

Effect of Lactobacilli sources on *Escherichia coli* and *Staphylococcus aureus* adherence to uroepithelial cells

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Abstract:

The inhibitory action of four lactobacilli isolates *Lactobacillus bulgaricus*, *L. acidophilus*, *L. plantarum* and *L. fermentum*, isolated from four different samples; yoghurt, vinegar, saliva and vagina respectively, on *Escherichia coli* and *Staphylococcus aureus* adhesion to uroepithelial cells were investigated. Results showed that all *Lactobacillus* isolates or their supernatant were able to reduce the number of the uropathogens attached to uroepithelial cells. However, inhibition level of lactobacilli cells was higher than their supernatant. Nevertheless, the human indigenous lactobacilli (*L. fermentum* and *L. plantarum*) were more competitive than food lactobacilli (*L. acidophilus* and *L. bulgaricus*).

Key words: lactobacilli, adherence, *E. coli*, *S. aureus*, epithelial cells.

Introduction:

Lactobacilli are believed to interfere with pathogens by different mechanisms. The first is competitive exclusion of genitourinary pathogens from receptors present on the surface of the genitourinary epithelium. Second, lactobacilli coaggregate with some uropathogenic bacteria, a process that, when linked to the production of antimicrobial compound, such as lactic acid, hydrogen peroxide, bacteriocin-like substances, and possibly biosurfactants, would result in inhibition of the growth of the pathogens [1].

Adherence of bacteria to epithelial cells has been shown to be an important factor in the colonization of mucous membranes [2]. Adhesion is believed to be a requirement for the realization of certain probiotic effects, such as immunomodulation and pathogen exclusion. However, the mechanisms of attachment are not understood. Recent studies have implicated the involvement of some surface proteins from lactobacilli in

adhesion to epithelial cells, mucin, and various extracellular matrix (ECM) proteins. The surface layer protein (SlpA) from other lactobacilli has also been shown to bind epithelial cells and ECM components [3].

Therefore, many researches have investigated the inhibitory effect of lactobacilli on pathogen adhesion; however, none of them have dealt with the effect of lactobacilli sources on this adhesion. The aim of this study is, therefore, to evaluate the *in vitro* inhibitory activities of four lactobacilli isolated from different sources on the adhesion of *Escherichia coli* and *Staphylococcus aureus* to uroepithelial cells.

Materials and Methods:

Isolation and identification

Uropathogenic *E. coli* and *S. aureus* were isolated from mid-stream urine specimens collected from young females presented with urinary tract infection. The identification was achieved according to Holt *et al.* [4].

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To isolate lactobacilli, four different samples were collected from vinegar, yoghurt, saliva and vagina, and streaked onto De Mann-Rogosa-Sharpe agar (MRSA) (pH 5.5, Himedia, India) plates and incubated at 37 °C for 48 h under anaerobic conditions. The lactobacilli were initially identified by their ability to grow on the selective MRSA, gram-positive staining, rod shape, and catalase-negative phenotype. Biochemical analyses, including sugar fermentation profile and gas production in MRS broth (pH 5.5, Himedia, India), were conducted as described in [4].

Quantification of bacterial suspensions (the lactobacilli and the uropathogens) was adjusted to approximately 1.5×10^8 CFU/ml by comparison to McFarland turbidity standards confirmed by enumeration using the spread plate technique [5].

Preparation of *Lactobacillus* spp. supernatants

Briefly, cultures of lactobacilli were grown in MRS broth at 37 °C for 48 h under anaerobic conditions. Overnight bacterial cultures contained 5×10^8 CFU/ml, and these cultures were centrifuged at 10,000 g for 15 min. at 4°C. The resulting supernatants were filtered through a 0.2 µm membrane filter to remove the remaining bacteria and debris [6]. Plating on MRS agar plates showed no evidence of lactobacilli growth.

Adherence assays

Uroepithelial cells were collected from 22 years old healthy young female, washed three times with phosphate buffered saline.

Overnight cultures of the uropathogen to be tested were suspended to 10^8 cells/ml in phosphate buffered saline. Equal volumes of the uropathogen suspensions and of

uroepithelial cells were mixed and incubated at 37 °C for 30 min. Afterward, the suspensions were washed with equal volume of phosphate buffered saline. Cells were retained by centrifugation (1000g for 10 min.) were placed on microscope slides, fixed with ethanol, and Gram stained. The assays were started within one hour of the collection of the uroepithelial cells, and each determination was performed in triplicate. As a negative control for adherence, uroepithelial cells incubated with bacteria free phosphate buffered saline [7]. The number of attached bacteria was calculated in 40 random uroepithelial cells.

Interference assays

Interference experiments were performed with *E. coli* and *S. aureus* since it was the potential genitourinary pathogens. The procedures described by Kwok *et al.* [7] were employed with the following modifications:

For displacement tests, uropathogen and uroepithelial cells were incubated together for 15 min., therefore, lactobacilli (10^8 cells/ml) or their supernatant were added, and incubation was continued for a further 30 min. The resulting suspensions were centrifuged (1000g for 10 min.), and cells observation was performed as indicated above.

Results and Discussion:

Results revealed that all *Lactobacillus* isolates or their supernatants were able to reduce the number of the uropathogens attached to the uroepithelial cells (table 1).

Generally, the lactic acid bacteria (LAB) are the most implicated of the probiotic organisms, particularly those of the genera *Lactobacillus* and *Bifidobacterium*, which stakes out their territory by secreting acids, thereby creating an environment which is inhospitable to disease-causing

bacteria. Lactobacilli change the oxidation-reduction potential through its production of metabolites by making the environment less conducive for organisms requiring oxygen. This action contributes to the overall inhibiting effect of these probiotic bacteria [8].

Ingrassia *et al.* [9] reported that the inhibitory effects of *L. casei* on adherent-invasive *E. coli* adhesion to differentiated and undifferentiated intestinal epithelial cells reached 75 %

to 84 % in coinubation and 43 % to 62 % in preincubation experiments, according to the cell lines used. Addition of *L. casei* culture supernatant to the incubation medium increased *L. casei* adhesion to intestinal epithelial cells and enhanced the inhibitory effects of *L. casei*. This effect was not due to a bactericidal effect on adherent-invasive *E. coli* or to a cytotoxic effect on epithelial intestinal cells.

Table 1: Number of attached uropathogen \pm SD (bacterial cells / uroepithelial cells) before and after the addition of *Lactobacillus* cells or supernatant.

<i>Lactobacillus</i> spp.	<i>E. coli</i>			<i>S. aureus</i>		
	before	after		before	after	
		cells	supernatant		cells	supernatant
<i>L. bulgaricus</i> ,	45 \pm 2	19 \pm 2.3	22 \pm 1.7	27 \pm 0.5	12 \pm 1.7	16 \pm 1.5
<i>L. acidophilus</i> ,	41 \pm 0.5	10 \pm 1.1	14 \pm 2.3	31 \pm 1.7	14 \pm 0.5	16 \pm 2.3
<i>L. plantarum</i>	39 \pm 1.7	3 \pm 1.1	13 \pm 1.3	29 \pm 2.3	3 \pm 0.5	11 \pm 1.1
<i>L. fermentum</i>	44 \pm 1.1	4 \pm 0.5	8 \pm 1.7	33 \pm 2.3	3 \pm 0.5	8 \pm 0.5

Lactobacillus plantarum isolated from sausages found to be bacteriocin producer is relatively heat stable with promising inhibitory spectra of antimicrobial activities [10].

However, *L. fermentum* and *L. plantarum* which isolated from human origin (indigenous flora) were more competitive than food lactobacilli (*L. acidophilus* and *L. bulgaricus*); a result agreed with Todoriki and coworkers [11] as they mentioned the ability of lactobacilli to adhere is strain specific. Also Aslim and Kilic [12] mentioned that only 10 strains from the 58 human vaginal isolates inhibited all the test bacteria. In their study, Tomas *et al.* [13] reported that only 6 of 134 isolated strains of vaginal lactobacilli were able to inhibit the growth of all the pathogens such as *E. coli*, *S. aureus*, *Enterococcus faecalis*, and *Klebsiella sp.*

Characteristics of *L. acidophilus* NCFMTM (a strain developed at North Carolina State University, Food

Science and Microbiology Departments, thus NCFM) were compared to *L. rhamnosus* GR-1 and *L. fermentum* RC-14, both known to colonize the vagina. Using standard screening methods, *L. acidophilus* NCFMTM adhered to urogenital cells and produced biosurfactants which significantly inhibited uropathogenic enterococci adhesion. However, the strain's ability to adhere, competitively exclude attachment of uropathogens, and inhibit the growth of uropathogens was not as high as *L. rhamnosus* GR-1 and *L. fermentum* RC-14 [14].

Almost certainly, the reason behind this result is *L. fermentum* and *L. plantarum* were isolated from human niches (vagina and oral cavities) equipped them with competing factors more effective than those of *L. acidophilus* and *L. bulgaricus* which were isolated from food where the competition is very low or negligible.

Furthermore, inhibition level of lactobacilli cells was higher than their supernatant. Ghalfi *et al.* [15] pointed out that *E. coli* O157 (VH21) was cocultured with *L. curvatus* CWBI-B28 in MRS broth. The results of the well diffusion assay suggested that the inhibition of *E. coli* O157 (VH21) was partially due to the bacteriocin; however, growth monitoring indicated that such inhibition is exclusively due to hydrogen peroxide. Therefore, the application of live cells *L. curvatus* CWBI-B28 in starter culture or as an adjunct starter would be more advantageous to food preservation than the purified bacteriocin. The H₂O₂ – producing ability of lactobacilli is thought to play a significant role in protecting the vaginal ecosystem from bacterial vaginosis infection [16].

Also an interesting observation was that coculture experiment showed significant inhibition of growth of *Aeromonas salmonicida*, which was mediated by competition for nutrients rather than secretion of inhibitory substances by the probiotic bacteria as measured in spent culture liquid [17].

Accordingly, the results of the present study strongly signify the source of lactobacilli as an important factor in the selection of probiotic microorganism.

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تأثير مصدر بكتريا *Lactobacilli* في التصاق بكتريا *Escherichia coli* و *Staphylococcus aureus* على الخلايا الطلائية البولية

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الخلاصة:

اختبر الفعل التثبيطي لأربع عزلات بكتريا حامض اللاكتيك وهي *L. bulgaricus* و *L. acidophilus* و *L. fermentum* و *L. plantarum* عزلت من اربعة مصادر مختلفة و هي اللبن و الخل و اللعاب و المهبل على التوالي لمنع التصاق *E. coli* و *S. aureus* بالخلايا الطلائية البولية. اظهرت النتائج ان عزلات *Lactobacillus* جميعها او طافي الزرع استطاعت خفض اعداد الممرضات البولية الملتصقة على الخلايا الطلائية البولية. في حين كان مستوى التثبيط بالنسبة لخلايا *Lactobacillus* اكثر من الطافي للخلايا نفسها. و من ناحية اخرى كانت بكتريا حامض اللاكتيك المعزولة من مصادر بشرية (*L. fermentum* و *L. plantarum*) قادرة على التنافس أكثر من تلك المعزولة من الاغذية (*L. acidophilus* و *L. bulgaricus*).