http://dx.doi.org/10.22037/afb.v4i4.17783

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2017, 4 (4):241-250
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

Antibiofilm Effects of Lactobacilli against Ciprofloxacin-Resistant Uropathogenic *Escherichia coli* strains in Pasteurized Milk

Mahsa Yeganeh[1], Hedayat Hosseini*[2], Sedigheh Mehrabian[1], Elham Siasi Torbati[1], Seyed Morteza Zamir[3]

1- Faculty of Biological Sciences, Tehran-North Branch, Islamic Azad University, Tehran, Iran.

2- National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

3- Biotechnology Group, Faculty of Chemical Engineering, Tarbiat Modares University, Tehran, Iran.

Abstract

Background and Objective: Uropathogenic *Escherichia coli*-induced urinary tract infections are the most common uropathogenic *Escherichia coli* etiological agent. In addition, most of biofilms created by these bacteria can be regarded as a serious problem in the food industry. Foodborne diseases have always been considered an emerging public health concern throughout the world. Many outbreaks have been found to be associated with biofilms. Thus, the aim of the present study is to investigate the anti-adhesive effects of lactic acid bacteria against strains of Ciprofloxacin-Resistant Uropathogenic *Escherichia coli* using microbial techniques in pasteurized milk.

Material and Methods: In this study, strains of *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus acidophilus* were provided from Pasteur Institute of Iran. Twenty strains of Uropathogenic *Escherichia coli*-Induced Urinary Tract Infections were isolated from patients with urinary tract infection in Shahid Labbafinejad hospital of Iran. Eight strains with ability of biofilm formation were selected for microbial tests. All of these eight strains were resistant to ciprofloxacin. Disk diffusion method was used to assess the susceptibility of all isolates to the ten common antibiotics. Eight samples of Uropathogenic *Escherichia coli* were inoculated in pasteurized milk. The microtitre plate 100 method was used to detect anti-adhesive activity of lactobacilli supernatant.

Results and Conclusion: Results showed that the eight human isolates were resistant to antibiotics. Isolate of number 4 was the most susceptible strains to antibiofilm effects of lactobacilli in the pasteurized milk. The anti-adhesive effects of lactobacilli on Uropathogenic were confirmed in all microbial tests. In this study, *Lactobacillus plantarum* revealed the highest inhibitory activity against Uropathogenic *Escherichia coli* 4 strain with inhibition zones of 42 mm. This strain was reported as a proper probiotic bacterium. According to the results, these lactobacilli have had spectacular effects on biofilm formation and pathogenicity of Uropathogenic strains to prevent the adhesion.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:
Received 9 July 2017
Revised 12 August 2017
Accepted 25 September 2017

Keywords:
- Antibiofilm effects
- Ciprofloxacin-resistant
- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Lactobacillus plantarum*

Uropathogenic *Escherichia coli*

***Corresponding author:**
Hedayat Hosseini,
National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Tel: +98-9123007655
Fax: +98-21-22376467
E-mail: hedayat@sbmu.ac.ir

1. Introduction

Uropathogenic *Escherichia (E.) coli* Induced urinary tract infections were identified in 1982 and introduced as the main cause of outbreak of infectious diseases around the world .This bacterium in food may cause spoilage and contribute to raised incidence of foodborne diseases.The emergence of multidrug resistant and disinfectant resistant bacteria such as Escherichia has increased rapidly. Antibiotic-resistant bacteria are worldwide death tolls [1,2]. Uropathogenic *E. coli* (UPEC) is one of the major concerns in the food industry. In some cases, dissemination of a single clonal group of UPEC isolates may occur within a community via contaminated food or other consumables [3-5]. Recent studies have shown that among all *E. coli* strains, only UPEC strains are able to survive in acidic conditions [6,7].

Many bacteria, including *E. coli*, *Staphylococcus* (*S.*) *aureus*, and *Pseudomonas* (*P.*) *aeruginosa* can form biofilm in the body tissues. The formed biofilm leads to the emergence of several deadly infectious diseases [8,9]. In addition, the biofilm created by *E. coli* can be regarded as a

serious problem in milk contamination in the post-pasteurization stage [10]. Also, this strain participates in the biofilm formation on the surfaces of a post-pasteurization unit in a dairy plant. Moreover, *E. coli* within the biofilm is resistant to many antibiotics compared to its free state (Planktonic) and almost is resistant to ciprofloxacin, carbenicillin, cloxacillin, cephaloridin, novobiocin, and vancomycin [11]. Antibiotic resistant microorganisms can increase mortality rates because they can survive through their ability to acquire and transmit resistance after exposure to antibiotic drugs, which are one of the therapies to infec-tious diseases. Therefore, the existence of drug-resistant bacteria in pasteurized milk could be considered a major problem in the antibiotic therapy and alternative remedies have to be applied for the treatment of many infectious diseases. One example of these common antibiotics is ciprofloxacin. Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. This includes bone and joint infections, intra-abdominal infections, certain type of infec-tious diarrhea, respiratory tract infections, skin infections, typhoid fever and urinary tract infections. For some infections, it is used in addition to other antibiotics [12,13].

One of the safe remedies for preservation of foods, is the use of lactic acid bacteria, which produces specific natural antibiotics. Lactobacilli are widespread in nature and reside in a variety of natural habitats, ranging from plants to the mammalian oral, gastrointestinal or vaginal cavities. Lactobacilli are characterized by their ability to inhibit the growth of bacteria throughout the production of antimicrob-ial materials such as bacteriocins and biosurfactants, lactic acid and etc. Thus, these probiotic bacteria prevent the formation of biofilm in pasteurized milk. Hence, the use of lactic acid bacteria through the production of natural antibiotics can be very useful and practical for the prevention of biofilm resulted from *E. coli* [14].

There is one report (in vitro) on antagonistic effects of lactobacilli against *Escherichia coli*. Previously, antagon-istic activity of probiotic lactobacilli against pathogens have been reported in Brain Heart Infusion agar medium [15]. However, there is lack of research in food stuff.

The present study was designed to evaluate the activity of lactobacilli against the biofilm production by ciprofloxacin-resistant UPEC strains in the pasteurized milk.

2. Materials and Methods

2.1 Bacterial strains

Twenty strains of Uropathogenic *E. coli* Induced Urin-ary Tract Infections were isolated from patients with urinary tract infection in Shahid Labbafinejad hospital of Iran. Eight strains with the ability of biofilm formation were selected for microbial tests. All of these eight strains were resistant to Ciprofloxacin. After Eosin Methylene Blue culture and observing the colonies with metallic luster, conventional biochemical tests including Gram's staining and micro-scopic observation was used to confirm the presence of *E. coli* strains. *E. coli* ATCC 25922 that was used as a control in this study was purchased from Persian Type Culture Collection. *Lactobacillus* (*L.*) *plantarum* ATCC 136H3, *L. acidophilus* ATCC 314 and *L. casei* ATCC 25598 were provided from Pasteur Institute of Iran. To activate the bacterial cultures, lactobacilli strains were cultured in MRS broth and MRS agar medium under anaerobic conditions and incubated at 37°C for 72 h and UPEC strains were cultured under aerobic conditions and incubated at 37°C for 24 h.

2.2 Antimicrobial drug susceptibility testing

The antimicrobial susceptibility profiles were performed via the antibiogram disk method. A volume of 100 µl of an overnight growth of each UPEC isolate on Mueller-Hinton broth with the turbidity of 0.5 McFarland (equal to 1.5×10^8 colony-forming units (CFU ml^{-1}) was streaked on Mueller-Hinton agar plates. The routinely used 10 antibiotic discs, including nalidixic acid, amikacin, ampicillin, sulfamethox-azole trimethoprim, ofloxacin, piperacillin tazobactam, imipenem, ciprofloxacin (Padtan teb, Iran) were placed on the surface of the inoculated plates. The plates were incubated at 37° C for 24 h. The inhibition zones were not found in resistant strains [16].

2.3 DNA extraction and polymerase chain reaction

To identify the resistance to ciprofloxacin in the most susceptible strain to antibiofilm effect of lactobacilli (UPEC 4), qnr A and qnrS gens in this isolate were analyzed by the PCR method. First, DNA extraction was performed using an optimized boiling method. UPEC 4 strain was grown in Luria-Bertani Broth (Merck, Germany) at 37°C overnight. Bacteria were pelleted from 1.5 ml Luria-Bertani broth and suspended in 200 µl of sterile distilled water, then incubated at 100° C for 10 min and centrifuged. Specific primers were used to amplify sequences of the qnrA, qnrS gens. Detection of adhesion-encoding genes (qnrA, qnrS) was done by multiplex PCR (Bio-Rad, America).

The reactions (25 µl) consisted of 10 pmol l^{-1} of each primer, 2 µl templates DNA, and 12.5 µl of a ready-to-use 2X PCR Master Mix Red by IBRC Taq DNA polymerase, with the following amplification conditions: an initial denaturation at 94°C for 10 min, followed by 35 DNA cycles of denaturation at 94°C for 2 min, annealing at a specific temperature and extesion at 72°C for 1 min. The PCR product (5 µl) underwent gel electrophoresis (Syngene G:BOX, America) on agarose (1% w w^{-1}) (Merck, Germany), followed by staining with ethidium bromide solution (Cinna colon, Iran). Amplified DNA

elements of specific sizes were detected by UV-induced fluorescence and the size of the amplicons was estimated by comparing them with the 1 kb DNA ladder (Thermo Scientific, America) included on the same gel [17-20].

2.4 Antimicrobial activity of Cell Free Supernatant of Lactic Acid Bacteria

Targeted colony of eight strains of UPEC were diluted using 0.1% w w^{-1} peptone water (Merck, Germany) to get 0.5 McFarland Turbidity Standard. All targeted Gram negative pathogenic bacteria being used were freshly spread onto Muller Hilton Agar respectively using Kirby Bauer technique. Then, 5 mm diameter size of wells were immediately made up in each plate. Overnight suspensions of inoculated lactobacilli in milk were centrifuged at 12000 × g for 30 min. The isolated supernatants were filtered by sterile filter 0.25 µ. Then, 80 µl of obtained supernatants were transferred to each well separately. Each plate was controlled by adding sterilized peptone water. All plates were incubated aerobically at 37°C for 24 h. The inhibition zones were measured in all of the plates. *E. coli* ATCC 25922 was used as controls in the experiments. The experiments were carried out and repeated three times [21,22].

2.5 Investigation of biofilm formation by UPEC strains using polystyrene microtiter plate 100

At first, 1 ml of fresh bacterial suspension was inoculated into a test tube containing 10 ml BHI broth medium. After inoculation of 100 µl of this medium into pasteurized milk, 350 µl of the above medium was poured into the sterile microtiter plates (Teb Amooze Sina, Iran) and pasteurized milk was poured into the control wells. After putting the plate caps, incubation was carried out for 24 h at 37°C. Then, the contents of the wells were emptied and rinsed 3 times with sterile serum. Ethanol 96% w w^{-1} (350 µl), was added to the wells to fix the cells. After 15 min, the contents of the wells were emptied and the plates were dried at the laboratory room temperature. Next, each well was stained with 350 µl of 2% w w^{-1} crystal violet for 5 min. The wells were gently washed with water and filled with 33% w w^{-1} acetic acid (Merck, Germany) as the solvent. After 15 min of incubation at 37°C, the optical absorbance was read at 492 nm using BioscreenC (DNV, Finland). Finally, UPEC samples with optical absorbance higher than control well were reported as biofilm former samples [23].

2.6 Investigation of the anti-adhesive effect of lactobacilli supernatant

To evaluate the anti-adhesive effect of probiotics, polystyrene microtiter plate 100 was used. First, 75 µl of the lactobacilli supernatant and then 75 µl of 0.5 McFarland inoculated suspension of UPEC in pasteurized milk were added to the wells. Polystyrene microtiter plate was incubated at 37°C for 24 h. Each of UPEC in pasteurized milk (without lactobacilli) was poured into the control wells. Then, the contents of the wells were removed and each well was washed three times by PBS. Ethanol 96% w w^{-1} (for 15 min) and 2% w w^{-1} crystal violet (for 10 min) were used for stabilizing the cells and staining, respectively. Then, the polystyrene microtiter plate was rinsed with a gentle stream of water. When the wells were dried by exposing to the air, 33% w w^{-1} acetic acid was added to the wells as a solvent, and optical absorbance was measured at 492 nm for each well using BioscreenC (DNV, Finland). The test was carried out in duplicate for each pathogen sample in the vicinity of both probiotics. Finally, UPEC samples with optical absorbance lower than control well were reported as susceptible samples to antibiofilm effects of lactobacilli [24,25].

Rao et al, have declared that cell free supernatant of both *L. plantarum* and *L. pentosus* strains showed good antibio-film activity against *P. aeruginosa* and *Klebsiella pneumonia*. Besides, they stated that these probiotics have good antimicrobial activity against some important pathogens such as *Escherichia coli*, *B. subtilis*, *P. aeruginosa*, and others [26].

2.7 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to examine the changes in biofilm structures caused by interactions between LAB and UPEC4 strains. For this assay, biofilms were allowed to form in 6-well polystyrene microtiter plates. After 48 h of incubation, the medium was aspirated and the non-adherent cells were removed by washing twice with PBS. The biofilms were then fixed according to Fischer et al. by adding a solution of glutaraldehyde (Merck, Germany) in 0.1 M phosphate buffer at a final concentration of 2.5% to the wells and storing the plate overnight at 4°C. The films were then dehydrated using an ethanol series (25, 50, 75 then 100% each for 15 min) and air dried for 20 min. The bottoms of the wells were then cut and kept in a desiccator before analysis. For examination, the discs were mounted onto aluminum stubs, sputter-coated with gold and imaged using a Scanning Electron Microscope (TE Scann MIRA2 LMU, Czech Republic) [27].

2.8 Statistical analysis

All experiments were conducted in triplicate. Statistical analysis was performed through analysis of variance using IBM SPSS & Duncan Statistics Software version 19. A $p \leq 0.05$ was considered to be statistically significant.

3. Results and Discussion

3.1 Identification of lactobacilli by morphology of colony, Gram reaction, catalase and oxidase activity tests

Gram staining and catalase test were conducted as an initial screening of lactobacilli. The isolates were Gram-positive, catalase-negative, and bacilli form and were maintained as stock cultures at -20° C in MRS broth supplemented with 15% (w w^{-1}) glycerol (Merck, Darmstadt, Germany) for further analysis.

3.2 Identification of *E. coli* strains by biochemical analysis

E. coli strains were identified by biochemical analysis (API). All the UPEC strains were Indol (+), MR(-)VP(+), Urea (-) and Simon citrate (-).

3.3 Antimicrobial drug susceptibility testing

Fifteen antibiotics were tested on eight strains of UPEC in vitro. The results were indicated that all of the strains with high biofilm formation capability were resistant to ciprofloxacin antibiotic.

3.4 Identification of qnr gene in UPEC 4 isolate:

To identify qnr A and qnrS genes in UPEC 4 isolate, Annealing temperature and other conditions were adjusted according to table 3.

Table 1. Identification of *E. coli* strains by biochemical analysis

TSI	Simon Sitrat	Urea	MR-VP	Indol	Motility	Number of strain
A/A	-	-	+	+	+	UPEC1
A/A	-	-	+	+	+	UPEC2
A/A	-	-	+	+	+	UPEC3
A/A	-	-	+	+	+	UPEC4
A/A	-	-	+	+	-	UPEC5
A/A	-	-	+	+	+	UPEC6
A/A	-	-	+	+	+	UPEC7
A/A	-	-	+	+	+	UPEC8

Table 2. Resistant and susceptible isolates against different antibiotic

Antibiotic	UPEC1	UPEC2	UPEC3	UPEC4	UPEC5	UPEC6	UPEC7	UPEC8
Nalidixic acid	S	S	R	R	R	S	R	S
Amikacin	S	S	S	S	S	S	S	S
Ampicillin	S	S	S	S	S	S	S	S
Sulfamethoxazole/Trimethoprim	R	R	*R*	R	S	R	S	R
Ofloxacin	S	S	R	S	S	S	S	S
Cefoxitin	S	S	S	S	S	S	S	S
Norfloxacin	S	S	R	R	S	S	R	S
Amoxicillin/Clavulanate	S	S	S	S	S	S	S	S
Tobromicin	S	S	S	S	S	S	S	S
Gentamicin	S	S	S	S	S	S	S	S
Piperacillin/Tazobactam	S	S	S	S	S	S	S	S
Imipenem	S	S	S	S	S	S	S	S
Ciprofloxacin	R	R	R	R	R	R	R	R
Ceftizoxime	S	S	S	S	S	S	S	S
Nitrofurantoin	S	S	S	S	S	S	S	S

R, resistant; S, susceptible; UPEC, urinary pathogenic *E. coli*; MDR, multiple drug resistance

Tabe 3. Acquired primer for PCR of qnr gene and annealing temperature

Annealing temperature	Identified gen	Primer sequencing	Company	Size
56° for 30 sec	qnrA	5-AGA GGA TTT CTC ACG CCA GG-3 5-TGC CAG GCA CAG ATC TTG AC-3	Cinna Gen	562 bp
55° for 30 sec	qnrS	5-ACG ACA TTC GTC AAC TGC AA -3 5- TAA ATT GGC ACC CTG TAG GC - 3	Cinna Gen	417 bp

Electrophoresis of PCR product for qnr A and qnrS genes on 1% w w^{-1} agarose gel have been shown in Figure 1. Among these eight isolates, an isolate had these two types of genes. None of the seven other isolates showed positive bands. Only UPEC 4 had two positive bands for qnr A and qnr S genes.

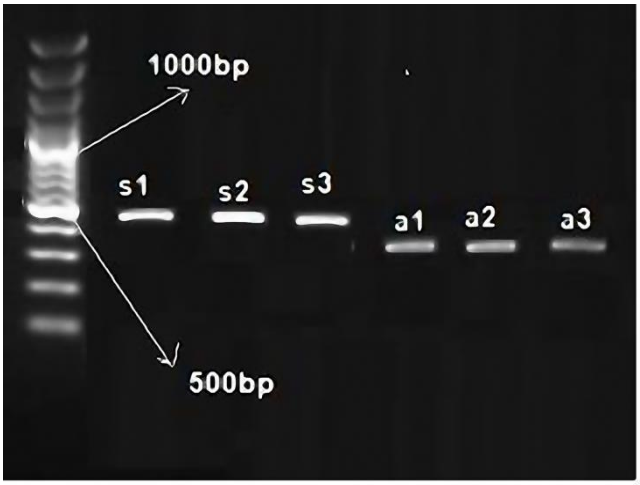


Figure 1. Electrophoresis of PCR product for qnr A and qnr S gens on 1% agarose gel.
s1,s2 ,s3 = qnr S
a1,a2,a3= qnr A

3.5 Antimicrobial activity of lactobacilli supernatants against UPEC strains using agar well diffusion assay

Antimicrobial effect of lactobacilli supernatant against UPEC showed that *L. plantarum* had maximum antimicrobial effect against UPEC4 (32 mm), *L. casei* had maximum antimicrobial effect against UPEC4 (29 mm) and *L. acido-philus* had maximum antimicrobial effect against UPEC4 (20 mm).

The antimicrobial effect of probiotic bacteria supernatant can be referred to the lactic acid production, because the medium acidity increases severely during the growth of probiotic bacteria. On the other hand, pathogenic bacteria are naturally sensitive to the acidic conditions and would be destroyed in low acidity [28].

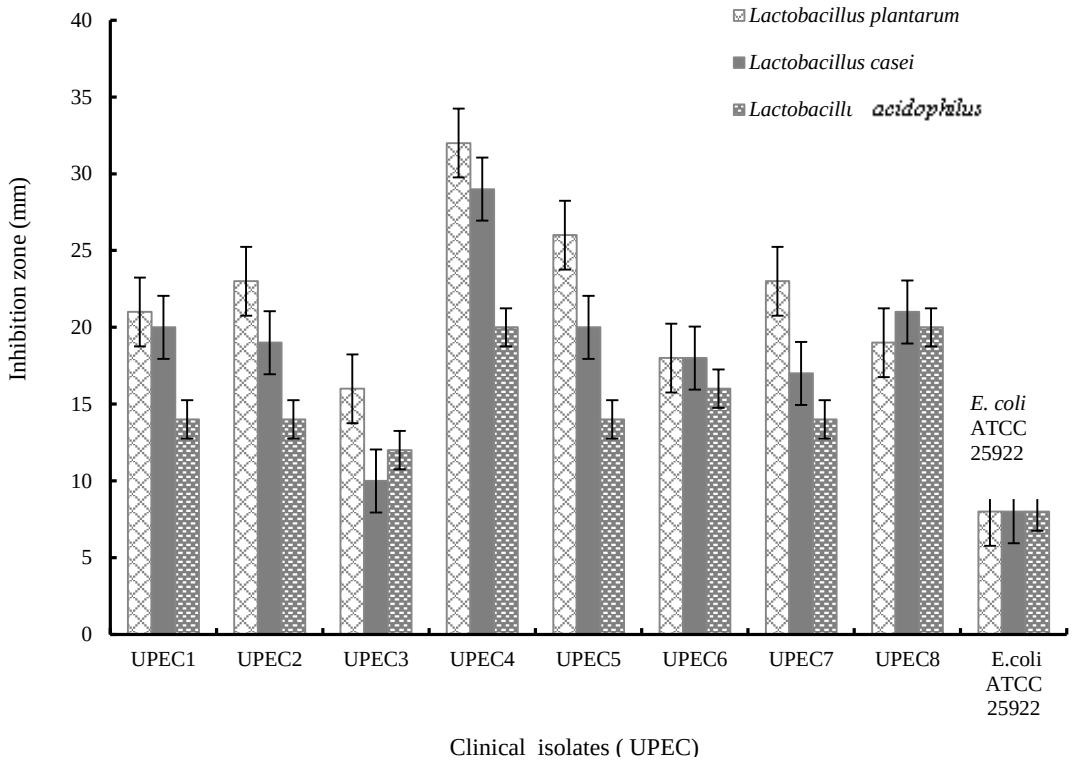


Figure 2. Antimicrobial effect of lactobacilli supernatants against UPEC strains by well diffusion method (mm)

Ogunbanwo et al. showed that supernatants resulted from the two probiotic *L. plantarum* and *L. brevis* have antimicrobial activity against *E. coli*, *Bacillus (B.) cereus* and *Yersinia enterocolitica*, and inhibit their growth [29].

Chen et al. and Taheur et al. showed that few of our curd lactobacilli CFSs displayed antagonistic activity against *S. mutans*, which is in agreement to recent studies that have claimed the antagonistic potential of lactobacilli against Streptococcus spp. [30,31]. In addition, human milk lactobacilli CFSs also showed varying antagonistic patterns against the tested pathogens. Similar to curd isolates, human milk Lactobacillus CFSs were inactive against *S. aureus*, *L. monocytogenes*, *E. coli* and *K. pneumonia* [32].

3.6 Biofilm formation produced by UPEC strains using microtiter plate 100

Biofilm formation test by using microtiter plate 100 showed that strains of **U**PEC3 had maximum ability in biofilm production. *E. coli* ATCC 25922 had the least capability for biofilm formation

3.7 Anti-adhesive effect of lactobacilli supernatant using microtiter plate 100

Results of anti-adhesive effect of *E. coli* strains showed that eight biofilm producer strains were susceptible to antibiofilm effects of lactobacilli. UPEC3 had maximum power for biofilm formation. Also, UPEC 3 was the most resistant isolate to lactobacilli.

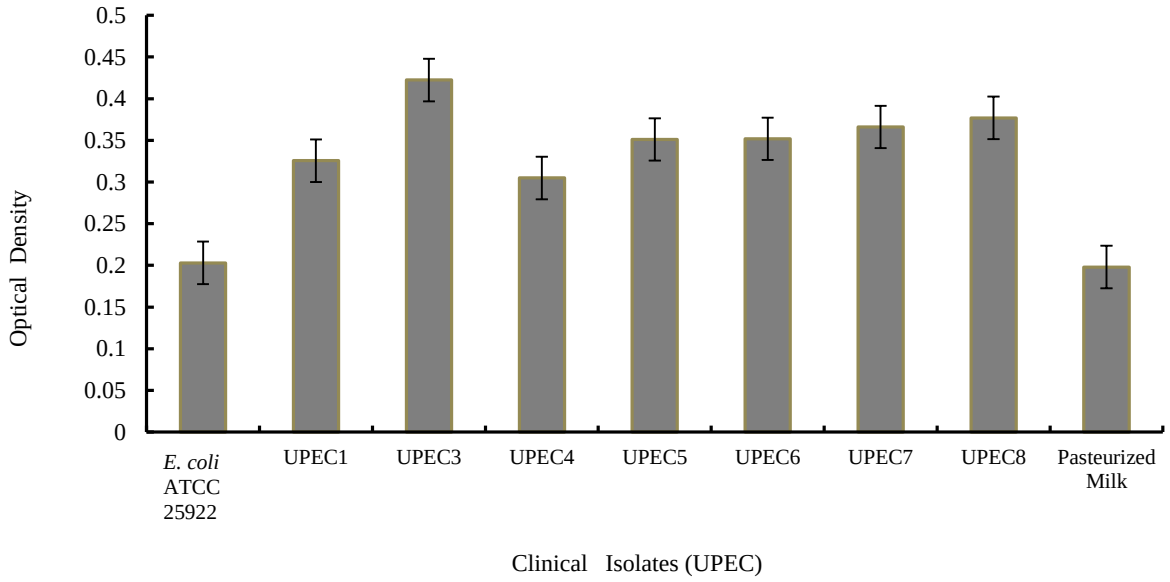


Figure 3. Biofilm formation produced by UPEC strains using microtiter plate in wavelength of 492 nm

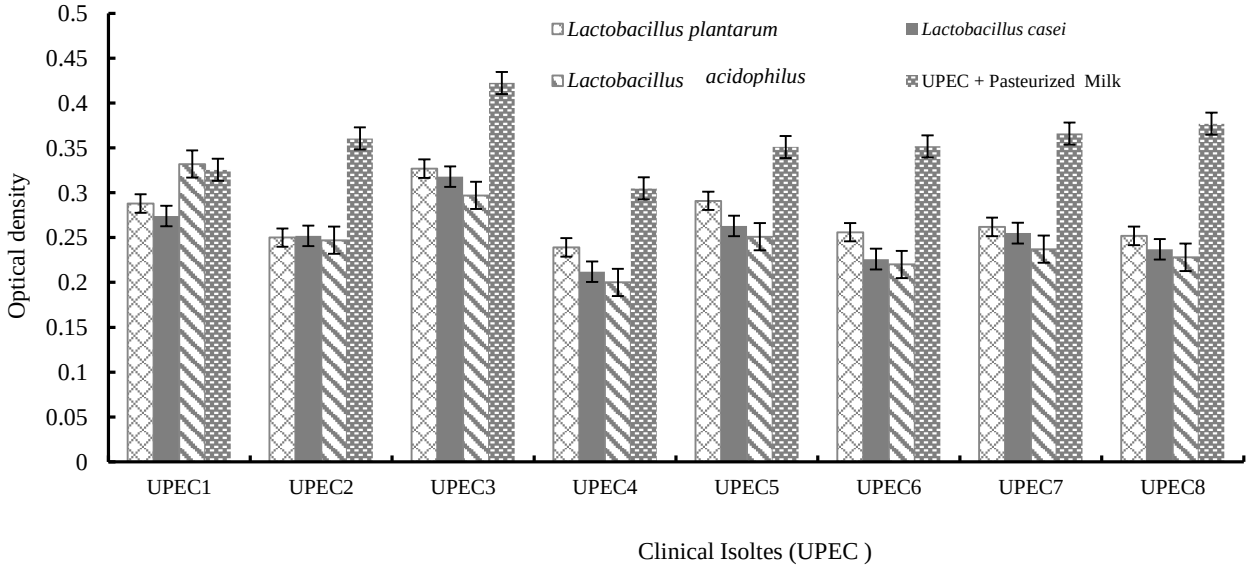
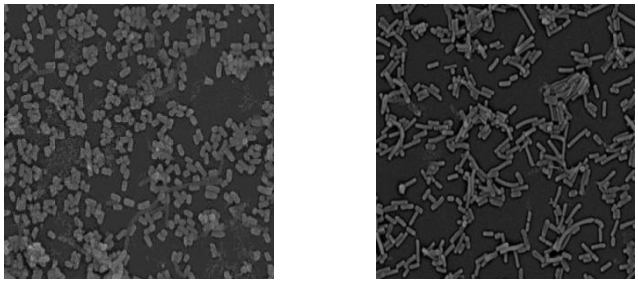


Figure 4. Anti-adhesive effect of lactobacilli supernatant using polystyrene microtiter plate 100

Abedi et al. found that the probiotic lactobacilli have anti-adhesive effect. According to their report, *L. delbrueckii* was able to prevent the binding of about 80% of *E. coli* strains to Caco-2 cells. One of the reasons for the difference between the results obtained in this study and those of Abedi et al. is the difference in adhesion levels. In the Caco-2 culture, the probiotic Lactobacillus was more successful than *E. coli* strains to adhere to the cellular receptor and prevent from pathogenic bacteria adhesion by occupying the place [33].

3.8 Scanning Electron Microscopy

The test pathogens in single-species biofilms and in the corresponding dual-species biofilms with LAB were examined using SEM to detect the effect of LAB on the pathogen biofilm formation upon co-culturing. The SEM images showed that LAB could largely replace the pathogens in their biofilms upon co-culturing as shown in Figure 5.



A **B**

Figure 5. Scanning electron micrographs of 48 h single-species biofilms of UPEC 4 strain and their corresponding dual-species biofilms with LAB. (**A**) *E. coli alone*, (**B**) UPEC 4 + *L. plantarum*,

4. Conclusion

Based on the findings of this study, it can be concluded that CFLS resulted from *L. plantarum*, *L. casei*, and *L. acidophilus* have inhibitory effects against Ciprofloxacin-Resistant UPEC strains in pasteurized milk. Therefore, cautions are necessary to decrease the incidence of multidrug resistant strains of *E. coli* in animals and people. In order to achieve this, good hygienic practices are necessary from the farm to the family.

Since the adhesion of UPEC strains to their host cells is well known as an important virulence factor in the pathogenicity, the CFLS could be suggested to control the infections caused by UPEC strains and prevented from biofilm production by these strains in pasteurized milk. However, further in vivo investigations are recommended to ensure this hypothesis.

5. Acknowledgements

We would like to thank the National Nutrition and Food Technology Research Institute (NNFTRI) affiliated with Shahid Beheshti University of Medical Sciences, and Faculty of Biological Science, Islamic Azad University, North-Tehran Branch, Tehran, Iran**.**

6. Conflict of Interest

The authors declare that no conflicts of any interest exist.

References

1. Miladi H, Zmantar T, Chaabouni Y, Fedhila K, Bakhrouf A, Mandouani K, Chaieb K. Antibacterial and efflux pump inhibitors of thymol and carvacrol against food-borne pathogens. Microb Pathog. 2016; 99: 95-100. doi: 10.1016/j.micpath.2016.08.008.

2. Griffiths M. *Escherichia coli*: Virulence, stress response and resistance. Pages in Understanding Pathogen Behavior. M.Griffiths, ed. CRC Press LLC, Boca Raton, FL, 2005: 240-278.

3. Price SB, Wright JC, DeGraves FJ, Castanie-Cornet MP, Foster JW. Acid resistance systems required for survival of *Escherichia coli* H7:O157 in the bovine gastrointestinal tract and in apple cider are different. Appl Environ Microbiol. 2004; 70: 4792-4799. doi: 10.1128/AEM-.70.8.4792-4799.2004.

4. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp Mol Pathol. 2008; 85(1): 11-19. doi: 10.1016/j.yexmp-.2008.03.007

5. Kanjee U, Houry WA. Mechanisms of acid resistance in *Escherichia coli*. Annu Rev Microbiol. 2013; 67: 65-81. doi: 10.1146/annurev-micro-092412-155708.

6. Oh DH, Pan Y, Berry E, Cooley M, Mandrell R, Breidt FJR. *Escherichia coli* H7:O157 strains isolated from environmental sources differ significantly in acetic acid resistance compared with human outbreak strains. J Food Prot. 2009; 72(3): 503-509. doi: 10.4315/0362-028X-72.3.503.

7. Kim G-H, Breidt F, Fratamico P, Oh. Acid resistance and molecular characterization of *Escherichia coli* H7:O157 and different non-O157 shiga toxin producing *E. coli* serogroups. J Food Sci. 2015; 80(10): 2257-2264. doi: 10.1111/1750-3841.12996.

8. Jensen PQ, Givskov M, Bjarnsholt T, Moser C. The immune system vs *Pseudomonas aeruginosa* biofilms. FEMS Immunol Med Microbiol. 2010; 59(3): 292-305. doi: 10.1111/j.1574-695X.2010.00706.x.

9. Sawhney R, Berry V. Bacterial biofilm formation, pathogenicity, diagnostics and control: An overview. Indian J Med Sci. 2009; 63(7): 313-321. doi: 10.4103/0019-5359.55113.

10. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol. 2000; 54: 49-79. doi: 10.1146/annurev.micro.54.1.49

11. Drenkard E, Ausubel FM. Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature. 2002; 416: 740-743. doi: 10.1038/416740a

12. Marchese A, Barbieri R, Sanches-Silva A, Daglia M, Nabavi S F, Jafari N J, Izadi M Ajami M, Nabavi S M. Antifungal and antibacterial activities of allicin: A review. Trends Food Sci Technol. 2016; 52: 49-56. doi: 10.1016/j.tifs.2016.03.010

13. Benavides AB, Ulcuango M, Yepez L, Tenea GN. Assessment of the in vitro bioactive properties of lactic acid bacteria isolated from native ecological niches of Ecuador. Rev Argent Microbiol. 2016; 38: 236-244. doi: 10.1016/j.ram.2016.05.003

14. Kenreigh CA, Wagner LT. Probiotic supplements ease diarrhea and other gastrointestinal disorders. Medscape Pharm. 2006; 12: 14-20. doi: 10.1089/act.2006.12.14

15. Soleimani NA, Kermanshahi RK, Yakhchali B, Sattari TN. Antagonistic activity of probiotic lactobacilli against *Staphylococcus aureus* isolated from bovine mastitis. Afr J Microbiol Res. 2010; 4(20): 2169-2173.

16. Bukh AS, Schonheyder HC, Emmersen JM, Sogaard M, Bastholm S, Roslev P. *Escherichia coli* phylogenetic groups are associated with site of infection and level of antibiotic resistance in community-acquired bacteraemia: A 10 year population-based study in Denmark. J Antimicrob Chemother. 2009; 64(1): 163-168. doi: 10.1093/jac/dkp156.

17. Bonkat G, Müller G, Braissant O, Frei R, Tschudin-Suter S, Rieken M, Wyler S, Gasser TC, Bachmann A, Widmer AF. Increasing prevalence of ciprofloxacin resistance in extended-spectrum beta-lactamase producing *Escherichia coli* urinary isolates. World J Urol. 2013; 31: 1427-1432. doi: 10.1007/s00345-013-1031-5.

18- Corkill JE, Anson JJ, Hart CA. High prevalence of the plasmid-mediated quinolone resistance determinant qnrA in multidrug- resistant Enterobacteriaceae from blood cultures in Liverpool, UK. J Antimicrob Chemotherap. 2005; 56:1115-1117. doi: 10.1093/jac/dki388

19- Putnam SD, Sanders JW, Tribble DR, Rockabrand DR, Riddle MS, Rozmajzl PJ, Frenck RW. Post-treatment changes in *Escherichia coli* antimicrobial susceptibility rates among diarrheic patients treated with ciprofloxacin. Antimicrob Agent Chemotherap. 2005; 49: 2571-2572. doi: 10.1128/AAC.49.6.2571-2572.2005

20. Saira B, Yasra S, Aamir A, Mashkoor M, Muhammad Azeem S, Ayesha T, and Abdul H. Multiple drug resistance patterns in various phylogenetic groups of Uropathogenic *E. coli* isolated from faisalabad region of Pakistan. Braz J Microbiol. 2011 Oct; 42(4): 1278-1283. doi: 10.1590/S1517-83822011000400005

21. Leisner JJ, Vancanneyt M, Rusul G, Pot B, Lefebvre K, Fresi A, Tee LK. Identification of lactic acid bacteria constituting the predominating microflora in an acid-fermented condiment (tempoyak) popular in Malaysia. Int J Food Microbiol. 2001; 63(1-2):149-157.

22. Shah NP, Dave R. Antimicrobial substances including bacteriocins produced by lactic acid bacteria. Biosci Microflora. 2002; 21(4): 217-223.

23. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, Ruzicka F .Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Acta Pathol Microbiol Immunol Scand. 2007; 115(8): 891-899. doi: 10.1111/j.1600-0463.2007.apm-630.x

24. Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, Versalovic J. Human derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. Anaerobe. 2008; 14(3): 166-171. doi: 10.1016/j.anaerobe.2008.02.-001.

25. Flint S, Palmer J, Bloemen K, Brooks J, Crawford R. The growth of *Bacillus stearothermophilus* on stainless steel. J Appl Microbiol. 2001; 90(2): 151-157. doi: 10.1046-/j.1365-2672.2001.01215.x.

26. Rao KP, Chennappa G, Suraj U, Nagaraja H, Raj CAP, Sreenivasa MY. Probiotic potential of Lactobacillus strains isolated from sorghum-based traditional fermented food. Probiotics Antimicrob Proteins. 2015; 7(2): 146-156. doi: 10.1007/s12602-015-9186-6.

27. Ventola CL. The Antibiotic Resistance Crisis. Pharm Ther. 2015; 40(4): 277-283.

28. Sarmast Ghahfarokhi E, Mobini Dehkordi M, Beheshtimaal K. Isolation and evaluation of lactic acid production content in native Lactobacillus of Chaharmahal va Bakhtiari province isolated from dairy products. Biological Journal of Microorganism. 2012; 1(3):41-52.

29. Ogunbanwo ST, Sanni AL, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. Afr J Biotechnol. 2003; 2(8): 219-227.

30. Chen CC, Chang CK, Duh PD, Wang PS, Wang SC. Antibacterial properties of *Lactobacillus plantarum* isolated from fermented mustards against *Streptococcus mutans*. 2013; Afr J Microbiol Res. 7: 4787-4793. doi: 10.5897/AJMR12.1885.

31-Taheur FB, Kouidhi B, Fdhila K, Elabed H, Slama RB, Mahdouani K, Bakhrouf A, Chaieb K. Anti-bacterial and anti-biofilm activity of probiotic bacteria against oral pathogens. 2016; Microb Pathog. 97:213-220. doi: 10.1016/j.micpath.2016.06.018.

32. Cotter PD, Hill C. Surviving the acid test: Responses of gram positive bacteria to low pH. Microbiol Mol Biol Rev. 2003; 67:429-453. doi: 10.1128/MMBR.67.3.429-453.2003.

33. Abedi D, Feizizadeh S, Akbari V, Jafarian-Dehkordi A. In vitro antibacterial and anti-adherence effects of *Lactobacillus delbrueckii* subsp *bulgaricus* on *Escherichia coli*. Res Pharm Sci. 2013; 8(4): 260-268.

http://dx.doi.org/10.22037/afb.v4i4.17783

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2017, 4 (4): 241-250
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

اثرات ضد زی‌لایه (Biofilm) لاکتوباسیل‌ها بر علیه سویه‌های *اشرشیا کلی* مولد عفونت ادراری مقاوم به سیپروفلوکساسین در شیر پاستوریزه

مهسا یگانه[۱]، هدایت حسینی[۲*]، صدیقه مهرابیان[۱]، الهام سیاسی تربتی[۱]، سید مرتضی ضمیر[۳]

۱- دانشکده علوم زیستی، دانشگاه آزاد اسلامی واحد تهران شمال، تهران، ایران.

۲- انستیتو تحقیقات تغذیه‌ای و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران.

۳- گروه بیوتکنولوژی، دانشکده مهندسی شیمی، دانشگاه تربیت مدرس، تهران، ایران.

چکیده

سابقه و هدف: *اشرشیا کلی* اوروپاتوژن (عامل بیماریزای دستگاه ادراری) شایع‌ترین عامل سبب‌شناسی عفونت‌های مجاری اداری می باشد. به علاوه، زی لایه‌های ایجاد شده توسط این باکتری‌ها می‌تواند منجر به یکسری مشکلات جدی در صنعت غذا شود. در سراسر جهان، بیماری‌های ناشی از غذا همیشه موجب نگرانی سلامت عمومی بوده است. موارد شیوع بسیاری مرتبط با این زی‌لایه‌ها یافت شده است. بنابراین، هدف تحقیق حاضر بررسی اثرات ضد چسبندگی گونه‌های باکتری‌های لاکتیک اسید بر *اشرشیا کلی* بیماریزای دستگاه گوارش مقاوم به سیپروفلاکسین با استفاده از فناوری‌های میکروبی در شیر پاستوریزه می باشد.

مواد و روش‌ها: در این مطالعه، سویه‌های *لاکتوباسیلوس پلانتاروم*، *لاکتوباسیلوس کازئی* و *لاکتوباسیلوس اسیدوفیلوس* از انستیتو پاستور ایران تهیه شدند. بیست سویه از مولد عفونت مجاری ادراری از بیماران مبتلا به عفونت مجاری ادرار در بیمارستان لبافی نژاد جمع آوری شدند. هشت سویه با توانایی تولید زی‌لایه با آزمون‌های میکروبی جدا شدند. تمام این هشت سویه به سیپروفلوکساسین مقاوم بودند. به منظور ارزیابی حساسیت این سویه‌ها به ده آنتی بیوتیک رایج از روش دیسک آنتی بیوگرام استفاده شد.از میان تمام گونه‌ها، هشت نمونه حساس *اشرشیاکلی* اوروپاتوژن به شیر پاستوریزه تلقیح شدند. روش میکرو پلیت ۱۰۰ چاهک برای تعیین فعالیت ضد زی‌لایه روماند (سوپرناتانت) لاکتوباسیل‌ها مورد استفاده قرار گرفت.

نتایج و بحث: نتایج نشان داد که هشت سویه جدا شده انسانی، مقاوم به آنتی بیوتیک هستند. سویه جدا شده شماره ۴ عامل عفونت ادراری، جزء حساسترین سویه‌ها به اثرات ضد زی‌لایه لاکتوباسیل‌ها در شیر پاستوریزه بودند. اثرات ضدچسبندگی لاکتوباسیل‌ها بر *اشرشیاکلی اوروپاتوژن* با تمام آزمون‌های میکروبی تایید شد. در این مطالعه، *لاکتوباسیلوس پلانتاروم* بیشترین فعالیت مهاری در برابر گونه UPEC 4 را نشان داد. این گونه به عنوان باکتری پروبیوتیک مناسب گزارش شد. براساس نتایج به‌دست آمده، لاکتوباسیل‌ها اثرات چشمگیری بر تشکیل زی‌لایه و بیماریزایی گونه‌های اوروپاتوژن در جلوگیری از چسبندگی می‌باشند..

تعارض منافع: نویسندگان اعلام می کنند که هیچ تعارض منافعی وجود ندارد.

تاریخچه مقاله

دریافت ۹ جولای ۲۰۱۷
داوری ۱۲ اگوست ۲۰۱۷
پذیرش ۲۵ سپتامبر ۲۰۱۷

واژگان کلیدی

- اثر ضد زی لایه
- *اشرشیا کلی* مولد عفونت مجرای ادراری
- *لاکتوباسلوس اسیدوفیلوس*
- *لاکتوباسیوس کازئی*
- *لاکتوباسیوس پلانتاروم*
- مقاوم به سیپروفلوکساسین

***نویسنده مسئول**

هدایت حسینی، انستیتو تحقیقات تغذیه ای و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی ، دانشگاه علوم پزشکی شهیدبهشتی، تهران، ایران.

تلفن: ۹۱۲۳۰۰۷۶۵۵-۹۸+
دورنگار: ۲۲۳۷۶۴۶۷-۲۱-۹۸+
پست الکترونیک:
Hedayat@sbmu.ac.ir