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Research Article

Competition of *Lactobacillus paracasei* with *Salmonella enterica* for Adhesion to Caco-2 Cells

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Competition of commensal and probiotic bacteria with pathogens for adhesion and colonization is one of the important protective mechanisms of gastrointestinal tract. In this study, we examined the ability of *Lactobacillus paracasei* to inhibit the adhesion of pathogenic *Salmonella enterica* to human colon adenocarcinoma Caco-2 cells. Caco-2 cells were grown for 6 or 21 days to obtain nondifferentiated or well-differentiated cells, respectively. In adhesion experiments, bacteria were added to the cells for 2 or 4 hours. The number of attached bacteria was expressed as colony-forming units (CFUs), Caco-2 cells were counted in hematocytometer. Both bacterial strains used adhered better to well-differentiated than to nondifferentiated Caco-2 cells, however, the amount of *Salmonella* adhered to Caco-2 after 2 hours of contact was 12-fold higher in comparison to *L. paracasei* and almost 27-fold higher after 4 hours of contact. Two types of experiments were done: coincubation (both bacteria were added to Caco-2 cells simultaneously), and preincubation (*L. paracasei* was incubated with Caco-2 cells first, and then *S. enterica* was added). In coincubation experiment, the presence of *L. paracasei* decreased *S. enterica* adhesion by 4-fold and in preincubation experiment even 7-fold. Generally, *Lactobacillus* spent culture supernatants (SCSs) acted weaker as inhibitors of *Salmonella* adhesion in comparison to the whole *L. paracasei* culture in coincubation experiment. In conclusion, the displacement of pathogens by lactic acid bacteria and its secretions showed here depends on the time of bacteria-epithelial cell contact, and also on the stage of Caco-2 differentiation.

1. INTRODUCTION

Adhesion to mammal's epithelial cells is a key process for bacteria to survive and colonize the gastrointestinal tract. For pathogenic bacteria, the adhesion to epithelium is a critical step, since it allows the release of enzymes and toxins initiating necrotic processes directly into the target cell, thereby facilitating the invasion.

The epithelial cells of gastrointestinal tract (GIT) are protected from pathogenic bacteria by a number of mechanisms. One of them is a reduction in pathogenic infections through competition of microbiota for adhesion sites with microbial pathogens and production of components with antimicrobial activity [1, 2]. To cause infection, pathogenic bacteria, after penetrating intestinal mucus, must adhere to enterocytes [3]. The initial step of adhesion in the case of *Salmonella* species is mediated by bacterial fimbriae which recognize certain receptors on eukaryotic cells [4]. Several studies indicate that lactic acid bacteria (LAB) could prevent the attachment of pathogens, in this way reducing colonization, and prevent infection [5–8].

Bacterial adhesion to intestinal epithelium has been studied in different experimental in vitro models involving polymer surfaces [9], intestinal mucus [10–12], or intestinal cell lines, for example, producing mucus HT29-MTX. In the present studies, we used human colon adenocarcinoma

epithelial Caco-2 cell monolayer [13] to investigate bacterial adhesion. The Caco-2 cells differentiate similarly to normal small intestinal epithelial cells expressing characteristic for immature as well as mature enterocytes with functional brush border microvilli and apical hydrolases [14–18]. Several studies have described adhesion to cultured cells of many different lactic acid bacteria [15, 19], *Salmonella* and other bacteria, as well as competition between the microbial species [20]. Chauvière et al. have shown that heat-killed *Lactobacillus* inhibits adhesion of diarrheagenic *Escherichia coli* (ETEC) to Caco-2 cells [14].

The aim of present in vitro study was to investigate the adhesion potency of gram-positive LAB, *Lactobacillus paracasei,* and gram-negative pathogen *Salmonella enterica* to nondifferentiated and well-differentiated Caco-2 cells monolayer and competitive exclusion of pathogenic bacteria by *Lactobacillus* or its secretions under different experimental conditions. The-isolated-from contamination food, *Salmonella* is an adequate example of common microbial pathogen causing GIT infection. *L. paracasei* was selected among three *Lactobacillus* and two *Lactococcus* strains tested as the only strain adhering better to well-differentiated than to nondifferentiated Caco-2 cells. This finding allowed to presume that *L. paracasei* better than other LAB will compete with *Salmonella* for adhesion to Caco-2 cells.

2. MATERIALS AND METHODS

2.1. Bacterial strains and adhesion to Caco-2 cells

Two bacterial strains were used, isolated from human stool *Lactobacillus paracasei* IBB2588 (IBB PAS, Warsaw, Poland) and isolated from instant soup pathogenic *Salmonella enterica* subsp. *enteritidis* sv Enteritidis KOS 1663 (purchased from The National Salmonella Centre, Poland). *L. paracasei* was cultured in MRS broth (de Man, Rogosa, Sharpe) or on MRS plates (MRS broth supplemented with 1.5% agar, Biocorp Ltd., Poland) under anaerobic conditions (in anaerobic jar, OXOID Ltd., UK) at 37°C for 18–20 hours. The pathogenic *S. enterica* was cultured in Luria-Bertani broth (Biocorp Ltd., Poland) or on LB plates (LB supplemented with 1.5% agar, Biocorp Ltd., Poland) at 37°C for 18–20 hours under aerobic conditions. For the experiments, the overnight culture was 100-fold diluted in medium for Caco-2 cells but devoid of antibiotics and antimycotics. Then bacteria were incubated with Caco-2 cells for 2 or 4 hours, washed 3 times with sterile PBS (pH 7.4) and, after trypsinization, number of adhered bacteria was quantified as well as number of Caco-2 cells as described below. The number of bacteria adhering to the Caco-2 cells was expressed as colony-forming units (CFU). The CFUs were determined by plating of diluted bacterial suspensions on MRS or LB plates depending on bacterial strain, see above.

2.2. Caco-2 cell cultures

For the adhesion assay, a 3-week-old (well-differentiated) Caco-2 cell (ECACC 86010202) culture was used. Caco-2 cells were cultured in Dulbecco's modified Eagle's minimal essential medium DMEM (Sigma, USA) supplemented with 10% heat inactivated foetal bovine serum (Gibco, Invitrogen Corporation, USA), and 1% nonessential aminoacids solution (Sigma, USA) and antibiotics antimycotics solution (10 IU/mL penicillin G, 100 μg/mL streptomycin sulphate, and 250 ng/mL amphotericin B; all antibiotics and antimycotics were from Sigma, USA).The medium was replaced by a new one every two days. The Caco-2 cells were grown at standard conditions (37°C, 5% CO_2, 95% humidity) on cover slides. After three weeks, cells were washed in PBS buffer and transferred into the culture medium without antibiotic and antymycotic solution, then used for adhesion experiments. After the experiment, cells were detached from cover glass and dispersed using trypsin-EDTA solution (0.5% porcine trypsin and 0.2% EDTA in PBS, Sigma, USA), and then counted in Bürker hematocytometer chamber (Merck, USA).

2.3. Competition studies

These studies were performed on *L. paracasei* IBB2588 and *S. enterica* KOS1663 submitted together to the Caco-2 cell culture. Overnight bacteria cultures were 100-fold diluted, up to about 1–2 $\times 10^7$ bacteria/mL, mixed and added to Caco-2 cells for 2- or 4-hour incubation at 37°C. After trypsinization, the mixture of bacterial cells was diluted and plated on MRS plates (for *Lactobacillus*) and LB plates (for *Salmonella*) to estimate their CFUs. *The following variants of the competition study* were performed.

(i) Coincubation of *L. paracasei* IBB2588 and *S. enterica* KOS1663 strains with Caco-2 cells.

(ii) Coincubation of *L. paracasei* devoided of MRS broth and *S. enterica* with Caco-2 cells. In this experiment, *L. paracasei* overnight culture was centrifuged (5000 rpm/min), the spent culture supernatant (SCS) was removed, bacterial pellet resuspended in isotonic salt solution, and, together with *S. enterica*, incubated with Caco-2 cells.

(iii) Coincubation of *L. paracasei* total SCS (obtained in above described manner) and *S. enterica* with Caco-2 cells.

(iv) Coincubation of different fractions of *L. paracasei* SCS and *S. enterica* with Caco-2 cells. The total SCS of *L. paracasei* was distributed by centrifugation in test-tube filters (Millipore, USA) onto 4 fractions according to the size of molecules: fraction I- >30 kDa, fraction II-30–10 kDa, fraction III- 10–5 kDa, and fraction IV-<5 kDa. The *S. enterica* was incubated at 37°C for 2 and 4 hours with 100-fold diluted supplementation of each fraction.

(v) Preincubation of Caco-2 cells with fresh MRS broth and subsequent addition of *S. enterica*.

(vi) Preincubation of *L. paracasei* with the Caco-2 cells for 2 hours, washing out the nonadhered lactobacilli and administration of *S. enterica* for further 2 or 4 hours.

2.4. Calculations and statistical analysis

Bacterial adhesion was expressed as the number of bacteria attached to one Caco-2 cell. Data were analyzed by one-way

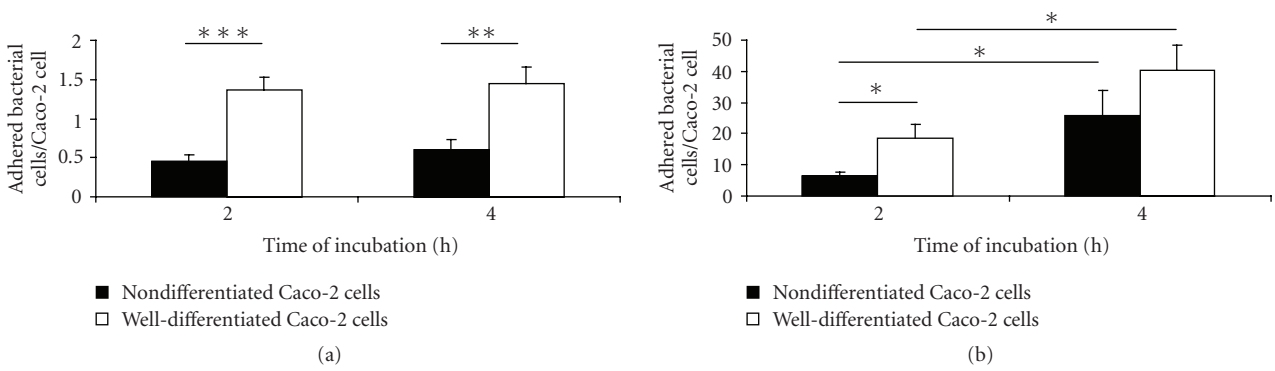

Figure 1: Adhesion of (a) *L. paracasei* and (b) *S. enterica* to nondifferentiated and well-differentiated Caco-2 cells in 2- and 4-hour experiments. Mean and SEM, *t*-test. Asterisks indicate the statistical differences: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

ANOVA followed by Tukey posthoc test, and unpaired *t*-test (GraphPad Prism v.3.0, GraphPad Software, San Diego, CA, USA) and shown as mean and SEM. In all statistical analyses, $P < .05$ was taken as the level of significance.

3. RESULTS

3.1. Adhesion of L. paracasei and S. enterica to Caco-2 cells

We studied the adhesion of five LAB strains (three *Lactobacillus* and two *Lactococcus*). These bacteria adhered to Caco-2 cells in the range of 0.5 to 5 bacteria per one cell. The adherence of *L. paracasei* to Caco-2 cells was low (Figure 1(a)). Only 0.6 bacteria adhered to one nondifferentiated (growing for 6 days) Caco-2 cells, and about 1.5 bacteria adhered to well-differentiated (growing for 21 days) cells. There was no significant difference between 2- and 4-hour incubation of *L. pracasei* with Caco-2. For competition experiments, the *L. paracasei* was selected since among all LAB strains tested *L. paracasei* was the only one adhering better to well-differentiated than to nondifferentiated Caco-2 cells exactly as pathogenic *S. enterica*. The adherence of *S. enterica* (Figure 1(b)) was high as compared to that of *L. pracasei* ($P < .01$). On the average, there were 6 and 25 bacteria per nondifferentiated Caco-2 cells after 2- and 4-hour incubation, respectively, and 18 and 40 bacteria per differentiated Caco-2 cells after 2- and 4-hour incubation, respectively (Figure 1(b)). Thus, the rule, the longer the contact the higher adhesion rate, was true for *S. enterica* but not for *L. pracasei*.

3.2. Competition between L. paracasei and S. enterica for adhesion to Caco-2 cells coincubation experiments

Coincubation of *L. paracasei* and *S. enterica* with Caco-2 cells for 2 and 4 hours resulted in about 4- and 2-fold decrease of *S. enterica* adhesion to Caco-2 cells, respectively, as compared to the experiment with incubation of *S. enterica* alone (Figures 2(b) versus 2(a) and Figures 3(b) versus 3)(a). A significant decrease ($P < .05$) in the number of *S. enterica* adhering to Caco-2 cells was also observed in a coincubation study when *L. paracasei* devoid of its growing medium and resuspended in salt solution was used (Figures 3(c) versus 3(a)). However, the decrease was statistically significant only after 4-hour incubation, and the effect was significantly weaker than that when *L. paracasei* was used with its incubation medium (Figures 2(c) versus 2(b) and Figures 3(c) versus 3(b)). The later indicated that not only *L. paracasei* by itself but also the substances secreted by *L. paracasei* to the medium might counteract the adhesion of *S. enterica* to Caco-2 cells. This was confirmed in the studies with the use of supernatant obtained after centrifugation of *L. paracasei* overnight culture (SCS). Four-hour incubation of *S. enterica* with SCS led to a significant decrease of adhesion of *S. enterica* to Caco-2 cells (Figures 3(d) versus 3(a)) while the 2-hour incubation did not show statically significant effect (Figures 2(d) versus 2(a)). The results of incubation of *S. enterica* with four separate *L. paracasei* SCS fractions are shown on Figures 2(e)–2(h). Besides fraction IV (<5 kDa), the presence of remaining fractions resulted in significant reduction of *S. enterica* adhesion to Caco-2 cells (Figures 3(e) versus 3(a), 3(f) versus 3(a) and 3(g) versus 3(a)). Among these fractions, the strongest inhibition was observed for fraction II (30–10 kDa). Fresh MRS-broth medium did not influence the adhesion of *S. enterica* to Caco-2 cells (data not shown).

3.3. Competition between L. paracasei and S. enterica for adhesion to Caco-2 cells pre-incubation experiments

Preincubation of *L. paracasei* with Caco-2 cells for 2 hours and subsequent addition of *S. enterica* resulted in over 2-fold stronger inhibition of *Salmonella* adherence than in coincubation experiment. This means that *Lactobacillus* inhibited *Salmonella* adherence 8- and 4-fold in pre- and coincubation experiment, respectively (Figures 2 and 4). The effect of preincubation was, however, statistically significant only in the 2-hour ($P < .05$) but not in the 4-hour coincubation experiment (Figure 4).

There were no statistically significant differences in *Salmonella* adherence to Caco-2 cells preincubated for 2 hours with *Lactobacillus* culture and to Caco-2 cells devoid of nonadhered *L. paracasei* cells (to get rid of nonadhered

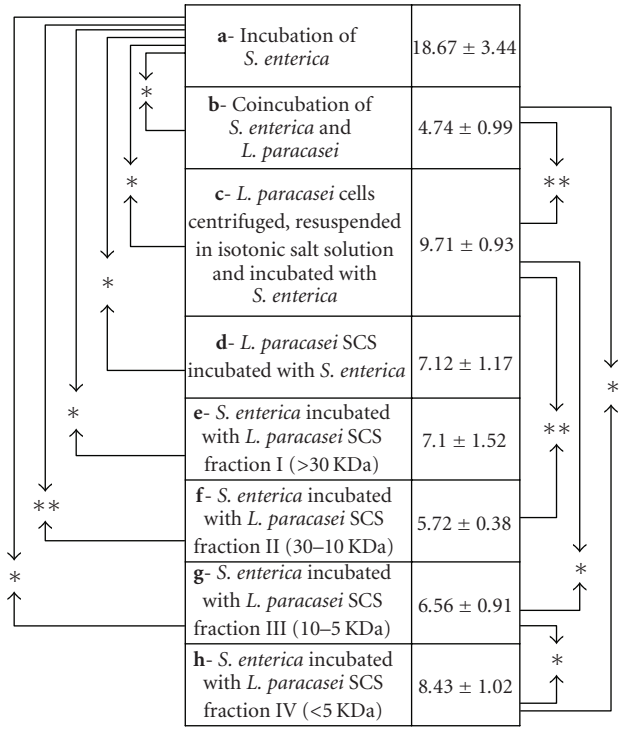

Figure 2: Two-hour coincubation experiments. Adhesion of *S. enterica* to Caco-2 cells under different conditions: (a) *S. enterica* was incubated with Caco-2 cells; (b) *S. enterica* and *L. paracasei* were coincubated; (c) *L. paracasei* overnight culture was washed with and resuspended in isotonic salt solution and coincubated with *S. enterica*; (d) *L. paracasei* overnight culture was centrifuged and spent supernatant (SCS) was incubated with *S. enterica*. *S. enterica* was incubated with SCS fractions: (e) I (>30 kDa); (f) II (30–10 kDa); (g) III (10–5 kDa); (h) IV (<5 kDa). Numbers represent the amount of adhered bacteria per 1 Caco-2 cell. Mean and SEM, *t*-test. Asterisks indicate the statistical differences: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

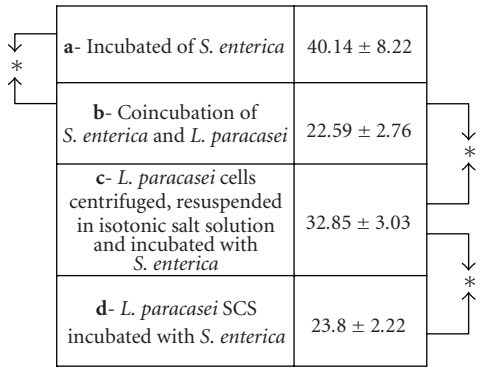

Figure 3: Four-hour coincubation experiments. Adhesion of *S. enterica* to Caco-2 cells under different conditions: (a) *S. enterica* was incubated with Caco-2 cells; (b) *S. enterica* and *L. paracasei* were coincubated; (c) *L. paracasei* overnight culture was washed with and resuspended in isotonic salt solution and coincubated with *S. enterica*; (d) *L. paracasei* overnight culture was centrifuged and spent supernatant (SCS) was incubated with *S. enterica*. Numbers represent the amount of adhered bacteria per 1 Caco-2 cell. Mean and SEM, *t*-test. Asterisks indicate the statistical differences: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

LAB after 2 hours of incubation, Caco-2 cells were washed with PBS).

In another type of preincubation experiment *L. paracasei* was incubated for 2 hours in DMEM (medium for Caco-2 cell culture), spoon down and the SCS was added to Caco-2 cell culture for 2 hours prior to *S. enterica* addition. Over 2-fold inhibition of *Salmonella* adhesion by this supernatant was observed (Figure 4).

4. DISCUSSION

Lactic acid bacteria of normal intestinal microbiota are known to counteract the pathogenic bacteria invasion. Such inhibitory effects of lactobacilli can be explained by a mechanism of nonspecific steric hindrance on the receptors for pathogens [21, 22]. Other inhibition mechanisms cannot be excluded, for example, some metabolic products from LAB, such as lactic acid, exopeptides, or exopolysaccharides, may inhibit the adherence of pathogenic bacteria.

In the present study, Caco-2 cell monolayer grown on the glass was used as a model for investigation of bacterial adhesion to the intestinal epithelium. Besides lack of mucus production, this model offers a number of interesting features ideal for investigation bacterial adherence. The nondifferentiated and well-differentiated Caco-2 cells were used as a model of nondifferentiated crypt and differentiated villi enterocytes, respectively [16–18]. The adhesion of pathogenic *S. enterica* KOS1663 to Caco-2 cells was examined in the presence of *L. paracasei* IBB2588 or secreted products of the LAB metabolism as a potential competitor or inhibitor. The examined salmonellae adhered to well-differentiated and to

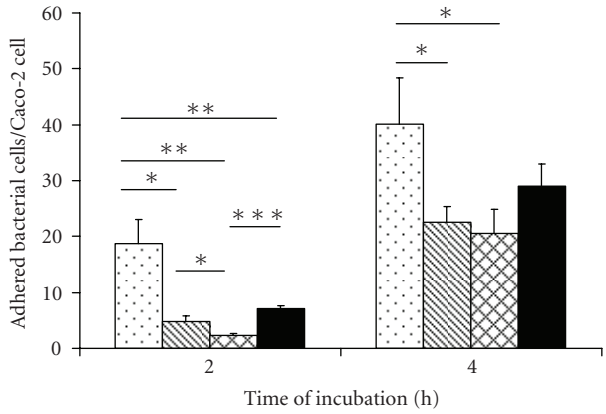

Figure 4: Adhesion of *S. enterica* to Caco-2 cells preincubated with *L. paracasei* or its culture spent supernatant (SCS) as indicated. Bars represent mean values with respective SEM. Asterisks indicate the statistical differences: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ (one-way ANOVA followed by Tukey posthoc test).

a lesser degree to nondifferentiated Caco-2 cells several times better than the lactobacilli (Figure 1). Ours [23] and others [24] studies have shown that the colonization of lactobacilli takes place mainly in the intestinal crypts, whereas the pathogenic bacteria encounter mostly the upper part of intestinal villi and thus adhere to well-differentiated enterocytes (i.e., expressing the brush border). Among 5 LAB strains tested, the *L. paracasei* IBB2588 was the only one that preferred to attach to well-differentiated Caco-2 cells (this strain attached to well-differentiated cells 2.5-fold better than to nondifferentiated). Most probably these bacteria, similarly to *S. enterica*, use receptor-type adhesion mechanism and receptors are better developed in well-differentiated cells. This feature of *L. paracasei* IBB2588 seemed to create conditions for stronger inhibitory effect, if present, on pathogen adhesion.

Our studies have shown that in the presence of *L. paracasei*, *S. enterica* adhered to Caco-2 cells with a 4-fold lower efficiency, and the adhesion was further reduced (8-fold) when *L. paracasei* was preincubated with Caco-2 cells prior to *Salmonella* addition. These results are especially impressive if one takes in consideration several-fold lower adhesion ability of *L. paracasei* in comparison to *Salmonella*. This finding also indicates that the phenomenon of adherence inhibition may involve not only competition for eukaryotic cell receptors but also an action of produced or secreted by *L. paracasei* antimicrobial compound(s). We have shown that *Lactobacillus* SCS and the bacteria devoid of SCS inhibit the adhesion of *Salmonella,* nevertheless, the effect was weaker than that of complete *Lactobacillus* culture containing bacteria in its growing medium with side products of bacterial metabolism. This indicates that both, *L. paracasei* cells and some bacterial products not defined in this study may act as inhibitors of *S. enterica* adhesion. Substances produced by lactobacilli show also other futures. Coconnier-Polter et al. have found that cell-free culture supernatant of *L. acidophilus* LB decreases intracellular ATP in *S. enterica* SL1344. It also releases lipopolysaccharide, increases permeability of the bacterial membrane and the sensitivity of *Salmonella* to sodium dodecyl sulfate [25]. The same authors have shown that *L. acidophilus* culture supernatant inhibits adhesion-dependent *Salmonella*-induced interleukin-8 production [6].

Several studies have been done on the nature of the secreted by *Lactobacillus* antibacterial substances. There are data indicating that the inhibitory molecules are of low molecular weight (not exciding 3 kDa) [26] or even as small as acting through pH modification lactic acid [27]. In our experimental system, this seems not to be the case, since the pH of Caco-2 cell medium was monitored during each experiment and it was stable in the range 7.2–7.5 due to the buffering properties of the medium. Moreover, we found that the SCS fraction containing molecules within a range 30–10 kDa produced the strongest inhibition of *S. enterica*. This suggests the relevance of some substance(s) much larger than the lactic acid. This is in agreement with other group of data that indicate on peptides as antimicrobial factors [28] or synergistic action of lactic acid and proteinaceous substances [29]. Recapitulate, it seems that individual LAB (probiotic) strains produce different and of diverse mechanisms of action antibacterial substances characteristics for a particular strain.

The important feature of the described here competition phenomenon is its transitory character. The strongest adhesion inhibition was observed in 2-hour experiment. Longer, 4-hour coincubation of *L. paracasei* and *S. enterica* led to a partial restoration of the pathogen adhesion to Caco-2 cells (Figures 3 and 4).

The ability of selected strains of *Lactobacillus* (probiotics) to inhibit the adhesion of pathogenic bacteria is highly specific, and depends on both the probiotic and pathogen strain [30, 31]. This indicates the need of a case-by-case characterization of the probiotic strains. Except specific antibacterial substances produced by LAB, the inhibition of adhesion could be related to the presence of specific adhesion molecules and receptors for which probiotic and pathogen are competing. It has to be taken under consideration that observed in vitro inhibitory effect of of probiotics on pathogen adhesion has to be confirmed in vivo.

REFERENCES

[1] A. C. Ouwehand and S. Vesterlund, "Health aspects of probiotics," *IDrugs*, vol. 6, no. 6, pp. 573–580, 2003.

[2] L. Baccigalupi, A. Di Donato, M. Parlato, et al., "Small surface-associated factors mediate adhesion of a food-isolated strain of *Lactobacillus fermentum* to Caco-2 cells," *Research in Microbiology*, vol. 156, no. 7, pp. 830–836, 2005.

[3] F. A. Sylvester, D. Philpott, B. Gold, A. Lastovica, and J. F. Forstner, "Adherence to lipids and intestinal mucin by a recently recognized human pathogen, *Campylobacter upsaliensis*," *Infection and Immunity*, vol. 64, no. 10, pp. 4060–4066, 1996.

[4] E. M. Lehto and S. J. Salminen, "Inhibition of *Salmonella typhimurium* adhesion to Caco-2 cell cultures by *Lactobacillus* strain GG spent culture supernate: only a pH effect?" *FEMS Immunology & Medical Microbiology*, vol. 18, no. 2, pp. 125–132, 1997.

[5] M.-H. Coconnier, M.-F. Bernet, S. Kernéis, G. Chauvière, J. Fourniat, and A. L. Servin, "Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by *Lactobacillus acidophilus* strain LB decreases bacterial invasion," *FEMS Microbiology Letters*, vol. 110, no. 3, pp. 299–305, 1993.

[6] M.-H. Coconnier, V. Lievin, M. Lorrot, and A. L. Servin, "Antagonistic activity of *Lactobacillus acidophilus* LB against intracellular *Salmonella enterica* serovar Typhimurium infecting human enterocyte-like Caco-2/TC-7 cells," *Applied and Environmental Microbiology*, vol. 66, no. 3, pp. 1152–1157, 2000.

[7] A. L. Servin, "Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens," *FEMS Microbiology Reviews*, vol. 28, no. 4, pp. 405–440, 2004.

[8] G. Zárate and M. E. Nader-Macias, "Influence of probiotic vaginal lactobacilli on in vitro adhesion of urogenital pathogens to vaginal epithelial cells," *Letters in Applied Microbiology*, vol. 43, no. 2, pp. 174–180, 2006.

[9] D. J. Stickler, J. C. Lear, N. S. Morris, et al., "Observations on the adherence of *Proteus mirabilis* onto polymer surfaces," *Journal of Applied Microbiology*, vol. 100, no. 5, pp. 1028–1033, 2006.

[10] P. V. Kirjavainen, A. C. Ouwehand, E. Isolauri, and S. J. Salminen, "The ability of probiotic bacteria to bind to human intestinal mucus," *FEMS Microbiology Letters*, vol. 167, no. 2, pp. 185–189, 1998.

[11] Y.-L. Ma, T. Guo, Z.-R. Xu, P. You, and J.-F. Ma, "Effect of *Lactobacillus* isolates on the adhesion of pathogens to chicken intestinal mucus in vitro," *Letters in Applied Microbiology*, vol. 42, no. 4, pp. 369–374, 2006.

[12] S. Vesterlund, J. Paltta, M. Karp, and A. C. Ouwehand, "Adhesion of bacteria to resected human colonic tissue: quantitative analysis of bacterial adhesion and viability," *Research in Microbiology*, vol. 156, no. 2, pp. 238–244, 2005.

[13] J. Fogh, J. M. Fogh, and T. Orfeo, "One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice," *Journal of the National Cancer Institute*, vol. 59, no. 1, pp. 221–226, 1977.

[14] G. Chauvière, M.-H. Coconnier, S. Kernéis, A. Darfeuille-Michaud, B. Joly, and A. L. Servin, "Competitive exclusion of diarrheagenic *Escherichia coli* (ETEC) from human enterocyte-like Caco-2 cells by heat-killed *Lactobacillus*," *FEMS Microbiology Letters*, vol. 70, no. 3, pp. 213–217, 1992.

[15] G. Chauvière, M.-H. Coconnier, S. Kernéis, J. Fourniat, and A. L. Servin, "Adhesion of human *lactobacillus acidophilus* strain LB to human enterocyte-like Caco-2 cells," *Journal of General Microbiology*, vol. 138, part 8, pp. 1689–1696, 1992.

[16] J. D. Greene and T. R. Klaenhammer, "Factors involved in adherence of lactobacilli to human Caco-2 cells," *Applied and Environmental Microbiology*, vol. 60, no. 12, pp. 4487–4494, 1994.

[17] H.-P. Hauri, E. E. Sterchi, D. Bienz, J. A. Fransen, and A. Marxer, "Expression and intracellular transport of microvillus membrane hydrolases in human intestinal epithelial cells," *Journal of Cell Biology*, vol. 101, no. 3, pp. 838–851, 1985.

[18] M. Pinto, S. Robine-Leon, M. D. Appay, et al., "Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture," *Biology of the Cell*, vol. 47, no. 3, pp. 323–330, 1983.

[19] C. N. Jacobsen, V. Rosenfeldt Nielsen, A. E. Hayford, et al., "Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans," *Applied and Environmental Microbiology*, vol. 65, no. 11, pp. 4949–4956, 1999.

[20] C. Solano, B. Sesma, M. Alvarez, et al., "Virulent strains of *Salmonella enteritidis* disrupt the epithelial barrier of Caco-2 and HEp-2 cells," *Archives of Microbiology*, vol. 175, no. 1, pp. 46–51, 2001.

[21] M.-F. Bernet, D. Brassart, J.-R. Neeser, and A. L. Servin, "Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions," *Applied and Environmental Microbiology*, vol. 59, no. 12, pp. 4121–4128, 1993.

[22] M.-F. Bernet, D. Brassart, J.-R. Neeser, and A. L. Servin, "*Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria," *Gut*, vol. 35, no. 4, pp. 483–489, 1994.

[23] D. Laubitz, A. Jankowska, W. Kazimierczak, et al., "The influence of intestinal myoelectrical activity-related electric fields of adhesion of bacteria to Caco-2 cells," *Bioelectromagnetics*. in press.

[24] G. K. Collington, I. W. Booth, M. S. Donnenberg, J. B. Kaper, and S. Knutton, "Enteropathogenic *Escherichia coli* virulence genes encoding secreted signalling proteins are essential for modulation of Caco-2 cell electrolyte transport," *Infection and Immunity*, vol. 66, no. 12, pp. 6049–6053, 1998.

[25] M.-H. Coconnier, V. Liévin-Le Moal, and A. L. Servin, "A *Lactobacillus acidophilus* strain of human gastrointestinal microbiota origin elicits killing of enterovirulent *Salmonella enterica* serovar Typhimurium by triggering lethal bacterial membrane damage," *Applied and Environmental Microbiology*, vol. 71, no. 10, pp. 6115–6120, 2005.

[26] C. Forestier, C. De Champs, C. Vatoux, and B. Joly, "Probiotic activities of *Lactobacillus casei rhamnosus*: in vitro adherence to intestinal cells and antimicrobial properties," *Research in Microbiology*, vol. 152, no. 2, pp. 167–173, 2001.

[27] A. Darfeuille-Michaud, D. Aubel, G. Chauvière, et al., "Adhesion of enterotoxigenic *Escherichia coli* to the human colon carcinoma cell line Caco-2 in culture," *Infection and Immunity*, vol. 58, no. 4, pp. 893–902, 1990.

[28] M. Hayes, R. P. Ross, G. F. Fitzgerald, C. Hill, and C. Stanton, "Casein-derived antimicrobial peptides generated by *Lactobacillus acidophilus* DPC6026," *Applied and Environmental Microbiology*, vol. 72, no. 3, pp. 2260–2264, 2006.

[29] P. K. Gopal, J. Prasad, J. Smart, and H. S. Gill, "In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxic *Escherichia coli*," *International Journal of Food Microbiology*, vol. 67, no. 3, pp. 207–216, 2001.

[30] M. Gueimonde, L. Jalonen, F. He, M. Hiramatsu, and S. Salminen, "Adhesion and competitive inhibition and displacement of human enteropathogens by selected lactobacilli," *Food Research International*, vol. 39, no. 4, pp. 467–471, 2006.

[31] Y.-K. Lee, K.-Y. Puong, A. C. Ouwehand, and S. Salminen, "Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli," *Journal of Medical Microbiology*, vol. 52, no. 10, pp. 925–930, 2003.