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Effect of Lactobacilli on *E. coli* Adhesion to Caco-2 Cells *in Vitro*

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ABSTRACT. Inhibitory effect of various lactobacilli against pathogenic strains of *E. coli* in model system Caco2 cells was determined by enumerating the number of adhering *E. coli* after pre-incubation (exclusion), post-incubation (displacement) or co-incubation (competition) with lactobacilli. Porcine *E. coli* strain F107 (F18ab, Stx2v) in the competition assay with porcine lactobacillus strain P10 gave bacterial counts 7.25 (log CFU per well); in the exclusion test it was only 7.05 while in displacement test it reached 7.29. The lowest *E. coli* counts adhering to Caco-2 cells were in exclusion assay (pre-incubation, *Lactobacillus* inoculated as the first). Pre-treatment of *E. coli* with our lactobacilli strains reduced the cultivable *E. coli* numbers.

Pathogenic strains of *E. coli* are a major cause of bacterial diarrhea in humans and animals (Vu-Khac *et al*. 2004; Holoda *et al*. 2004). Lactobacilli and other commensal bacteria (*e.g*., some bifidobacteria, enterobacteria) provide the protection of the host against possible colonization by the pathogenic bacteria (Reid *et al*. 1998; Mego *et al*. 2005) by inhibiting the pathogen–cell association and invasion (Chauviere *et al*. 1992; Coconier *et al*. 1992; Bujňáková *et al*. 2004). Lactobacilli are believed to interfere with pathogens by different mechanisms, *e.g*., by the production of antimicrobial compounds, such as lactic acid, dihydrogen peroxide, or bacteriocin-like substances (Mc-Croarty *et al.* 1988); some of the *Lactobacillus* strains co-aggregate with pathogenic bacteria (Kmeť *et al*. 1999; Bujňáková and Kmeť 2002; Bujňáková *et al*. 2004).

The important criteria for a prospective probiotic strain are the ability to adhere to mucosal surfaces of the gastrointestinal tract (Bernet *et al*. 1993). As the studies of colonization and inhibitory effect of commensal bacteria *in vivo* are expensive and time-consuming, *in vitro* methods are required for the selection of promising strains (Strompfová *et al*. 2004). Enterocyte-like Caco-2 cells were successfully used for *in vitro* studies of the mechanism of cellular adhesion of nonpathogenic lactobacilli (Chauviere *et al*. 1992; Tuomola *et al*. 1998). Here we examine the inhibition of adhesion provided by four selected lactobacilli strains against known pathogenic strains of *E. coli*.

MATERIALS AND METHODS

Bacterial strains, growth conditions and Caco-2 cell lines. *Lactobacillus* spp. strains were isolated from pig feces (strains 3, 10), poultry (strain STL 2/1) and human vagina (HV 28) and grown in Rogosa agar (*Oxoid*) in 100 % CO_2 at 37 °C. *E. coli* strains were: 602/2, S44 (fedA-positive, Sta) obtained from pig post-weaning diarrhea, F107 (Stx2v, F18ab), and KRS (human aerobactine-positive strain). The Caco-2 cell lines were grown in DMEM (Matijasic *et al*. 2003) containing L-glutamine and 10 % fetal calf serum (Feta-clone II; *Hyclone*, Germany) and antibiotics. Monolayers of Caco-2 cells were seeded at a concentration of 1.6×10^6 cells per cm^2 in 24-well Nunc tissue plates (*PolyLabo*, France). Maintenance of cells and all experiments with cell lines were carried out in an atmosphere of 5 % CO_2 at 37 °C.

Adherence inhibition assay. Three different procedures were used in order to differentiate between exclusion, competition or displacement of the pathogens by lactobacilli. Exclusion test: Caco-2 cell monolayers were cultured with lactobacilli (10^8 CFU/mL) for 1 h. Nonadhering lactobacilli were removed by washing with phosphate-buffered saline; then *E.* coli (10^8 CFU/mL) was added and incubated for another 1 h. For competition test, lactobacilli and *E. coli* were incubated simultaneously for 1 h. For displacement tests, the pathogenic *E. coli* were incubated with monolayers for 1 h and, after removing nonadhering pathogens, lactobacilli were added and incubation was continued for another 1 h. The number of bacteria adhering to the intestinal cell was determined by the macrodilution agar method.

RESULTS AND DISCUSSION

The results of interaction experiment between pathogenic *E. coli* and lactobacilli are shown in Table I. KRS strain was incubated together with strain HV 28; the number of adhering *E. coli* strain KRS was lower in exclusion test (7.19 ± 0.57 log CFU) in comparison with displacement (7.61 ± 0.25 log CFU) or competition test (7.64 ± 0 log CFU). Porcine pathogenic *E. coli* strains 602/2 and S44 were suppressed by lactobacilli isolated from pig feces (strains 3, 10), values of adhering *E. coli* were the lowest in the exclusion test (6.78 ± 0.37 log CFU for strain 602/2, and 7.32 ± 0.13 for S44). Pre-incubation (exclusion) of *Lactobacillus* strain STL 2/1 produced the highest reduction in *E. coli* F107 counts (7.05 ± 0.08 log CFU) comparing with co-incubation (competition) 7.44 ± 0.22 and post-incubation (displacement) 7.29 ± 0.01. Pre-incubation of lactobacilli decreased the counts of adhering pathogenic *E. coli* in all four cases.

Table I. The adhesion[a] of four *E. coli* pathogenic strains (KRS, 602/2, S44, F107) to Caco-2-cell lines in simultaneous incubation (competition; Com), pre-incubation (exclusion; Exc) and post-incubation (displacement; Dis) with strains of *Lactobacillus* spp.

Strain[b]		Com[c]	Exc[d]	Dis[e]
E. coli	lactobacilli			
KRS	HV 28	7.64 ± 0	7.19 ± 0.57	7.61 ± 0.25
602/2	3, 10	6.83 ± 0.04	6.78 ± 0.37	7.03 ± 0.01
S44	STL 2/1	7.44 ± 0.22	7.32 ± 0.13	7.62 ± 0.09
F107	3, 10	7.25 ± 0.02	7.05 ± 0.08	7.29 ± 0.01

[a]Total number of *E. coli* (log CFU ± SD per well) associated with Caco-2 cells.
[b]For strain characteristics *see Materials and Methods*.
[c]Competition test: *E. coli* and *Lactobacillus* spp. were applied to Caco-2 cells at the same time.
[d]Exclusion test: *Lactobacillus* spp. was applied first, followed by *E. coli.*
[e]Displacement test: *E. coli* was applied first, followed by *Lactobacillus* spp.

Pre-treatment of *E. coli* with our lactobacilli strains reduced the cultivable *E. coli* numbers. Our findings confirm that good adhesion to intestinal epithelial cells is necessary for health benefit of probiotic strains.

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