriori* test was $P < 0{\cdot}01$.

RESULTS

Effect of gastric and intestinal digestion on the viability of human lactobacilli

As can be seen in Fig. 1, at pH 3 any *Lactobacillus* present a decrease in the viability at least during 3 h of incubation, whereas at pH 2 *Lact. acidophilus* and *Lact. gasseri* survive during 90 min. When milk was added (pH 2·5) the viability was recovered in all cases.

With respect to the intestinal fluid, *Lact. acidophilus* seem to be slightly more resistant than *Lact. gasseri*. As we stated previously, the presence of milk stimulated survival in all the strains and situations (Fig. 1).

Adherence to Caco-2 cells

Lactobacillus gasseri strongly adhered to Caco-2 cells similar to the positive control, an intestinal isolate, *Lact. delbrueckii* subsp. *lactis,* whereas *Lact. acidophilus* adhered in least numbers (Table 1). These results were confirmed by a radiolabelled method. The results are expressed as the adherence rate, i.e. the ratio between the number of the bacteria added to the cell monolayer and the remaining adhered bacteria. This is 3·23 ± 0·99 % in *Lact. gasseri* and 1·39 ± 0·55 % for *Lact. acidophilus*.

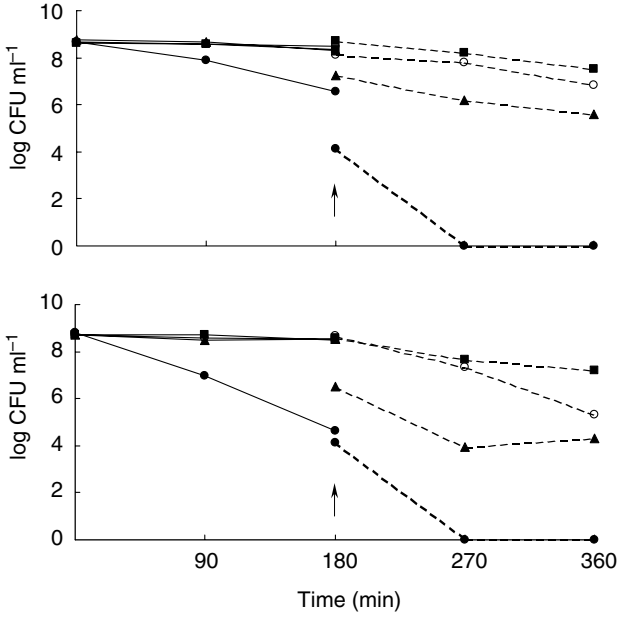

Fig. 1 Effect of digestion by gastric (solid lines) and intestinal fluids (dotted lines) on the survival of *Lact. acidophilus* UO 001 (a) and *Lact. gasseri* UO 002 (b) at pH 2 (●), pH 3 (▲), and pH 7 (■) and in gastric juice pH 2 plus skimmed milk (○)

Table 1 Ability of *Lactobacillus acidophilus* UO 001 and *Lact. gasseri* UO 002 to adhere to Caco-2 cells

Strain	No. of adherent cells (mean ± SD)
Lact. delbrueckii subsp. *lactis* (intestinal isolate)	213·9 ± 17·5
Lact. gasseri	233·9 ± 55·4
Lact. acidophilus	168·6 ± 39·9
Lact. delbrueckii subsp. *bulgaricus* (dairy origin)	35·08 ± 13·02

The biochemical characterization of the adhesion (Table 2) revealed that this involved protease- and metaperiodate-sensitive factors on the bacterial surface (possibly glycoproteins) in *Lact. gasseri,* whereas in the case of *Lact. acidophilus* heat-labile carbohydrates requiring divalent cations are involved in the adherence.

Interaction with normal microbiota

When each *Lactobacillus* strain is incubated in mixed culture with *Ent. faecalis,* no differences in the viability were observed with respect to the control (Fig. 2). Similar results were obtained for *E. coli* (data not shown). In the case of *B. bifidum* and *Bact. fragilis,* the agar spot test described in Methods was used. No inhibition was observed with any *Lactobacillus* (data not shown).

Inhibition of enteropathogens

In relation to the interaction between the lactobacilli and some enteropathogens, complete inactivation was observed after 7 h of mixed incubation of *Lact. acidophilus* or *Lact. gasseri* with *Salm. choleraesuis, Y. enterocolitica* or *E. coli*

Table 2 Nature of the adhesins of *Lactobacillus* sp. for Caco-2 cells

Treatment	No. of adherent cells (mean ± SD) of: *Lact. acidophilus*	*Lact. gasseri*
None (control)	168·6 ± 39·9 (100%)	233·9 ± 55·4 (100%)
Trypsin (1 mg/ml^{-1})	164·8 ± 40 (97·8%)	25·4 ± 10·6 (10·8%)†
Lipase (1 mg/ml^{-1})	182·7 ± 31 (108%)	174·2 ± 52·8 (74·5%)
Sodium metaperiodate (10 mg/ml^{-1})	46·9 ± 23·1 (27·8%)*	5·3 ± 1·9 (2·3%)†
Heat (100°C)	55·9 ± 25·3 (35·5%)*	208·9 ± 24·8 (89·3%)
EDTA (20 mM)	54·5 ± 18·5 (32·3%)*	ND

*$P < 0{\cdot}001$ compared with the *Lact. acidophilus* control.
†$P < 0{\cdot}001$ compared with the *Lact. gasseri* control.
EDTA, ethylenediaminetetraacetic acid; ND, nor determined; SD, standard deviation.

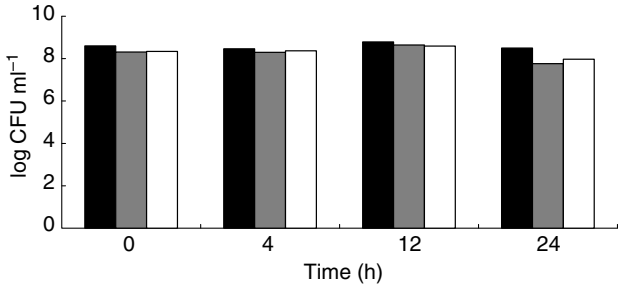

Fig. 2 Effect of *Lactobacillus* sp. on *Enterococcus faecalis* growth. Control culture (black bar) mixed culture with *Lact. acidophilus* UO 001 (patterned bar), and with *Lact. gasseri* UO 002 (white bar)

O111. Only slight inhibition was detected in the case of *S. aureus* (Fig. 3).

On the other hand, when the spot agar method was used for *Clostridium* sp., *Campylobacter*, or *Listeria*, a strong inhibition was observed (data not shown).

In order to characterize the inhibitory compound produced by *Lactobacillus,* its culture supernatant was assayed for the presence of bacteriocin and organic acids. The active component of the supernatants was small, as judged by its solubility in up to 85% ammonium sulphate, its pass-through dialysis tubing with a pore exclusion limit of 1200 Da and its resistance to heat (100°C for 30 min). In addition, its polar nature was confirmed because it was not

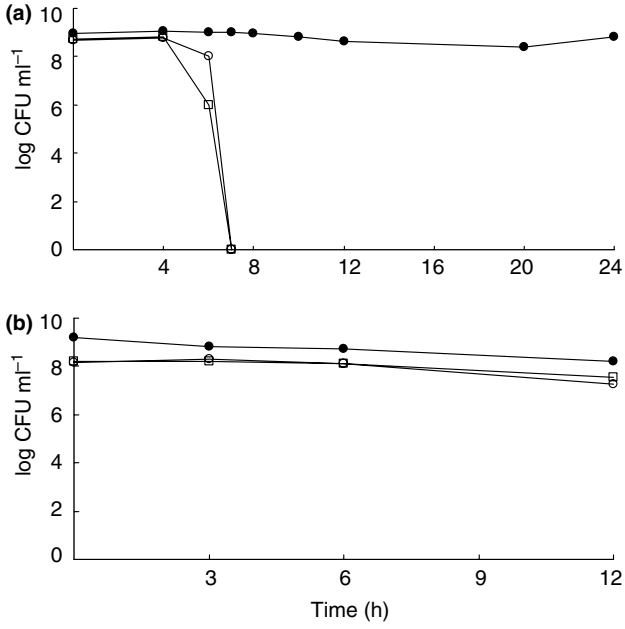

Fig. 3 Interaction between *Lactobacillus* sp. and enteropathogenic bacteria. Mixed culture assay with (a) *Salmonella choleraesuis* serotype Enteritidis; (b) *Staphylococcus aureus* (●), control culture, (○), enteropathogen with *Lact. acidophilus* UO 001, (□), enteropathogen with *Lact. gasseri* UO 002

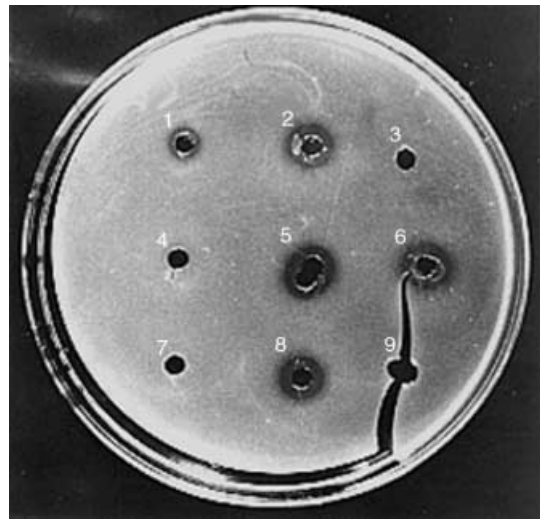

Fig. 4 Analysis of the inhibitor produced by *Lactobacillus acidophilus* UO 001 against *E. coli* O111. (1) *Lactobacillus* cells washed with distilled water; (2) concentrated supernatant of the culture; (3) redisolved sediment after precipitation of the supernatant with 85% ammonium sulphate; (4) supernatant dialysed through a membrane with a pore limit of 1200 Da; (5) supernatant treated with proteinase K; (6) supernatant heated at 100°C; (7) supernatant treated with lactate dehydrogenase; (8) aqueous and (9) organic fractions after chloroform extraction

extractable with chloroform. Finally, it was resistant to proteinase K, which discarded the assumption that it was a bacteriocin. Similar results were obtained from supernatants of lactobacilli incubated in aerobiosis, in a 5% CO_2 atmosphere or anaerobically, which indicated that the inhibitor was not H_2O_2. All these data were compatible with an organic acid and most probably lactic acid, given the homofermentative metabolism of the lactobacilli under study and the loss of antibacterial activity of *Lact. acidophillus* after L-lactic dehydrogenase treatment (Fig. 4).

Adhesion interference of intestinal pathogens

A large reduction in adherence of *E. coli* was observed when *Lact. gasseri* was incubated with Caco-2 monolayers before the addition of the pathogen, indicating that a phenomenon of exclusion exists, presumably by having the same cellular receptors. This fact was statistically significant as can be seen in Table 3.

DISCUSSION

The first requirement for a probiotic bacterium is its ability to survive transport to the active site in which its beneficial action is expected. Hence, the bacteria destined to benefit intestinal functions must survive passage through the acidic environment to the stomach. The gastric pH in healthy

Table 3 Effect of *Lactobacillus gasseri* on the attachment of *E. coli* O111 to Caco-2 cells under conditions of exclusion, competition and displacement

	Percentage of adherence (mean ± T.D.)	
Condition	Trial 1	Trial 2
Control	1·52 ± 0·07	3· 45 ± 0·35
Competition	1·20 ± 0·64	3·02 ± 0·62
Exclusion	0·16 ± 0·14*	1·48 ± 0·53*
Displacement	1·60 ± 0·33	2·77 ± 0·88

*Adhesion levels significantly different ($P < 0·01$).

humans is about 2–2·5. As demonstrated, *Lact. acidophilus* and *Lact. gasseri* survive under these conditions for at least 90 min, which could be enough to reach their action site in the intestine.

The resistance to bile salts is another important character, which could explain the inefficiency of some commercial preparations of probiotics. It is quite difficult to suggest a precise concentration to which the selected strain should be tolerant (Havenaar *et al.* 1992). The lactobacilli tested in this study were not lysed in the presence of bile salts (0·15%) and seem to be resistant to gastric and intestinal conditions, above all, in the presence of skimmed milk, as also was observed for *Lact. casei* 212·3 (Charteris *et al.* 1998) and for propionibacteria (Velraeds *et al.* 1996), showing that Emmental cheeses exerted a protective effect. This is important in order to use these strains as dietary adjuncts in dairy functional foods.

Adhesion of the probiotic microorganisms to the intestinal mucosa is a prerequisite for colonization and for antagonistic activity against enteropathogens (Owehand 1998). Upon reaching the intestine, the probiotics must attach to the brush border of microvilli or adhere to the mucus layer in order not to be swept from the colon by peristalsis. The adherence assay was carried out using the human intestinal cell line Caco-2, a well-characterized cellular lineage established from a human colonic adenocarcinoma (Fogh *et al.* 1977). The ability of *Lactobacillus* to adhere to the differentiated Caco-2 cells varies considerably between species, as different authors (Chauviére *et al.* 1992; Coconnier *et al.* 1992; Bernet *et al.* 1993; Green and Kleanhammer 1994) have reported. In our case, *Lact. gasseri* is the most adherent.

Multiple components of the bacterial cell surface seem to participate in the adherence of the strains to intestinal epithelial cells. In *Lact. gasseri,* adherence involved proteins and carbohydrates (possibly glycoproteins), while *Lact. acidophilus* seemed to depend on carbohydrates; in this case, divalent cations, probably Ca^{2+}, were also involved in adherence. The results are consistent with previous findings in which it was reported that lactobacilli adhere to human intestinal cells by means of a mechanism that involves different combinations of carbohydrate and protein factors on the bacterial cell surface (Chauviére *et al.* 1992; Coconnier *et al.* 1992; Bernet *et al.* 1993; Green and Kleanhammer 1994). The same components are also involved in lactobacilli adherence to vaginal cells (Boris *et al.* 1998). In contrast, lipoteichoic acid is responsible for *Lactobacillus* adherence to uroepithelial cells (Chan *et al.* 1985).

Lactic acid bacteria have been shown to inhibit the *in vitro* growth of many enteric pathogens and have been used in both humans and animals to treat a broad range of gastrointestinal disorders (Rolfe 2000). *Lact. acidophilus* and *Lact. gasseri* have been shown *in vitro* to strongly inhibit same enteropathogenic bacteria without interfering with the normal bacterial residents of the gastrointestinal tract, lactic acid could account for this antimicrobial activity. Similar results were reported for other strains of *Lactobacillus* isolated from chickens that inhibited *Salmonella* and *E. coli* growth (Jin *et al.* 1996) and a strain of *Lact. acidophilus* that inhibited *Camp. pylori* (McGroarty and Reid 1988), also because of the production of lactic acid.

Competitive inhibition for bacterial adhesion sites on intestinal epithelial surfaces is another mechanism of action for probiotics. The results of competitive binding assays clearly showed that *Lact. gasseri* effect the attachment of enteropathogen *E. coli* to Caco-2 cells under the condition of exclusion; Coconnier (Coconnier *et al.* 1992) also observed that lactobacilli have been shown to exclude enterotoxigenic *E. coli* from Caco-2 cells.

Recently great interest has arisen in the possibility of deliberately feeding beneficial microorganisms to humans as an alternative to antibiotic therapy in gastrointestinal disorders, probiotics being an attractive treatment alternative (Rolfe 2000). There have been hundreds of publications describing the use of probiotics to prevent and treat a variety of gastrointestinal disorders. However, only a relatively few studies have contributed convincingly to the health effects of probiotics in humans.

The results obtained *in vitro* do not allow direct extrapolation on the behaviour of lactobacilli, although they are an indication of the possible ability of the strains as probiotics. In particular, the inhibition of pathogens observed *in vitro* might be a method for screening potentially efficient strains for sanitary purposes. We conclude that these organisms possess a number of interesting properties that constitute the basis for their use as health-promoting bacteria and warrant further clinical investigations. Studies *in vivo* are under investigation.

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