RESEARCH ARTICLE

The antimicrobial and antibiofilm activity of *Lactobacillus salivarius* and *Lactobacillus casei* against *Escherichia coli*

Rania M. Al-Groom[1,2*]
[1]Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Zarqa University, 2000 Zarqa 13110, Jordan
[2]Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, 2000 Zarqa 13110, Jordan

Abstract: Background: Lactobacilli have been crucial for the production of fermented products for centuries. They are also members of the mutualistic microbiota present in the human gastrointestinal and urogenital tract. Recently, increasing attention has been given to their probiotic, health-promoting capacities. **Objectives:** The purpose of this study was to (a) evaluate the antibacterial and antibiofilm activities of *Lactobacillus salivarius* (ATCC 11741) and *Lactobacillus casei* (ATCC 9595) against *Escherichia coli*; and (b) assess the anti-virulence potential of these probiotics, by examining their impacts on the expression of selected genes in the test organism. **Materials and Methods:** The antibacterial, antibiofilm and antivirulence activities of *L. salivarius* and *L. casei* against *E. coli* were investigated by agar well diffusion, microtiter plate, crystal violet assay, quantitative real-time polymerase chain reaction (qPCR) analysis. **Results:** Susceptibility testing indicated antibacterial and antibiofilm activities of *L. salivarius* and *L. casei* against *E. coli*. Agar inhibition assay showed that *L. salivarius* and *L. casei* has antibacterial activity against *E. coli* with an inhibition zone of 21±2 mm and 24±1 mm respectively. The *L. salivarius* and *L. casei* were found to degrade and inhibit *E. coli* biofilm. All biofilm-forming cells treated with *L. salivarius* and *L. casei* supernatants showed reduced expression of genes involved in biofilm formation and quorum sensing. The expression of *yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS* genes of *E. coli* was decreased, 0.75-fold, 0.65-fold, 0.5-fold, 0.73-fold, 1.2-fold and 0.85-fold respectively after exposure to *L. salivarius*, while the expression of *yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS* genes of *E. coli* was decreased, 1.0-fold, 0.75-fold, 0.5-fold, 0.82-fold, 1.4-fold and 0.9-fold respectively after exposure to *L. casei*. **Conclusion:** The results of this study indicate that *L. salivarius* and *L. casei* strains showed a good antibacterial and antibiofilm against *E. coli*. Generally, present study suggested that the *L. salivarius* and *L. casei* strains exhibits a good antimicrobial activity.
Keywords: Probiotics, biofilm, gene expression, *E. coli*

Correspondence to: Rania M. Al-Groom, Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Zarqa University; Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Jordan; E-mail: rgroom@zu.edu.jo; raniaalgroom@bau.edu.jo

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1 Introduction

A new alternative therapy against multidrug-resistant bacteria is required to treat infectious diseases, as biofilm formation is a global public health concern (Aloush et al., 2006; Lara et al., 2010; Subramani et al., 2017). Biofilm is a type of self-produced extracellular matrix, which is embedded by the bacteria to provide a protective environment for them to grow (Colvin et al., 2011; Flemming and Wingender, 2010; Jaffar et al., 2016). One of the resistance abilities of bacteria is achieved by biofilms formation. By definition biofilm is a community of microorganisms usually adhered to a surface and encased in an extracellular polysaccharide matrix (EPS). Biofilms are highly problematic especially in clinical settings due to their disadvantage that causes refractory chronic infections (Bjarnsholt, 2013) especially their ability to tolerate antimicrobial therapy at concentrations up to 1,000 times greater than those required to inhibit planktonic cells (Dosler and Karaaslan, 2014; Kapoor et al., 2011; Spoering and Lewis, 2001). Therefore, biofilm prolongs the duration of bacterial infections, increases tolerance to antibiotics and provides resistance against phagocytic cells. In addition, biofilm can be formed in a wide range of surfaces both on biotic and abiotic surfaces in humans (Shrout et al., 2011). In the clinic, microbial biofilms through colonization on implants (prosthetic heart valves, catheters and joint replacement) and medical devices, account for hospital-acquired infections that make the patients easily infected by certain pathogens. Moreover, biofilm infections lead to different disorders, for instance, diabetes mellitus, dental caries, medical implants and wound infections that significantly affect the quality of life, cancer development, and subsequently, increase the global morbidity rate (Bjarnsholt et al., 2018). Recent evidence indicates that one of the strongest options for fighting pathogenic biofilms would be probiotics (Barzegari et al., 2020). Probiotics are defined as live microorganisms which

when administered in adequate amounts confer a health benefit on the host (Fang et al., 2018). The possible mechanisms by which probiotics may inhibit enteric pathogens include modification of the host intestinal environment and immune system, competition for nutritional substrates as well as sites of adhesion on intestinal epithelial cells, secretion of antimicrobial compounds and inactivation of toxins (Birošova and Mikulašova, 2009). Earlier studies have reported the use of probiotics in the prevention and treatment of gastrointestinal infections caused by Salmonella (Alcaine et al., 2007).The most extensively studied probiotic strains are reported from genera Lactobacillus and Bifidobacterium, which are also included in many functional foods and dietary supplements (Frick et al., 2007; Macpherson and Harris, 2004). Probiotics are living bacteria that confer a health-related profit to the host when administered in acceptable doses. This action of probiotics is mediated by interacting with host gut microbiota (Barzegari et al., 2020). Lactobacillus (lactic Acid Bacteria, LAB) and Bifidobacterium are the most important microbial genera that are generally used in the preparations of probiotics (Barzegari et al., 2020). These strains support a balanced immune function, healthy gut microbiome and improved nutrient absorption and lead to a healthy host (Sánchez et al., 2017). They are also capable to potentially modulate the microbial ecology of biofilms by pathogens' growth inhibition, adhesion and co-aggregation (Barzegari et al., 2020). Furthermore, probiotics exert antimicrobial activities against the gastrointestinal (GI) tract pathogens via declining luminal pH, competing for adhesion sites and nutrients and producing antimicrobial agents such as bacteriocins, hydrogen peroxide and organic acids (Barzegari et al., 2020). Based on these properties, probiotics present effectiveness in managing biofilms. To date, some articles have been published on the beneficial effects of probiotics on the pathogenic biofilms formation in the wound as well as oral and infectious diseases (Barzegari et al., 2020). Thus, treatment for *E. coli*, infections often becomes a challenge due to the ability of these bacteria to be resistant to antibiotics via producing strong biofilm (Subedi et al., 2018). Therefore, recent studies are focusing alternative antimicrobial strategies to treat bacterial infections. However, there is a lack of information on the biofilm-associated infections involved in altered virulence properties of *E. coli*. Therefore, this study aimed to evaluate the impact of *Lactobacillus salivarius* (ATCC 11741) and *Lactobacillus casei* (ATCC 9595) on the growth, biofilm formation and gene expression profile of *E. coli*.

2 Materials and Methods

2.1 Bacterial strains and culture conditions

A standard reference of *Escherichia coli* (ATCC 8739) was purchased from American Type Culture Collection (ATCC, USA) and used throughout this study. *E. coli* was streaked on nutrient agar (NA) plate and incubated at 37°C for 24 hours. Then the strain was suspended in brain heart infusion (BHI) broth and incubated at 37°C for 24 hours and stored at 80°C in broth with 30% glycerol (Alfarrayeh et al., 2021; Jeong et al., 2018; Prabhurajeshwar and Chandrakanth, 2019; Shaaban et al., 2020; Wasfi et al., 2018; Yonezawa et al., 2015). The following two probiotic LB strains were used in this study: *Lactobacillus salivarius* (ATCC 11741) and *Lactobacillus casei* (ATCC 9595). The strains were cultured in deMan, Rogosa, and Sharpe (MRS) and brain-heart infusion (BHI) media (BD Difco, Franklin Lakes, NJ) at 37°C for 24 hours (Al-kafaween et al., 2021; Wu et al., 2015).

2.2 Agar diffusion assay

The agar diffusion method for antibacterial screening of probiotics. The antibacterial activity of probiotics on *E. coli* was assessed using an agar diffusion method. *E. coli* was incubated in BHI broth at 37°C for 24 hrs. Melted BHI agar medium held at 45°C was inoculated with *E. coli* at a concentration equivalent to McFarland 0.5 standard (1.5×10^8 CFU/ml). Wells of 7 mm diameter were filled by 150 μl of each probiotic. Zones of Inhibition was measured using digital callibir after incubating the plates at 37°C for 24 hrs. The experiment was performed in triplicate (Bidossi et al., 2018; Jeong et al., 2018; Lin et al., 2015; Prabhurajeshwar and Chandrakanth, 2019; Wasfi et al., 2018).

2.3 Antibacterial testing of treated and untreated probiotic

To determine the antibacterial activity of each probiotic, *E. coli* was grown overnight at 37°C in BHI broth. The *E. coli* culture was diluted with BHI broth medium to a turbidity equivalent to McFarland 0.5 (1×10^8 cells/ml). Subsequently, 150 μl of the *E. coli* suspension and 150 μl of untreated supernatants were added to the wells of 96-well plate for each probiotic. The plates were incubated at 37°C for 24 hrs. In control wells, the probiotic was replaced by sterile MRS broth. The OD_{600} nm was recorded after incubation using microplate reader. The experiment was performed in triplicate (Jeong et al., 2018; Prabhurajeshwar and Chandrakanth, 2019; Shaaban et al., 2020; Wasfi et al., 2018).

2.4 The effect of probiotics on *E. coli* adherence

This test was performed in a similar manner as the antimicrobial test using BHI medium supplemented with 0.2% sucrose and the reduction in biofilm formation was evaluated by crystal violet assay as previously described. Initially, after incubation, supernatants were removed and media was then removed by invertip the plate and tapping the plate. The plate was washed three times with PBS to remove free-floating planktonic bacteria and drained for drying. The plate was stained with 200 μl of 0.1% crystal violet for 5 min. Then, the plate was carefully rinsed under running tap water to

remove excess stain, dried at room temperature before solubilizing the biofilm with 95% of ethanol. The absorbance was measured by using a microplate reader at OD_{570}. The experiment was performed in triplicate (Alfarrayeh et al., 2021; Bidossi et al., 2018; Carvalho et al., 2021; Fang et al., 2018; He and Ahn, 2011; Ishikawa et al., 2020; Jeong et al., 2018; Lin et al., 2015; Olson et al., 2018; Sánchez et al., 2017; Wasfi et al., 2018).

2.5 The effect of probiotics on *E. coli* biofilm

An overnight culture of *E. coli* was diluted to McFarland 0.5 in BHI supplemented with 0.2% sucrose. This culture was distributed in the 96-well plate by the volume of 200 μl and incubated at 37°C for 24 hrs. Culture supernatant was removed, and wells were washed with sterile saline. A volume of 200 μl of untreated supernatant was added in each well and incubated at 37°C for 24 hrs. The absorbance was measured by using a microplate reader OD_{570}. The reduction in biofilm formation was determined as previously described. The experiment was performed in triplicate (Alfarrayeh et al., 2021; Bidossi et al., 2018; Carvalho et al., 2021; Fang et al., 2018; He and Ahn, 2011; Ishikawa et al., 2020; Jeong et al., 2018; Lin et al., 2015; Olson et al., 2018; Sánchez et al., 2019; Wasfi et al., 2018).

2.6 Extraction of total bacterial RNA

The effect of probiotics on *E. coli* in the planktonic form and the biofilm form. *E. coli* was grown overnight at 37°C in BHI broth and was diluted to McFarland 0.5. A volume of 200 μl *E. coli* suspension and 200 μl of each probiotic were added to 1 ml of BHI broth and were incubated at 37°C for 24 hrs. In control wells, each probiotic supernatant was replaced by MRS broth. After incubation, culture suspension was removed from wells for RNA extraction from planktonic bacteria. Cells adhering to the plate wells were washed twice by sterile saline and then dislodged and suspended in saline by scraping into a centrifuge tube. The total RNA was isolated from *E. coli* planktonic and adherent cells using kit SV Total RNA Isolation System (Promega, UK) according to the manufacturers instructions. The remaining DNA in RNA samples was treated by RNase-free DNase I to eliminate DNA contamination. Agarose gel electrophoresis of RNA samples verified its integrity. RNA concentration and purity were determined by the ND1000 spectrophotometer (NanoDrop). Finally, Total RNA was converted to cDNA following the manufacturers instructions kit (Promega, UK) (He and Ahn, 2011; Ishikawa et al., 2020; Jeong et al., 2018; Prabhurajeshwar and Chandrakanth, 2019; Sánchez et al., 2017; Wasfi et al., 2018; Wasfi et al., 2016).

2.7 Reverse transcription quantitative real-time PCR and data analysis

RT-qPCR was used to examine the effect of probiotics on the expression levels of six target genes [*yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA* and *rpoS*] involved in biofilm formation, quorum sensing, and stress survival) in *E. coli*. The primers for the qPCR used in the current study (Table 1). Reverse transcription quantitative real-time PCR was performed by Applied Biosystems StepOne. All reactions (20 μl) were performed using three technical replicates. Each reaction mixture contained 100 ng cDNA and 300 nM primers per reaction. The RT-qPCR cycling conditions were as follows: one cycle with 95°C for 2 min.; then 40 cycles of denaturation at 95°C for 5 sec., annealing at 52-62°C (depending on primers used) for 10 sec., and extension and fluorescent data collection at 72°C for 20 sec. A dissociation curve was generated at the end of each reaction. In all qPCR runs, negative controls without template were run in parallel. The 16s rRNA gene (housekeeping gene) was selected as the internal control. The relative mRNA levels of genes of interest were determined and normalized to the expression of the housekeeping gene using the $2^{-\Delta\Delta}$ Ct value analysis. The qPCR data were expressed as the fold change in expression levels of genes in *E. coli* cells exposed to each probiotics as compared to their levels in the untreated cells. The changes in gene expression were tested in the *E. coli* cells in the planktonic form and the biofilm-forming state. The experiment will be performed in triplicate (He and Ahn, 2011; Shaaban et al., 2020; Jeong et al., 2018; Prabhurajeshwar and Chandrakanth, 2019; Sánchez et al., 2017; Wasfi et al., 2018; Wasfi et al., 2016).

Table 1. Gene specific primers of *E. coli* used for RT-qPCR analysis

Gene name	Amplicon size (bp)	Annealing temp (C^0)	Direction primer sequence (5' → 3')
yjfO (bsmA)	76	53	For: CGCCAGTAACGGACCATC Rev: GTGCTTACGCTACCTATTCG
csgA	191	56	For: ATGGCGGCGGTAATGGTG Rev: GTTGACGGAGGAGTTAGATGC
ycfR (BhsA)	81	54	For: CGAAGTTCAGTCAACGCCAGAAG Rev: TCCAGCGATCCCAGATTTGTCC
tnaA	174	54	For: CTGGATAGCGAAGATGTG Rev: CGGAATGGTGTATTGATAAC
lsrA	178	54	For: TACTCATAACCTTCGTGGATTCTG Rev: TACTTGCGGCGAGGCTTC
rpoS	199	54	For: CTCAACATACGCAACCTG Rev: GTCATCAACTGGCTTATCC
16s rRNA Reference gene	101	52	For: CCTACGGGAGGCAGCAGTAG Rev: CAACAGAGCTTTACGATCCGAAA

2.8 Statistical analysis

For all assays, all experiments were performed in triplicate. All data were expressed as mean ± standard deviation. Independent student t-test from (SPSS version 20) was used to compare between treated and untreated groups. The statistical analyses performed were considered significant when $P < 0.05$.

3 Results

3.1 Agar diffusion assay

The zone of inhibition produced by whole bacterial culture (concentration 1.5 $\times 10^8$ cells/ml) was larger than that pro-

duced by spent culture supernatant produced by same concentration of cells. This indicates the higher antibacterial effect of whole bacterial culture as compared to the cell-free filtered supernatant. According to the zone of inhibition diameter, the highest antibacterial activities of probiotics was observed with *Lactobacillus casei*, whereas the lowest antibacterial activities was observed with *Lactobacillus salivarius* (Table 2).

Table 2. Growth Inhibition zone (mm) of probiotic against *E. coli*

Strain	Zone of inhibition (mm)	
	Whole bacterial culture *	Spent culture supernatant *
Lactobacillus salivarius	21±2	15±2
Lactobacillus casei	24±1	19±1

Note: The values of means ± S.D. of inhibition zones (mm). *All results were significantly different from control ($P < 0.05$).

3.2 Antibacterial testing of treated and untreated probiotic

The average of optical density (OD) for control sample and tested sample was calculated. As shown in Figure 1, the probiotics (*L. salivarius* and *L. casei*) showed significant inhibitory effect on the growth of *E. coli* ($P < 0.05$). After treated with probiotics the growth of *E. coli* was reduce by measuring the absorbance. There was significant difference in the potency of the inhibitory effect between the two samples ($P > 0.05$). After neutralizing the supernatant, the antimicrobial effect was significantly reduced ($P < 0.05$) compared with untreated supernatant, yet still showing significant reduction ($P < 0.05$) in *E. coli* growth. *L. salivarius* and *L. casei* were showed significant reduction ($P < 0.05$) in its antimicrobial effect on *E. coli* indicating that both probiotics contribute in its antimicrobial effect against *E. coli* (Figure 1).

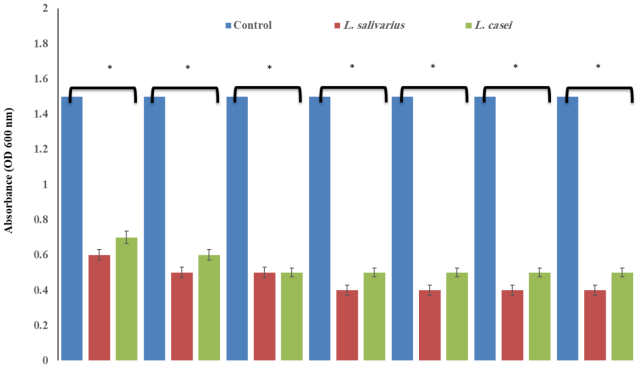


Figure 1. Optical density (OD) of *E. coli* growth in the presence of *L. salivarius* and *L. casei*. Data are expressed as the mean ± S.D., *$P < 0.05$ compared with *E. coli* growth in broth as control.

3.3 Effect of probiotics on *E. coli* adherence and preformed biofilm

Growth of *E. coli* biofilms in the presence of two probiotics was significantly ($P < 0.05$) reduced relatively to the untreated control. The preformed biofilm was decreased compared to the untreated control. *L. salivarius* and *L. casei* supernatant caused significant reduction ($P < 0.05$) in *E. coli* adherence and preformed biofilm. Reduction percentages were 78% and 67%, respectively. The effect of *L. casei* supernatant was the least among tested supernatants on adherence as it showed significant effect on the preformed biofilm. The *L. salivarius* and *L. casei* supernatant caused reduction in adherence with percentages of 78% and 67% respectively and reduction in preformed biofilm with percentage of 22% and 33% respectively (Figure 2).

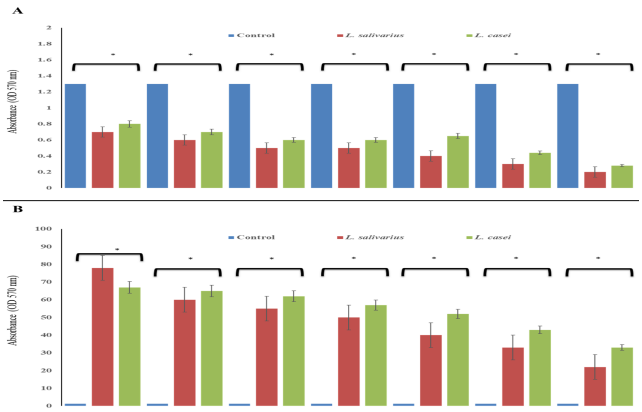


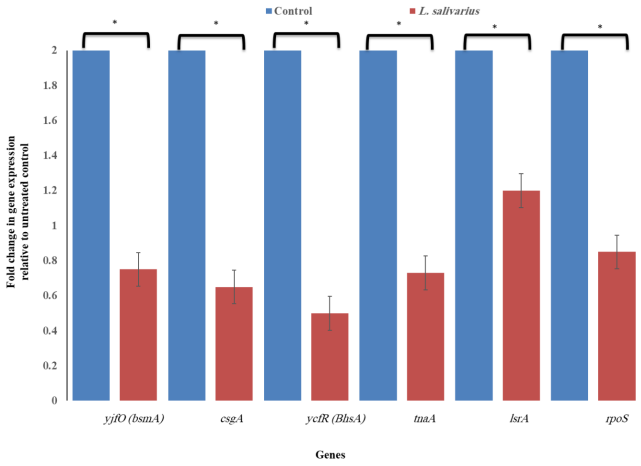
Figure 2. Effect of probiotics on *E. coli* biofilm. (*L. salivarius* and *L. casei*). Control: *E. coli* grown in broth. Data are expressed as the mean ± S.D. *$P < 0.05$, compared with control.

3.4 RT-qPCR analyses

In the current study, qPCR was used to evaluate and compare the impact on *E. coli* cells after exposure to two probiotics (*L. salivarius* and *L. casei*) overnight. The levels of expression of six genes, that have been previously shown to be involved in virulence of the *E. coli* in the planktonic and biofilm-forming cells, were compared to the control untreated cells prepared under the same conditions with and without probiotics. The selected genes included three genes involved in biofilm formation [*yjfO* (*bsmA*), *csgA*, and *ycfR* (*BhsA*)], two genes involved in quorum sensing (*tnaA* and *lsrA*), and one gene associated with stress survival *rpoS*. The ct values between biological replicas were standardized against the reference gene and changes in relative expression to untreated cells were analysed.

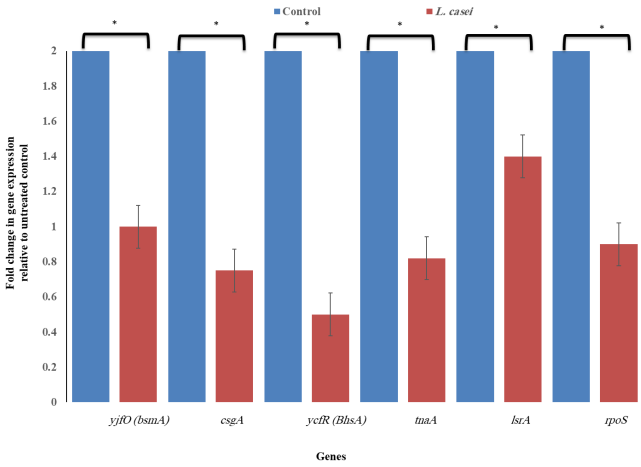
As revealed by the independent student t-test from (SPSS version 20), there was a significant overall difference ($P < 0.05$) in the expression of each of the tested genes between the exposed and control group, in both planktonic forms and biofilm-forming cells. All genes, [*yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS*] were downregulated following exposure to *L. salivarius* and *L. casei* (Figure 3 and Figure 4). Although different degrees of downregulation were observed following exposure to the *L. salivarius* and *L. casei*. Significant reduction in gene expression of *yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS* forming genes was observed in the *E. coli* cells in the presence of *L. salivarius* and *L. casei*.

In the case of *yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS* genes, its expression was downregulated following exposure to the *L. salivarius* and *L. casei*. The expression of *yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS* genes of *E. coli* were decreased, 0.75-fold, 0.65-fold, 0.5-fold, 0.73-fold, 1.2-fold and 0.85-fold respectively after exposure to *L. salivarius* (Figure 3), while the expression of *yjfO* (*bsmA*), *csgA*, *ycfR* (*BhsA*), *tnaA*, *lsrA*, and *rpoS* genes of *E. coli* were decreased, 1.0-fold, 0.75-fold, 0.5-fold, 0.82-fold, 1.4-fold and 0.9-fold respectively after exposure to *L. casei* (Figure 4)



Note: Mean values of fold changes (± SD) are shown in relation to untreated (control) *E. coli* cells. Asterisks indicate statistically significant differences in the expression of each gene between treated samples and control ($^*P < 0.05$).

Figure 3. Alterations in gene expression profiles associated with exposure of *E. coli* to *L. salivarius* as determined by qPCR.



Note: Mean values of fold changes (± SD) are shown in relation to untreated (control) *E. coli* cells. Asterisks indicate statistically significant differences in the expression of each gene between treated samples and control ($^*P < 0.05$).

Figure 4. Alterations in gene expression profiles associated with exposure of *E. coli* to *L. casei* as determined by qPCR.

4 Discussion

Selected probiotics particularly *L. salivarius* and *L. casei* had good inhibitory effects against *E. coli* pathotype (Karimi et al., 2018; Wasfi et al., 2018). Many studies showed growth inhibitory effects of probiotics against different pathogens. Study by Karimi et al., 2018 showed that yogurt consumption causes intestinal colonization of probiotic bacteria such as Lactobacillus, and provided conditions to prevent colonization of EHEC (Karimi et al., 2018). Another study showed growth inhibitory effects of probiotic *Lactobacillus casei* and *Enterococcus fascium* against *Listeria monocytogenesis*, *Escherichia coli* bacillus cereus and *Salmonella enteritidis* (Hassanzadazar et al., 2014; Hassanzadazar et al., 2012). Obtained results of the present study showed growth inhibitory effects of two probiotics against *E. coli*. Similar studies confirmed antimicrobial effects of culture supernatant of probiotics, for example previous study showed growth inhibitory effects of *L. plantarum* and *L. curvatus* against different pathogens with well diffusion method (Karimi et al., 2018). Matsusaki studied growth inhibitory effects of probiotic Lactobacillus with colony count method (Karimi et al., 2018). All of the probiotic tested using plate assays inhibited *E. coli*. The extent of inhibition was dependent on the probiotic strain, such that *L. salivarius* tended to inhibit *E. coli* growth to a greater extent than that observed for the *L. casei*. Based on the results of this study, present probiotic bacteria in natural resources can be used for inhibition and reduction of pathogens, including enteric pathogens and antibacterial effects of their metabolites are active and stable under different conditions of temperature and acidity. A variety of genes have been shown to be important in *E. coli* fitness and pathogenicity, and thus modulating the expression of these genes can add to the effectiveness of antimicrobial therapy. Six of these genes, which are involved in biofilm formation, quorum sensing, and stress survival in *E. coli*, were selected for this study, and their differential gene expression profiles in response to exposure to the tested probiotics were determined using qPCR.

A number of genes included three genes involved in biofilm formation [*yjfO* (*bsmA*), *csgA*, and *ycfR* (*BhsA*)], two genes involved in quorum sensing (*tnaA* and *lsrA*), and one gene associated with stress survival *rpoS* in *E. coli* (Alkafaween et al., 2021). The current results showed that all genes were downregulated after exposure to *L. salivarius* and *L. casei* with different digress of downregulation. This pattern of expression was the same regardless of the probiotics. Therefore, the current findings may suggest that the both probiotics under study can prevent or disrupt *E. coli* biofilms. It has to be noted that the biological relevance of downregulating the above-mentioned genes may not be strictly limited to biofilm disruption, with a possibility to affect multiple cellular processes. Previous study mentioned that the mutation of the *yjfO* gene in *E. coli* has been shown to cause alteration of cell motility, increased sensitivity to pH

and oxidative stresses, and reduction of viability, rather than only affecting the biofilm formation (Lee et al., 2011). A set of genes have been previously shown to play an important role in the quorum-sensing network in *E. coli*, such as the *tnaA* and *lsrA* genes (Wasfi et al., 2016). The present results showed that both genes were downregulated in response to all the tested probiotics. It is tempting to speculate that the tested probiotics may act as quorum-sensing inhibitors, and thus may have the potential to decrease the virulence of pathogens like *E. coli*, by interrupting their cellular communication system.

5 Conclusion

In conclusion, with increasing rates of antimicrobial resistance in important pathogens, there is a growing interest in the targeted application of lactobacilli against pathogens. This study has shown that both lactobacilli *L. salivarius* and *L. casei* can inhibit *E. coli*. The current study suggested that the *Lactobacillus* strains in the present study displayed potential probiotic properties. These strains had significant antimicrobial effect against *E. coli*. Moreover, we showed the antibiofilm effect of *Lactobacillus* strains against *E. coli*. The effects of *L. salivarius* and *L. casei* probiotics provides antibacterial effect against pathogenic bacteria. The results of this study indicated that *L. salivarius* and *L. casei* probiotics directly inhibit growth and biofilm of *E. coli* by reduced the level of gene expression of various genes in *E. coli*. However, further studies are needed to investigate probiotic characteristics of various Lactobacillus strains.

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Conflict of Interest

There is no conflict of interest to be declared.

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