# In vitro effect of vaginal lactobacilli on the growth and adhesion abilities of uropathogenic Escherichia coli

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ORIGINAL PAPER

# In vitro effect of vaginal lactobacilli on the growth and adhesion abilities of uropathogenic *Escherichia coli*

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**Abstract** *Escherichia coli* is one of the main causes of uncomplicated urinary tract infections and responsible of vaginal infections. Lactobacilli can inhibit this pathogen by the production of antimicrobial substances as organic acids, hydrogen peroxide and/or bacteriocins. The aim of this work was to study the effects of beneficial vaginal lactobacilli on *E. coli* through in vitro experiments. The inhibitory activity of three vaginal *Lactobacillus* strains against *E. coli* was assessed using the agar plate diffusion. Moreover, the effect of *Lactobacillus reuteri* CRL (Centro de Referencia para Lactobacilos Culture Collection) 1324 on the adhesion and internalization capabilities of *E. coli* was studied on HeLa cells. Two *Lactobacillus* strains inhibited the growth of the pathogens by production of organic acids. *L. reuteri* CRL 1324 reduced the adhesion and internalization of *E. coli* 275 into HeLa cells. The results obtained suggest that *L. reuteri* CRL 1324 can be considered as a probiotic candidate for further in vivo studies for the prevention or treatment of urinary tract infections caused by *E. coli*.

**Keywords** *Lactobacillus* · *Escherichia coli* · Organic acids · Adhesion · Internalization

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## Introduction

Urinary tract infections (UTI) are one of the most common infectious diseases worldwide and represent a very high economic cost to the public health system (Pitout and Laupland 2008). *Escherichia coli* is one of the most frequently isolated pathogen in nosocomial and community-acquired bacterial infections, including urinary tract, intestinal and systemic infections in humans (Pitout 2013), while uropathogenic *Escherichia coli* (UPEC) is the main cause of uncomplicated urinary tract infections. UPEC presents different virulence factors including adhesion determinants, motility, acquisition of metals, production of toxins and immune evasion (Mobley et al. 2009; Nielubowicz and Mobley 2010; Ejrnaes et al. 2011). Furthermore, some UPEC strains are able to generate intracellular bacterial communities and quiescent intracellular stores (Mysorekar and Hultgren 2006; Anderson et al. 2010). On the other hand, *E. coli* is associated with vaginal infection, such as vaginitis (Razzak et al. 2011).

The standard therapies for UTI include different antibiotics, which can alter the autochthonous microbiota and generate resistant strains (Beerepoot et al. 2012). Thus, alternative strategies for their treatment are urgently required because of the high level of resistance of microorganisms to conventional antimicrobials (Geerlings et al. 2014). In women with recurrent UTI, which often show persistent vaginal colonization with *E. coli* (Johnson and Russo 2005), the absence of *Lactobacillus* strains producing hydrogen peroxide appears to be determinant in the pathogenesis of recurrent UTI, facilitating the colonization with *E. coli* (Gupta et al. 1998; Atassi and Servin 2010).

The normal vaginal microbiome is dominated by lactobacilli (Human Microbiome Project Consortium 2012; Witkin 2015). The use of probiotic products containing vaginal

lactobacilli has been proposed as an alternative treatment against infections by *E. coli* (Stapleton et al. 2011). Probiotics are defined as "live microorganisms which, when administered in adequate amounts, exert a beneficial physiological effect in the host health" (FAO/WHO 2001; Hill et al. 2014). Stapleton et al. (2011) reported the association of a reduction in recurrent urinary tract infection by the use of Lactin-V (*Lactobacillus crispatus* intravaginal suppository probiotic) after the standard treatment for cystitis.

Probiotics can exert beneficial effects by different mechanisms, such as production of antimicrobial substances (organic acids, hydrogen peroxide and bacteriocins) (Nader-Macías et al. 2008; Martín and Suárez 2010; Mirmonsef et al. 2012; Stoyancheva et al. 2014) and biosurfactants (Gudiña et al. 2010), modulation of immune system (Oelschlaeger 2010; Joo et al. 2011; De Gregorio et al. 2016) and biofilm formation (Martín et al. 2008; Leccese Terraf et al. 2012). Colonization capability, including adhesion to epithelial cells or mucosal surfaces that contribute to the beneficial effect through the time, is also one of the mechanisms suggested for probiotics (Nader-Macías et al. 2008; Bouchard et al. 2015). The aims of this work were: to evaluate the susceptibility of *E. coli* to antagonistic substances produced by lactobacilli, to determine the *Lactobacillus reuteri* CRL 1324 ability of adhesion to and internalization into human cervical HeLa cells, and to assess the *L. reuteri* CRL 1324 capability to counteract UPEC adhesion to and internalization into human cervical HeLa cells.

## Materials and methods

### Bacterial strains and culture conditions

Three vaginal *Lactobacillus* strains and two *E. coli* strains were used (Table 1). The three *Lactobacillus* strains were previously selected for their in vitro beneficial characteristics, such as production of hydrogen peroxide, inhibition of urogenital pathogens, auto-aggregation, adhesion to mucin and ability to form biofilm in culture media without Tween 80 (Juárez Tomás et al. 2005; Juárez Tomás et al. 2011; Leccese Terraf et al. 2012; Leccese Terraf et al. 2014). Lactobacilli were stored in yeast extract milk at −20 °C and sub-cultured twice in De Man–Rogosa–Sharpe (MRS) broth (De Man et al. 1960), for 12 h at 37 °C, under static conditions. A third sub-culture was performed in MRS and MRS without Tween 80 (MRS-T) and incubated for 12 h at 37 °C (Leccese Terraf et al. 2012). MRS-T was used because this medium favored biofilm formation and allowed the growth of most of the *Lactobacillus* strains evaluated (Leccese Terraf et al. 2012).

*Escherichia coli* strains were isolated from human urine samples in Tucumán, Argentina, and identified by standard phenotypic techniques (Table 1). These microorganisms were stored at −20 °C in BHI (Brain Heart Infusion, composition in g/l: 200 infusion brain, 250 heart infusion, 10 peptone, 5NaCl, 2 glucose and 2.50 $Na_2HPO_4$) added with 20% glycerol. Before the experiments, each *E. coli* strain was sub-cultured in BHI broth for 12 h at 37 °C, under aerobic conditions without agitation. A second sub-culture was performed in BHI or LAPTg broth (Raibaud et al. 1973) prior to use.

### Susceptibility of *E. coli* to antagonistic substances produced by vaginal lactobacilli

The antagonistic effect of the culture supernatants of vaginal lactobacilli on uropathogenic *E.coli* was determined by the diffusion technique on agar plate (Juárez Tomás et al. 2011). *Lactobacillus* supernatants (from the third sub-culture), obtained by centrifugation (6000×*g*, 10 min) were divided into three fractions. One fraction was assayed without treatment (crude supernatant), the second was neutralized with NaOH (2 mol/l), and the third one was neutralized and treated with catalase (1000 U/ml). Crude and treated supernatants (25 µl) were seeded in agar plate holes (4 mm) containing the different *E. coli* strains at three concentrations (Table 2). Each one of the *E. coli* strains was plated in BHI-1% agar and LAPTg-1% agar.

**Table 1** *Lactobacillus* and *E. coli* strains used in this work

| *Lactobacillus* strains | Source of strains |
|---|---|
| *Lactobacillus reuteri* CRL 1324 | Isolated from human vagina (Ocaña et al. 1999a, b) |
| *Lactobacillus rhamnosus* CRL 1332 | |
| *Lactobacillus gasseri* CRL 1263 | |
| Escherichia coli strains | Source of strains |
| *Escherichia coli* 275 | Isolated from a patient with cystitis |
| *Escherichia coli* 36 | Isolated from a patient with pyelonephritis |

*CRL* from the Centro de Referencia para Lactobacilos Culture Collection

**Table 2** Growth inhibition of *E. coli* strains by vaginal lactobacilli with beneficial properties

| Culture media for lactobacilli[a] | | | Vaginal *Lactobacillus* strains | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *L. reuteri* CRL 1324 | | *L. rhamnosus* CRL 1332 | | *L. gasseri* CRL 1263 | |
| | | | MRS | MRS-T | MRS | MRS-T | MRS | MRS-T |
| | | pH[b] | 4.27 | 4.58 | 4.08 | 5.08 | 4.68 | 5.42 |
| *E. coli* strains | Culture media[c] | Inoculum | Inhibitory halos in mm[d] | | | | | |
| 36 | LAPTg | $3.21\times10^8$ | 7.5 | 8 | 8 | 0 | 0 | 0 |
| | LAPTg | $3.21\times10^7$ | 6.5 | 6.5 | 8 | 0 | 0 | 0 |
| | LAPTg | $1.61\times10^7$ | 8 | 6.5 | 8 | 0 | 0 | 0 |
| | BHI | $3.21\times10^8$ | 5 | 0 | 6.5 | 0 | 0 | 0 |
| | BHI | $3.21\times10^7$ | 5 | 5 | 5.5 | 0 | 0 | 0 |
| | BHI | $1.61\times10^7$ | 0 | 5 | 5 | 0 | 0 | 0 |
| 275 | LAPTg | $3.10\times10^7$ | 5.5 | 5.5 | 6.5 | 0 | 0 | 0 |
| | LAPTg | $3.10\times10^6$ | 7 | 7 | 10 | 0 | 0 | 0 |
| | LAPTg | $1.55\times10^6$ | 10 | 8 | 9.5 | 0 | 0 | 0 |
| | BHI | $3.10\times10^7$ | 4 | 3 | 4 | 0 | 0 | 0 |
| | BHI | $3.10\times10^6$ | 5 | 3 | 6 | 0 | 0 | 0 |
| | BHI | $1.55\times10^6$ | 5 | 4 | 6 | 0 | 0 | 0 |

[a]Culture media used for the growth of vaginal lactobacilli: MRS and MRS without Tween 80 (MRS-T)

[b]Final pH of *Lactobacillus* cultures after 12 h incubation in MRS broth and MRS-T, at 37 °C.

[c]Culture media used for the growth of *E. coli* strains: LAPTg and BHI broths

[d]Halos of inhibition (in mm) in agar plates produced by untreated supernatants of vaginal lactobacilli. Each value represents the average value of repetitions performed on different days

The plates were incubated for 5 h at room temperature and then for 24 h at 37 °C. The inhibition halos of the pathogen growth originated by the *Lactobacillus* supernatants were determined as millimeters.

## HeLa cells assay

### *Bacterial culture conditions*

*L. reuteri* CRL 1324 was used to assess the in vitro inhibitory capability against *E. coli* 275 infection in eukaryotic HeLa cells. Bacteria from the third sub-culture in MRS were washed once with phosphate-buffered saline (PBS) and resuspended at different concentrations in Dulbecco's modified Eagle's medium (DMEM; pH 7.4; D. Dutscher, Brumath, France). Bacterial numbers were estimated by a VWR V-1200 spectrophotometer at 650 nm (VWR, France) and by the viable cell numbers using a micro method previously described (Baron et al. 2006). The *E. coli* 275 population (in CFU/ml) was determined on MacConkey agar plates after 24 h of incubation at 37 °C. The *L. reuteri* CRL 1324 viable cells number was counted on MRS agar plates (pH 5.4) incubated for 48 h at 37 °C.

### *HeLa cells and culture conditions*

HeLa cells were cultured in T75 cell culture flasks using DMEM medium containing 10% heat-inactivated fetal calf serum (FCS). Cells were incubated at 37 °C in a humidified incubator with 5% $CO_2$. The HeLa cells were cultured to obtain a confluent monolayer, treated with 0.05% trypsin (Gibco-BRL, Grand Island, NY) and resuspended in fresh medium at a concentration of $2\times10^5$ cells/ml. Cells were counted in a Malassez chamber. For adhesion and internalization assays, cells were then seeded in 12-well plates ($2\times10^5$ cells/well) and incubated overnight at 37 °C in 5% $CO_2$ to obtain a confluent monolayer.

### *Adhesion assays*

The adhesion assays were adapted from Almeida et al. (1996) and Bouchard et al. (2013), and modified as follows. Confluent monolayers of HeLa cells ($2.5\times10^5$ cells/well) were washed four times with PBS and incubated at 37 °C in 5% $CO_2$ with 1 ml of *E. coli* 275 suspension in DMEM at $2.5\times10^7$ CFU/ml to achieve a 100:1 multiplicity of infection (MOI; ratio of *E. coli* cells to HeLa cells). Adhesion assays with *L. reuteri* CRL 1324 were performed by adding 1 ml of *L. reuteri* at $1.0\times10^8$ CFU/ml or $5.0\times10^8$ CFU/ml

to achieve a 400:1 or 2000:1 ratio of interaction (ROI; ratio of *L. reuteri* cells organisms to HeLa cells). Adhesion of *E. coli* and *L. reuteri* was quantified at 1 h post-infection.

For the inhibition of adhesion assays, HeLa cells were incubated with *L. reuteri* CRL 1324 at a ROI of 400:1 or 2000:1 and *E. coli* 275 (MOI 100:1) for 1 h at 37 °C with 5% $CO_2$. After the incubation steps, HeLa monolayers were washed four times with PBS and treated with 0.05% trypsin for 10 min at 37 °C. HeLa cells were centrifuged for 5 min at 800×*g* and lysed using 100 µl of 0.01%Triton in sterile water. The population of bacteria adhered (CFU/ml) was determined using the micromethod as described before.

The adhesion assay of *E. coli* was used as a reference. Adhesion rates were defined as the adhered *E. coli* population in the presence of *L. reuteri* referred to the adhered *E. coli* population in the experimental procedure.

*Internalization assays*

Internalization assays were adapted from Almeida et al. (1996) and Bouchard et al. (2013) and modified as follows. Confluent monolayers of HeLa cells ($2.5 \times 10^5$ cells/well) were washed twice with PBS and incubated in 5% $CO_2$ at 37 °C with 1 ml of *E. coli* 275 and/or *L. reuteri* CRL 1324 suspension in DMEM, at a MOI of 100:1 for *E. coli* and a ROI of 400:1 or 2000:1 for *L. reuteri* CRL 1324. *E. coli* 275 and *L. reuteri* CRL 1324 internalization was quantified 2 h post-infection. For the inhibition of internalization assays, *L. reuteri* CRL 1324 and *E. coli* were simultaneously added to HeLa cells for 2 h.

*E. coli* and *L. reuteri* CRL 1324 internalization was quantified at 2 h post-infection following an additional 2 h incubation step with DMEM supplemented with gentamicin (100 µg/ml). This step produced the killing of extracellular bacteria and allowed only the quantification of the internalized bacterial population. Subsequently, HeLa monolayers were washed four times with PBS, treated with trypsin, centrifuged for 5 min at 800×*g*, and lysed in 0.01% Triton. *E. coli* and *L. reuteri* populations were determined as described above.

The internalization assay of *E. coli* was used as a reference. Internalization rates were then defined as the internalized *E. coli* population in the presence of *L. reuteri* CRL 1324 referred to the internalized *E. coli* population in the experimental protocol.

*Statistical analysis*

Each experiment was done in triplicate (biological repeats). Statistical analysis was performed using R software (R development Core Team 2013). The differences in adhesion and internalization capacities of *E. coli* in the presence of *L. reuteri* were assessed using one-way analysis of variance, considering as statistically significant a $p$ value lower than 0.05.

## Results and discussion

### Susceptibility of *E. coli* to antagonistic substances by lactobacilli

The crude supernatants from *L. reuteri* CRL 1324 and *L. rhamnosus* CRL 1332 cultures inhibited the growth of UPEC strains, as shown in Table 2. *L. reuteri* CRL 1324 supernatants produced higher zones of inhibition when the pathogen was grown in LAPTg (halo size = 5.5–10 mm) instead of BHI (halo size = 0–5 mm). In general, the inhibition produced by *L. reuteri* CRL 1324 on the pathogens grown in LAPTg was higher when the inoculum of the pathogen in agar plates was lower. Similar inhibition patterns were obtained when *L. reuteri* CRL 1324 was grown in MRS and MRS-T. The *L. rhamnosus* CRL 1332 supernatants from MRS inhibited *E. coli* growth and the inhibition was higher when the pathogen was cultured in LAPTg. In the present work, the neutralized and catalase-treated supernatants did not show the inhibition of the pathogen, indicating that the inhibitory effect is by the production of organic acids (data not shown). *L. gasseri* CRL 1263 did not inhibit any of the two *E. coli* strains assayed.

The results reported in this work are in partial agreement with those published by several authors reporting that the inhibition of UPEC by lactic acid bacteria (LAB) isolated from human vagina is related to lactic acid production. However, production of hydrogen peroxide by these LAB strains was frequently published. Cadieux et al. (2009) have evidenced that the culture supernatants of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 (isolated from human vagina and distal urethra and vagina, respectively) inhibited the growth of UPEC C1212 by lactic acid and hydrogen peroxide production. Also the expression of type 1 fimbriae and P (adhesion factors) was affected. Atassi and Servin (2010) showed that *L. gasseri* KS120, isolated from human vagina, produced lactic acid and hydrogen peroxide, acting cooperatively to inhibit enteric (*Salmonella enterica* serotype Typhimurium SL1344), vaginal (*Gardnerella vaginalis* DSM 4944) and urinary tract pathogens (*E. coli* CFT073). The potential inhibition of pathogens by LAB isolated from the urogenital tract has sometimes been associated to bacteriocin production. For example, Razzaket al. (2011) have published that four isolates of lactobacilli (from vaginal swabs) showed potent bacteriocin activity against *E. coli* and *Staphylococcus aureus* indicator isolates, while previous results have shown the inhibitory effect of salivaricin CRL 13218 (Ocaña et al. 1999a, b; Vera Pingitore et al. 2012) on pathogens.

In healthy human vagina, a low pH limits the growth of different urogenital pathogens. *E. coli* colonization is not frequently isolated at low pH, but the risk of infections increases when vaginal pH is close to neutrality (Falagas et al. 2006). O'Hanlon et al. (2013) suggested that when lactobacilli dominate the vaginal microbiota, women have more lactic acid mediating the protection against pathogens.

### Adhesion and internalization of *L. reuteri* CRL 1324

*Lactobacillus reuteri* CRL 1324 was the strain that evidenced the highest *E. coli* inhibition. Therefore, this *Lactobacillus* strain was selected to assay its adhesion and internalization abilities into HeLa cells. *L. reuteri* (a heterofermentative bacterium) is considered as one of the *Lactobacillus* species autochthonous to humans and animals hosts (Hou et al. 2015).

The results demonstrated that the adhesion abilities were significantly higher at a ROI of 2000:1 ($7.33 \times 10^5$ CFU/well) than at a ROI of 400:1 ($1.53 \times 10^5$ CFU/well) (Fig. 1). *L. reuteri* CRL 1324 exhibited poor internalization capabilities into the HeLa cells at the two ROI assayed ($6 \times 10^2$ CFU/well at a ROI of 2000:1, and $1.28 \times 10^2$ CFU/well at an ROI of 400:1), which corresponds to less than 1 internalized bacteria per 400 HeLa cells (data not shown).

Adhesion of lactobacilli to HeLa cells has been widely reported. For example, Joo et al. (2012) reported the adhesion of *Lactobacillus helveticus* HY7801 (a human vaginal isolate) to HeLa cells. Martín et al. (2012) showed that five human *Lactobacillus* strains strongly adhered to HeLa cells and suggested that aggregation-promoting factor-like proteins participate in the phenomenon. Verdenelli et al. (2014) evidenced that *L. rhamnosus* IMC 501, *Lactobacillus paracasei* IMC 502 and their SYNBIO (intestinal isolates) combination can adhere to HeLa cells, while these strains were able also to exert antimicrobial activity against pathogenic *Candida* strains. Parolin et al. (2015) demonstrated that the adhesive properties were different in several strains, with *Lactobacillus crispatus* BC1, *L. crispatus* BC3 and *L. gasseri* BC8 being the most adherent strains in their assay. The adherence of beneficial bacteria to eukaryotic cells is strain- and specie-specific usually being the mucus-binding proteins associated (Walsham et al. 2016; Hou et al. 2015). Walsham et al. (2016) showed that CmbA and MUB increase the binding of *L. reuteri* to both HT-29 and LS174T cells. In a previous work, the presence of genes encoding for a mucus adhesion-promoting protein (MapA), a mucus-binding protein (MubI) and a cell and mucus-binding protein (CmbA) was evidenced in *L. reuteri* CRL 1324 (Leccese Terraf et al. 2014), and further studies will be carried out to evaluate the involvement of these proteins in the adhesion process.

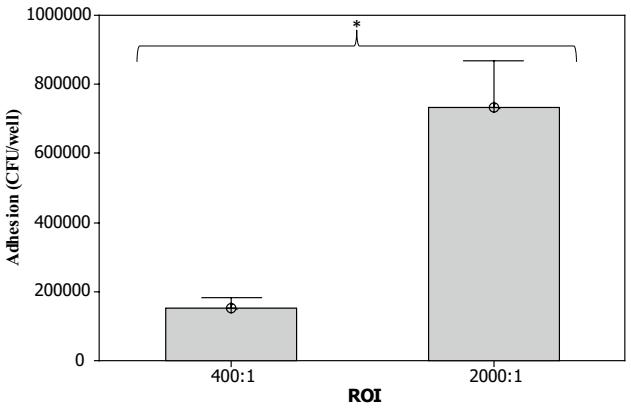


**Fig. 1** Adhesion to HeLa cells of *L. reuteri* CRL 1324. *L. reuteri* CRL 1324 (ROI of 400:1 and 2000:1) populations adhered to HeLa cells were determined after 1 h of interaction. Data are presented as mean populations per well (i.e., corresponding to $2.5 \times 10^5$ HeLa cells) + standard error. Each experiment was performed in triplicate, and differences between groups were compared using Student's *t* test

### Effect of *L. reuteri* CRL 1324 on the adhesion and internalization of *E. coli* to HeLa cells

The adhesion of lactobacilli to the vaginal mucosa is significant to promote and help in the probiotic action (Martín et al. 2013). The effects of lactobacilli on different urogenital pathogens were demonstrated on HeLa cells, including *Gardnerella vaginalis, Candida albicans* by different authors (Joo et al. 2011, 2012; Parolin et al. 2015; Santos et al. 2016) and *E. coli* (Atassi et al. 2006).

In this study, *L. reuteri* CRL 1324 and *E. coli* 275 were co-incubated with HeLa cells to determine if the *Lactobacillus* strains can affect the pathogen adhesion. *L. reuteri* at a ROI of 2000:1 reduced the adhesion rate of *E. coli* at a MOI of 100:1, decreasing by 30% the adhesion observed with *E. coli* 275 (Fig. 2). However, *L. reuteri* CRL 1324 at a ROI of 400:1 did not significantly affect the adhesion rate of *E. coli*.

UPEC can invade host uroepithelial tissue and once internalized can persist in quiescence for long periods without causing clinical symptoms (Mulvey et al. 2001). The co-incubation of *L. reuteri* CRL 1324 and *E. coli* 275 with HeLa cells partially inhibited the pathogen internalization as shown in Fig. 3. *L. reuteri* CRL 1324 with a ROI of 400:1 and 2000:1 led to a significant decrease of *E. coli* internalization rates by 51–57%. In the present work, the mechanism of competition between *L. reuteri* CRL 1324 and *E. coli* 275 for HeLa cell adhesion was assayed (co-incubation of lactobacilli, HeLa cells and pathogen) and demonstrated. However, other researchers evaluated the mechanism of exclusion, through the incubation of lactobacilli and epithelial cells or mucus followed by pathogen

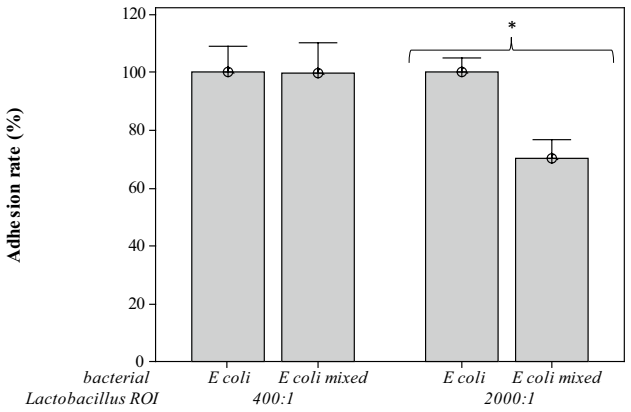


**Fig. 2** Inhibition of *E. coli* 275 adhesion to HeLa cells by *L. reuteri* CRL 1324. Data show adhesion rates of *E. coli* 275 after 1 h of interaction with HeLa cells in the presence of *L. reuteri* CRL 1324 at a ROI of 400:1 and 2000:1. *E. coli* 275 was used at a MOI of 100:1. An adhesion assay of *E. coli* 275 alone was used as a reference. The adhered *E. coli* population in the reference experiment (corresponding to 100% adhesion rate) corresponds to $2.3\times10^6$ CFU/well and $2.72\times10^6$ CFU/well for the assay with *L. reuteri* at ROI 400:1 and 2000:1, respectively. Adhesion rates were then defined as the adhered *E. coli* 275 population in the presence of *L. reuteri* relative to the adhered *E. coli* 275 population in the reference experiment. Data are presented as means + standard error. Each experiment was done in triplicate, and differences between groups were compared using Student's *t* test

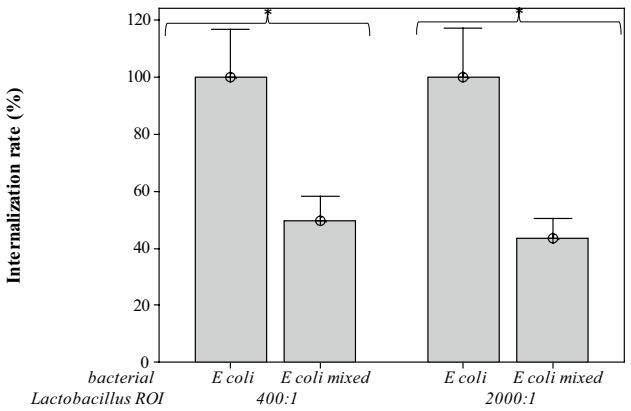


**Fig. 3** Inhibition of *E. coli* 275 internalization into HeLa cells by *L. reuteri* CRL 1324. Data show internalization rates of *E. coli* 275 strain after 2 h of interaction with HeLa cells in the presence of *L. reuteri* CRL 1324 at a ROI of 400:1 and 2000:1. *E. coli* 275 was used at a MOI of 100:1. An internalization assay of *E. coli* 275 alone was used as a reference. The adhered *E. coli* population in the reference experiment (corresponding to 100% adhesion rate) corresponds to $2.24\times10^2$ CFU/well and $4.60\times10^2$ CFU/well for the assay with *L. reuteri* at ROI 400:1 and 2000:1, respectively. Internalization rates were then defined as the internalized *E. coli* 275 population in the presence of *L. reuteri* relative to the internalized *E. coli* 275 population in the reference experiment. Data are presented as means + standard error. Each experiment was done in triplicate, and differences between groups were compared using Student's *t* test

addition (Li et al. 2008; Walsham et al. 2016). Walsham et al. (2016) demonstrated that the pre-incubation of gut symbiont *L. reuteri* strains inhibited enteropathogenic *Escherichia coli* (EPEC) binding to intestinal epithelial cells in a strain- and cell-dependent manner. *L. reuteri* ATCC PTA 6475 (a human isolate) and ATCC 53608 (a pig isolate) significantly inhibited EPEC binding to HT-29, but not to LS174T cells. In addition, these strains affected EPEC adherence to small intestinal biopsy epithelium.

During all the adhesion experiments and subsequent internalization assays, the density of the HeLa cells monolayer was maintained as controlled by cell counting. Thus, the lower adhered population of *E. coli* 275 to HeLa cells did not result from a smaller amount of attached HeLa cells in the wells when incubated with *L. reuteri* CRL 1324. The results obtained suggest that *L. reuteri* CRL 1324 could be able to compete with *E. coli* 275 for binding to some receptors on Hela cells. The ability of *L. reuteri* CRL 1324 to significantly reduce the adhesion and internalization of *E. coli* 275 is clinically relevant, because the pathogen adhesion and invasion on the host cells are main steps in the infection process.

Few studies have investigated the ability of LAB to modulate the internalization of pathogens on host cells. The results presented in this work are similar to those reported by Atassi et al. (2006), who demonstrated the inhibition of adhesion to and internalization into HeLa cells of *E. coli* IH11128 by adhering vaginal *Lactobacillus* strains. Delley et al. (2015) showed that *L. johnsonii* NCC533, *L. johnsonii* NCC2917 and *L. rhamnosus* NCC4007 (commercial probiotics) reduced the adhesion of two UPEC strains (*E. coli* UTI89 and *E. coli* CFT073) to human bladder cancer cell line UM-UC-3; however, the results were not significant.

## Conclusions

The results obtained in this work indicate that *L. reuteri* CRL 1324 and *L. rhamnosus* CRL 1332 were able to in vitro inhibit the growth of UPEC strains by organic acids production. Also *L. reuteri* CRL 1324 inhibited *E. coli* 275 adherence to and internalization into HeLa cells. In this way, this strain can be proposed as a beneficial *Lactobacillus*, which could generate some type of protection against urogenital infections. In vivo trials are being performed to advance in the design of probiotics formulations for the prevention and/or treatment of urinary tract infections.

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de Argentina) and ECOS-SUD (France). The strains were licensed through a CONICET-BIOLIFE agreement.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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