
ORIGINAL ARTICLE

Cell Surface Properties and Biofilm formation ability of Lactic Acid Bacteria from the Oral Cavity

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ABSTRACT

*Extensive research on the surfactant properties of lactic acid bacteria strains has unveiled a multitude of intriguing physicochemical and biological characteristics, encompassing antimicrobial, antibiofilm, and therapeutic attributes. Strains *Lactiplantibacillus salivarius* OC 1 (46%), *Pediococcus acidilactici* OC1 (48%), *Lacticaseibacillus rhamnosus* OC1 (48%), *Lactiplantibacillus acidophilus* 2 (54%) showed moderate autoaggregation activity. It was found that strains with the highest autoaggregation ability (*P. acidilactici* OC1, *L. rhamnosus* OC1, *L. plantarum* mal, *L. fermentum* X) showed strong coaggregation interaction with respect to opportunistic microorganisms. Among the 37 strains of lactobacilli studied, 18 had the ability to form biofilms. *Pediococcus acidilactici* B2, *Lactiplantibacillus plantarum* K2, *Lactiplantibacillus plantarum* 42, *Streptococcus salivarius* OC1 showed that the biofilm formed by the cultures effectively inhibits the growth of both planktonic cells and cells in the biofilm of *Staphylococcus aureus* and *Klebsiella pneumoniae*. Beyond their probiotic properties, the capacity of lactobacilli to impede the formation of biofilms by opportunistic microorganisms positions them as promising candidates for future applications in the development of next-generation antimicrobial drugs or as supplementary agents to complement existing antibiotics.*

Keywords: lactic acid bacteria strains, cell surface properties, aggregation, coaggregation, adhesion, biofilm formation, oral cavity.

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INTRODUCTION

Biofilms refer to organized microbial communities that adhere to surfaces, enclosed within a protective extracellular matrix [1]. Biofilm formation accompanies approximately 65% of all human microbial infections, making it imperative to understand the role, composition, and impact of microbial biofilms on human health. Within biofilms, fungi and bacteria, known as "residents," exhibit reduced sensitivity or even insensitivity to antimicrobial drugs. The ability of microorganisms to adhere to diverse surfaces promotes biofilm development in clinical settings, such as catheters, prosthetic heart valves, joints, and various host tissues. This adherence leads to effective colonization, contributing to persistent drug resistance [2]. Therefore, comprehending the dynamics of biofilm formation becomes crucial in addressing the challenges posed by persistent microbial infections and drug resistance.

The term "biofilm" was coined by the renowned American researcher J. Costerton, who initiated in-depth investigations into this phenomenon [3]. According to contemporary understanding, a biofilm is a meticulously organized community of microorganisms enclosed within a polymeric matrix synthesized by community members. This matrix attaches to living or inert surfaces. Biofilms exhibit structural heterogeneity, genetic diversity (formed by representatives of various microorganism species, including bacteria, protozoa, fungi, and algae), intricate regulatory mechanisms governing "social relationships" within the community, and the presence of an extracellular exopolymeric matrix (EPS). This EPS, comprising polysaccharides, proteins, lipids, and nucleic acids (RNA and extracellular DNA, eDNA), shields microorganisms from external factors, whether environmental or immune defense factors within

the body [4]. The matrix or extracellular polymeric substance (EPS) is a polymeric substance released by adherent cells into the external environment. It consists of 97% bound water, maintaining high hydration. With a porous structure, it allows the passage of low molecular weight compounds while trapping proteins, large molecules, and particles from the medium. The production of this specific link is a crucial condition for the development of a mature biofilm. Some researchers underscore the importance of a polysaccharide component for all biofilms, with sugars varying in structure and composition, primarily represented by β -1,6-N-acetyl-D-glucosamine and cellulose. Proteins make up 40% of the total biofilm matrix, with amyloid-like proteins (curli fibers in Gram-negative bacteria and TasA/TapA proteins in bacilli) representing a significant portion. Additionally, specific proteins such as bap-family proteins, lectins, sugar-binding proteins, and autotransporters are isolated in the biofilm. These proteins contribute to intercellular contacts, cell-to-cell and cell-to-substrate attachment, and participate in binding polysaccharides for biofilm organization [5, 6]. Moreover, water channels within the biofilm structure facilitate the delivery of dissolved substances from the medium to the underlying cell layers. These water channels have the potential to serve as a means of delivering antibacterial agents, including relatively large objects such as bacteriophages [7].

Antibiotics, the primary treatment for bacterial infections, exhibit limited efficacy against microbes within biofilms. Currently, there is no antibiotic specifically designed for combating biofilms, and the multidrug resistance observed in biofilms is surpassing the increasing bacterial resistance to antibiotics [8, 9].

Consequently, infections associated with bacterial biofilms pose a pressing challenge for modern medicine. Ongoing efforts are dedicated to developing approaches for their prevention and treatment. However, there is still no solution for completely eradicating mature biofilms, particularly those formed on the surfaces of implantable medical devices. As a result, there is an urgent need to expand the array of tools and explore alternative methods to combat pathogens capable of forming biofilms, making this a critical issue in contemporary medicine.

The purpose of the investigation is to study the cell surface properties and mechanisms of biofilm formation in lactic acid lesions isolated from the oral cavity, with the aim of discovering their role in maintaining oral health and the possibility of application in the medicine.

MATERIAL AND METHODS

Aggregation ability of lactic acid bacteria (LAB) strains. The aggregation ability of LAB strains was studied using the method described by Collado et al. (2008), with some modifications [10]. Bacterial cells from an overnight culture were harvested by centrifugation (5 000 g, 20 min, 4°C), washed twice in phosphate buffer with pH7.1 (10 mM Na₂HPO₄, 1 mM KH₂PO₄, 140 mM NaCl, 3 mM KCl) and suspended in this solution. The absorbance of the cell suspension was adjusted to 0.25 ± 0.05 (A_{600 nm}), at which the titer of live cells is 10^7 - 10^8 CFU/mL. The optical density of homogenized bacterial suspension was measured after 2, 5 and 24 hours without shaking. % aggregation was calculated by the formula $[1 - (A_{time}/A_0)] \times 100$. Where A_{time} is absorbance at 2, 5 and 24 hours, A₀ absorbance at 0.

Coaggregation ability. To determine the coaggregation ability of LAB strains we used suspensions of daily cultures of the studied strains prepared according to the method of studying the aggregation ability. Strains of opportunistic microorganisms: *Enterobacter cloacae* 1, *Acinetobacter pittii* 1, *Klebsiella pneumoniae*, *Staphylococcus aureus* 3, *Staphylococcus epidermidis*, *Enterobacter bugandensis* 22, *Shewanella putrefaciens* 1, *Echerichia coli* 1 served as test cultures.

The mixtures were incubated at 37°C without stirring and the optical density (OD_{600 nm}) was measured after 24 hours at 37°C. The percentage of co-aggregation was calculated as $[(A_{pat} - A_{lact}) / 2(A_{mixture}) / (A_{pat} - A_{lact}) / 2] \times 100$, where A_{pat} and A_{lact} represent the absorbance in tubes containing only the pathogen or *Lactobacillus* strain, respectively, and A_{mixture} represents the absorbance of the mixture after 24 hours [11].

Adhesive ability of lactobacteria strains isolated from the oral cavity. The adhesive properties of lactobacteria were evaluated using the method of V.I. Brilis [12]. Human erythrocytes of blood group 0 (I) Rh+ served as a substrate for lactobacilli adhesion.

The adhesive properties of the studied lactobacteria cultures were evaluated under a light microscope by determining the average adhesion index, which is equal to the average number of lactobacteria attached to one erythrocyte. The criteria for assessing the adhesive properties of microorganisms according to the method of V.I. Brilis were as follows: high degree of adhesion - 4.01 and more bacteria, medium degree of adhesion - from 2.01 to 4.0 bacteria, low degree of adhesion - from 1.01 to 2.0 bacteria, zero degree of adhesion - 0-1.0 bacteria on the surface of one erythrocyte.

Method for determination of biofilm formation by lactobacteria strains. Determination of biofilm formation by lactic acid bacteria was performed according to the previously described method with some

modifications [13]. Overnight cultures of each strain were diluted in freshly prepared MRS-bouillon at a ratio of 1:100 and grown in 96-well microtiter plates at 37°C for 48 h under aerobic conditions. The negative control wells contained only MRS broth. Total cell mass was measured as absorbance at 630 nm in a spectrophotometer (Bio-Rad, Hercules, CA). After incubation, the plates were decanted and the wells were washed three times by immersion in sterilized distilled water. Microtitration plates were dried for 30 min and then stained with 0.1% aqueous solution of crystal violet. The biofilms were incubated for 20 min at room temperature and then washed six times with distilled water until unbound crystal violet was removed. The plates were then air-dried once more for 30 min. One hundred microliters of 95% ethanol were added to each well and the absorbance was measured after 30 min. The threshold value (ODC) was determined as the mean of the OD of the negative control. Based on the OD, strains were classified as no biofilm formers ($OD \leq ODC$), weak biofilm formers ($ODC < OD \leq 2x ODC$), moderate ($2x ODC < OD \leq 4x ODC$) or strong biofilm formers ($4x ODC < OD$) [14].

Antagonistic activity of biofilms of lactobacteria cultures. The antagonistic activity of biofilms of lactobacteria cultures was studied according to the previously described method with some modifications [15]. Overnight cultures of all strains grown in MRS broth were diluted to an optical density of 0.1 in freshly prepared MRS broth medium. Five microliters of inoculum were added to the wells of a sterile 96-well polystyrene flat-bottom plate filled with 100 ml of fresh broth. Seeds were cultured for 48 h at 37°C under aerobic conditions and then unattached cells were gently removed using a pipette and biofilms visually present on the bottom and sides of the plate were washed with 2 mL of phosphate buffer at pH7.1 (10 mM Na_2HPO_4 , 1 mM KH_2PO_4 , 140 mM NaCl, 3 mM KCl) to remove planktonic and poorly attached cells. Absorbance (A600 nm) of suspensions of pathogenic bacteria in TSE-broth was adjusted to 0.25-0.05 to standardize the number of bacteria (10^7 - 10^8 CFU/ml), added to biofilms and incubated at 30°C for 24, 48 and 72 hours. Every 24 hours, half of the broth in the wells was replaced with fresh broth. After incubation, the planktonic cultures were carefully removed and the biofilm cells were suspended by scraping and thorough shaking. To estimate the number of attached bacteria in the biofilm three wells of each strain were washed three times and scraped as previously described. The resulting suspensions were transferred into sterile tubes and mixed by vortexing for 30 s. Next, dilutions in saline (0.85% (wt./vol.) NaCl) were prepared, seeded on Miller-Hinton agar medium, incubated at 37°C for 24-48 hours, and bacterial counts were performed.

RESULTS

Aggregation capacity of strains. To initially screen the probiotic potential of strains and to determine the correlation between cell surface indices and adhesion, we tested the autoaggregation and coaggregation abilities of 12 lactobacteria strains.

The autoaggregation capacity (AC) of 12 *Lactobacillus* strains isolated from the oral cavity was found to range from 26 to 52% after 5 h of incubation at 37°C (Fig. 1). To exclude the activity of secreted extracellular compounds promoting autoaggregation, the autoaggregation capacity of cells suspended in their own supernatant was determined. Based on the % AC, strains can be divided into three groups: high degree of autoaggregation capacity (HAC) $\geq 70\%$, medium degree of autoaggregation capacity (MAC) 20-70% and low degree of autoaggregation capacity (LAC) $\leq 20\%$ [9]. As shown in Fig. 1, all 12 strains belong to the group with a medium degree of AC. Within this group, strains *Lactiplantibacillus salivarius* OC 1 (46%), *Pediococcus acidilactici* OC1 (48%), *Lacticaseibacillus rhamnosus* OC1(48%), *Lactiplantibacillus acidophilus* 2 (54%) showed relatively high autoaggregation activity.

Leuconostoc lactis Sh and *Lactiplantibacillus fermentum* F strains showed the lowest autoaggregation activity, 29% and 26%, respectively. The observed autoaggregation ability of the tested LAB strains is strain dependent and may be related to cell surface components, as it was not lost after washing and suspending the cells in phosphate buffer.

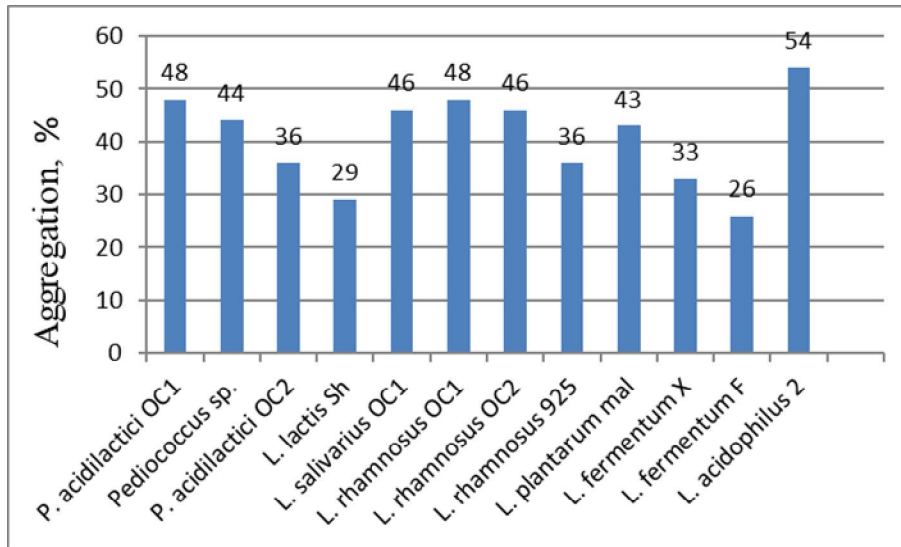


Fig. 1. Autoaggregation ability of lactobacteria strains

Autoaggregation is important in biofilm formation. In many cases, the aggregation ability is related not only to the adhesive properties of the cells, but also to their ability to survive and persist in the gastrointestinal tract [16]. The relationship between autoaggregation and adhesive abilities has been described in the case of several species of bifidobacteria [17].

Coaggregation ability. Coaggregation rates between strains of lactobacteria and opportunistic microorganisms isolated from patients with acute tonsillitis were determined. The results are presented as the percentage of decrease in absorption of the mixed suspension compared to the individual suspension. All the strains studied showed coaggregation ability with opportunistic microorganisms isolated in acute tonsillitis. But this property has strain specificity. Fig. 2, 3, 4 and 5 present data of the strains that showed the highest coaggregation ability. Among the studied strains of *Pediococcus* genus, *P. acidilactici* OC1 strain showed strong coaggregation ability against 3 strains of tested opportunistic microorganisms: *Enterobacter cloacae* 1, *Staphylococcus aureus* 3, *Enterobacter bugandensis* 22 (Fig. 2). The % of coaggregation after 24 hours is 26%, 28% and 32%, respectively.

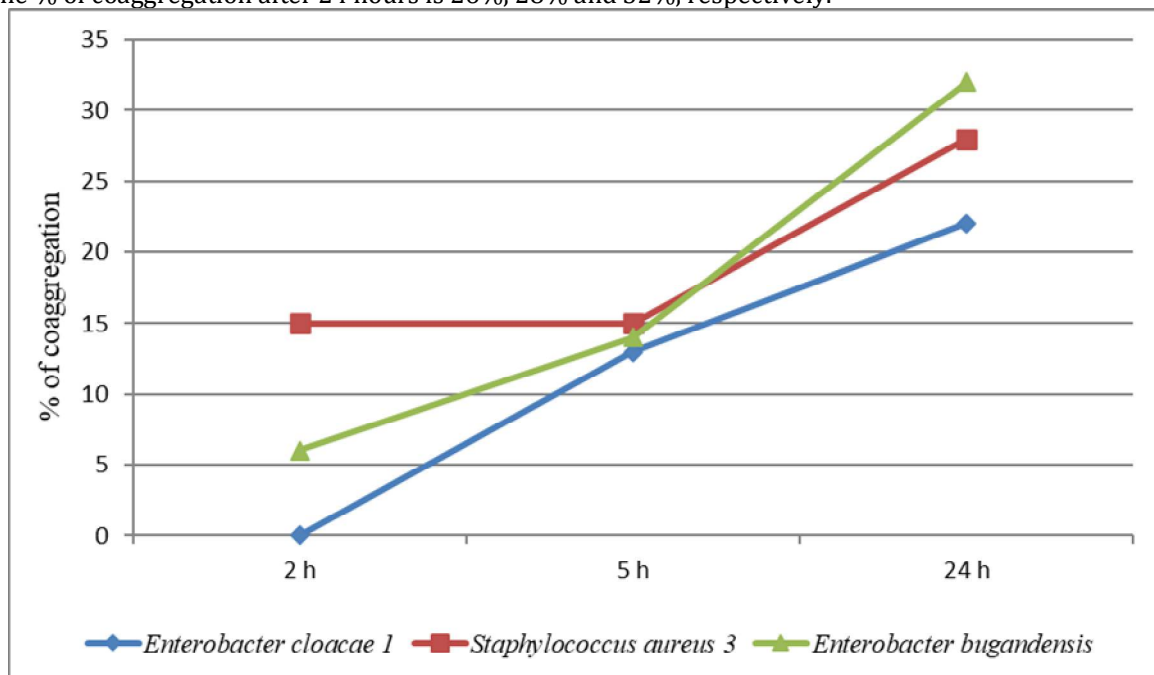


Fig. 2. Coaggregation ability of *Pediococcus acidilactici* OC1 strain

Among 3 strains of *L. rhamnosus* OC1 strain showed the strongest coaggregation ability against the tested opportunistic microorganisms: *Enterobacter cloacae* 1, *Klebsiella pneumonia*, *Staphylococcus aureus* 3,

Staphylococcus epidermidis (Fig. 3). The % of coaggregation after 24 hours was 32%, 27%, 29% and 32%, respectively.

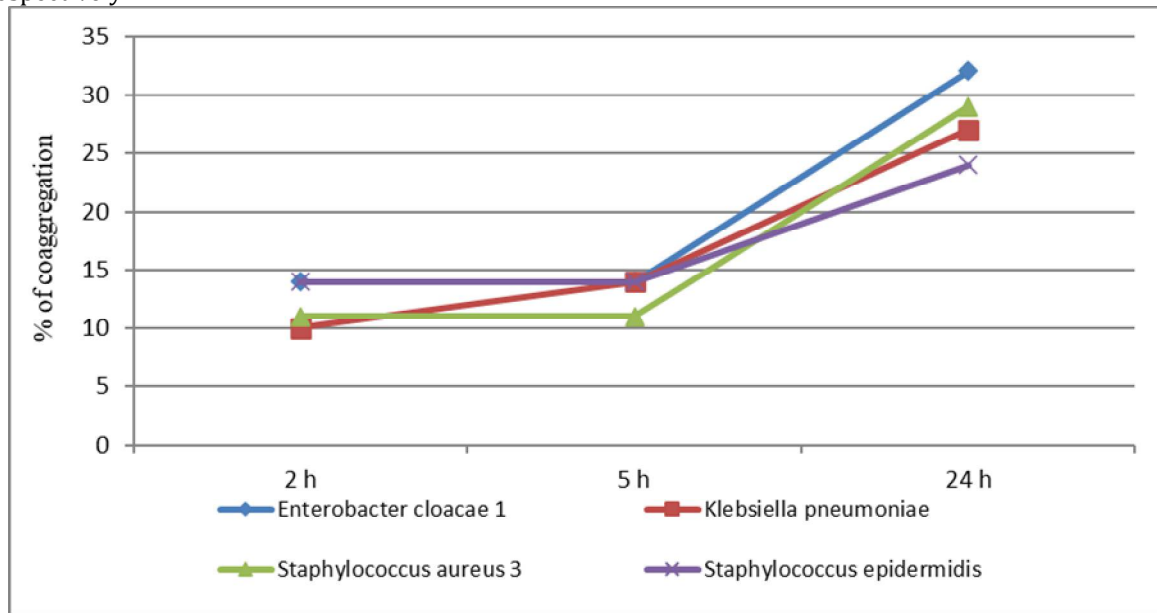


Fig. 3. Coaggregation ability of *L. rhamnosus* OC1 strain

L. plantarum mal strain showed strong coaggregation ability against 6 tested opportunistic microorganisms: *Enterobacter cloacae* 1, *Escherichia coli* 1, *Acinetobacter pittii* 1, *Klebsiella pneumoniae*, *Staphylococcus aureus* 3, *Staphylococcus epidermidis* (Fig. 4). The % of coaggregation after 24 hours was 24%, 24%, 27%, 28%, 28% and 27%, respectively.

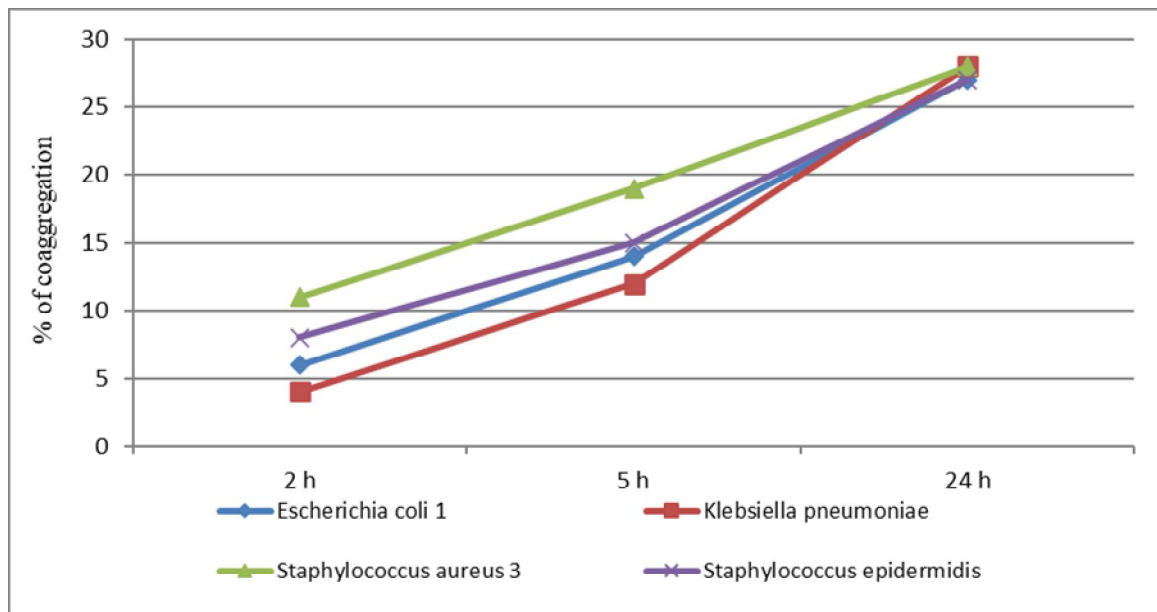


Fig. 4. Coaggregation ability of *L. plantarum* mal strain

L. fermentum X strain showed coaggregation ability against 3 tested opportunistic microorganisms: *Enterobacter cloacae* 1, *Klebsiella pneumoniae*, *Staphylococcus epidermidis* (Fig. 5). The % of coaggregation after 24 hours amounted to 27%, 27% and 25%, respectively.

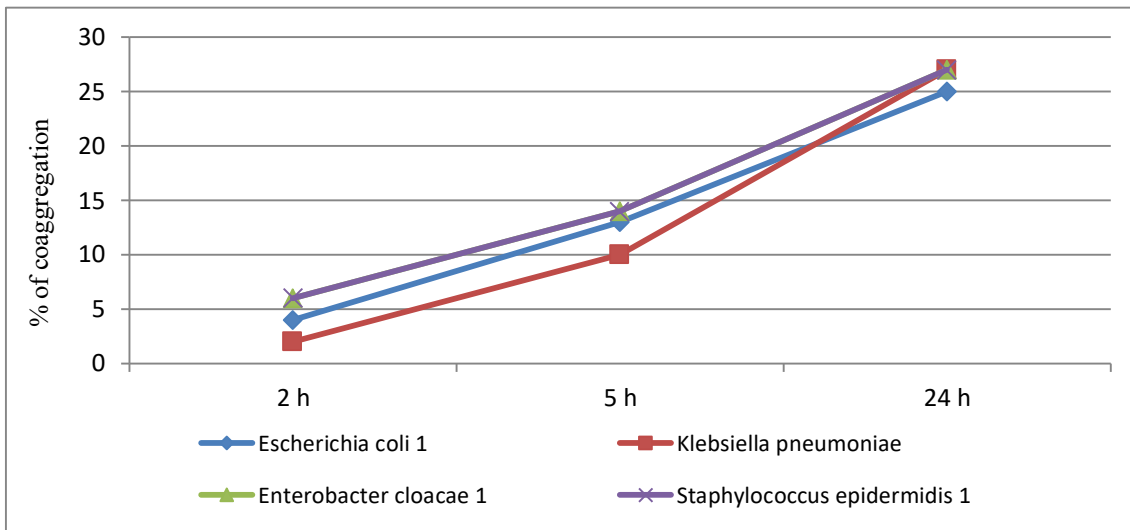


Fig. 5. Coaggregation ability of *L. fermentum* X strain

It was found that strains with the highest autoaggregation ability (*P. acidilactici* OC1, *L. rhamnosus* OC1, *L. plantarum* mal, *L. fermentum* X) showed strong coaggregation interaction with respect to opportunistic microorganisms. Including the *Lactiplantibacillus acidophilus* 2 strain, which has high autoaggregation ability, showed coaggregation activity against 6 strains of pathogens: *Enterobacter cloacae* 1, *Acinetobacter pittii* 1, *Klebsiella pneumoniae*, *Staphylococcus aureus* 3, *Staphylococcus epidermidis*, *Enterobacter bugandensis* 22. It is important to note that the coaggregation ability of this culture reaches a high degree already after 5 hours of joint incubation (Fig. 6).

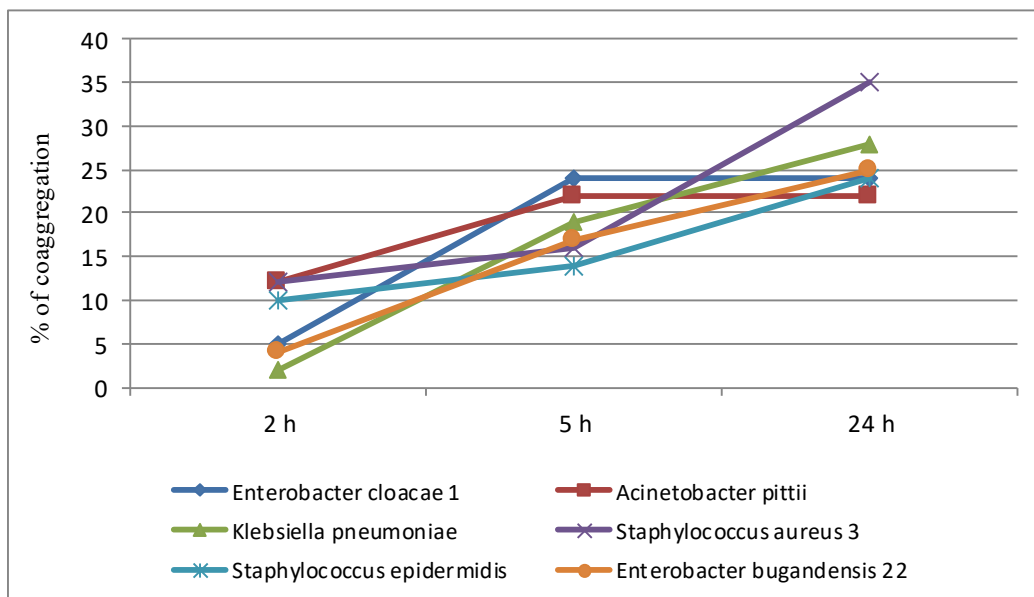


Fig. 6. Coaggregation ability of *L. acidophilus* 2 strain

Lactiplantibacillus strains can form a barrier that prevents colonization by pathogenic bacteria through coaggregation [18]. Coaggregation of lactobacteria strains with potential pathogens promotes the production of antimicrobial compounds while interacting very closely with them. Aggregation properties together with coaggregation ability can be used in the primary selection of probiotic bacteria [18, 19]. Autoaggregation and coaggregation are important in biofilm formation to protect the host from pathogen colonization. It is believed that when probiotic lactobacteria are present in sufficient numbers in the host, a healthy balance between beneficial and potentially harmful microflora is created [20, 21].

Adhesive activity of lactobacteria strains isolated from the oral cavity. Adhesive ability is recognized as an important attribute of probiotics. When probiotics attach to the epithelium, they function stably in the intestine. To initially screen the probiotic potential of strains and to determine the correlation between cell surface indices, we tested the adhesive capacity of strains that showed high autoaggregation capacity.

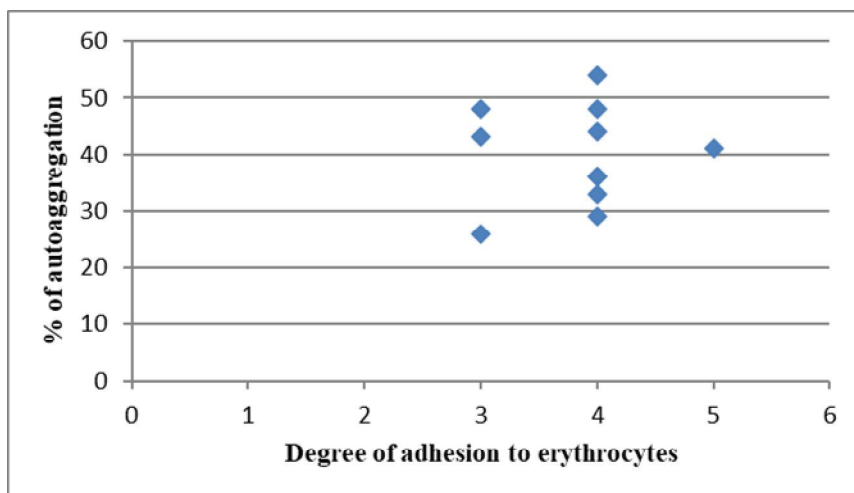


Fig. 7. Correlation between autoaggregation and adhesive abilities of *Lactobacillus* strains

Adhesive activity is one of the important criteria in assessing the prospects for the use of strains in probiotic preparations, because it directly affects the duration of persistence of lactobacteria in the body [17]. The analysis of the data on the adhesive properties of lactobacteria with respect to human erythrocytes presented in fig. 7 shows that the strains studied have a medium degree of adhesive activity with respect to human erythrocytes. Adhesive activity and autoaggregation ability of lactobacteria strains have a positive correlation.

Biofilm formation by lactic acid bacteria isolated from the oral cavity and strains from a collection of microorganisms. The ability of biofilm formation by 37 strains of lactic acid bacteria was studied: 10 of which were isolated from the oral cavity and 27 strains were obtained from the collection of microorganisms, with a time interval of 24 hours, 48 hours and 72 hours.

The results showed that incubation time had a significant effect on biofilm formation. The process of biofilm formation over time; with increasing incubation time, the rate of biofilm formation increased and reached the maximum value after 72 h. Strains of *Pediococcus* genus were characterized by high biofilm formation ability. Out of 7 strains studied, 4 strains had biofilm-forming ability. The biofilm-forming ability index ranged from 2.3xODC to 4.7xODC.

Also, among the strains of *Lactiplantibacillus plantarum* species, high biofilm formation ability was observed. Out of 7 strains studied, 4 strains had biofilm-forming ability The index of biofilm-forming ability ranged from 2.6xODC to 5.1xODC (Table 1).

Table 1: Biofilm formation by lactic acid bacteria isolated from oral and microbial collection strains

Types of LAB	Number of strains tested	Number of strains forming biofilm	A strain with a high biofilm-forming capacity
<i>Pediococcus acidilactici</i>	7	4	<i>Pediococcus acidilactici</i> B2
<i>Lactiplantibacillus plantarum</i>	7	4	<i>Lactiplantibacillus plantarum</i> mal
<i>Lactocaseibacillus rhamnosus</i>	4	2	<i>Lactiplantibacillus rhamnosus</i> 925
<i>Lactiplantibacillus brevis</i>	4	4	<i>Lactiplantibacillus brevis</i> Fr2
<i>Lactobacillus fermentum</i>	3	1	<i>Lactiplantibacillus fermentum</i> OC1
<i>Enterococcus durans</i>	2	1	<i>Enterococcus durans</i> OC1
<i>Streptococcus salivarius</i>	2	1	<i>Streptococcus salivarius</i> 17P
<i>Lactiplantibacillus acidophilus</i>	2	0	-
<i>Apilactobacillus kunkeei</i>	1	1	<i>Apilactobacillus kunkeei</i> 1
<i>Lactiplantibacillus paracasei</i>	2	0	-
<i>Lactiplantibacillus reuteri</i>	1	0	-
<i>Leuconostoc lactis</i>	1	1	<i>Leuconostoc lactis</i> Sh
<i>Enterococcus faecium</i>	1	0	-
Total	37	18	

Among the strains of *Lacticaseibacillus rhamnosus* species a high biofilm formation ability was found. Of the 4 strains studied, 2 strains have the strongest biofilm-forming ability. The biofilm forming ability index ranged from 2.6xODC to 5.1xODC. And also, all the studied strains of *Lactiplantibacillus brevis* (4 strains) showed biofilm forming ability

Biofilm formation by opportunistic microorganisms isolated from the oral cavity. The ability of biofilm formation of 37 strains of opportunistic microorganisms with time intervals of 24 hours, 48 hours and 72 hours was studied.

It was revealed that the ability to form biofilms of opportunistic microorganisms is significantly affected by incubation time.

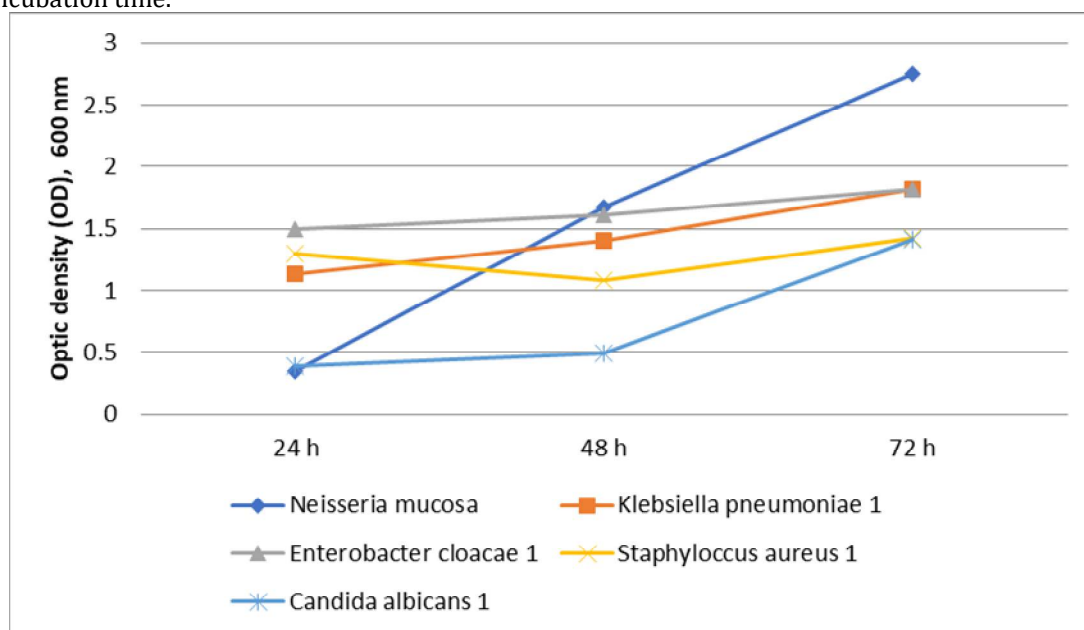


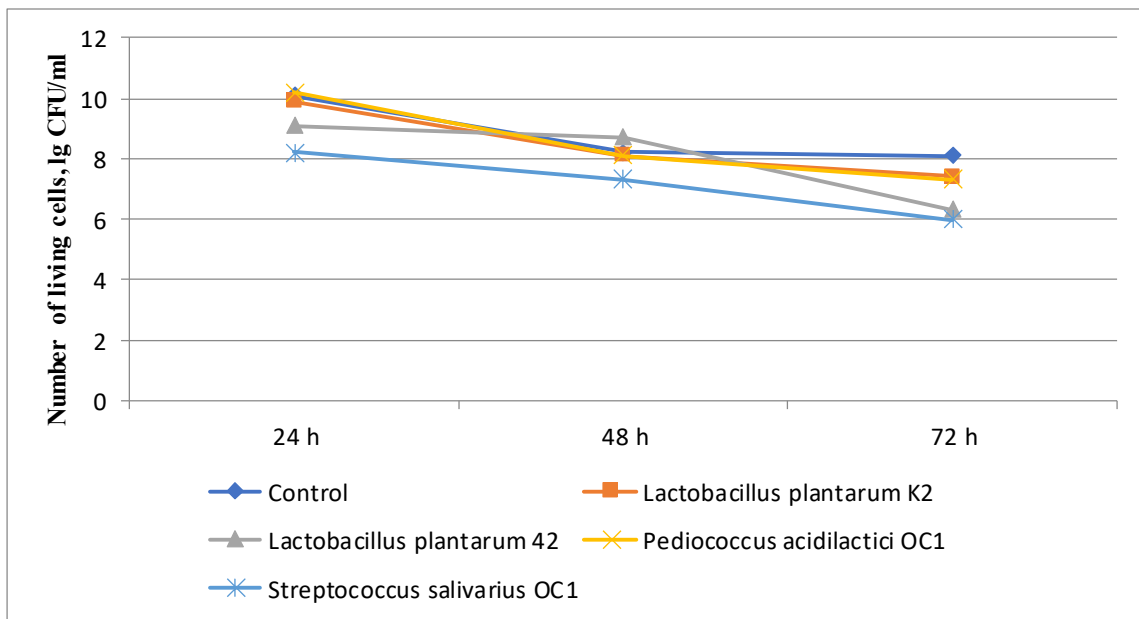
Fig. 1. Ability of opportunistic microorganisms to form biofilms over time

Fig. 1 shows the process of biofilm formation over time; with increasing incubation time, the rate of biofilm formation increased and reached its maximum value after 72 h. Strains with high biofilm-forming ability were found among microorganisms *Staphylococcus aureus*, *Enterobacter cloacae*, *Neisseria mucosa*, *Acinetobacter pittii*, *Klebsiella pneumoniae* and *Candida albicans* (Table 2).

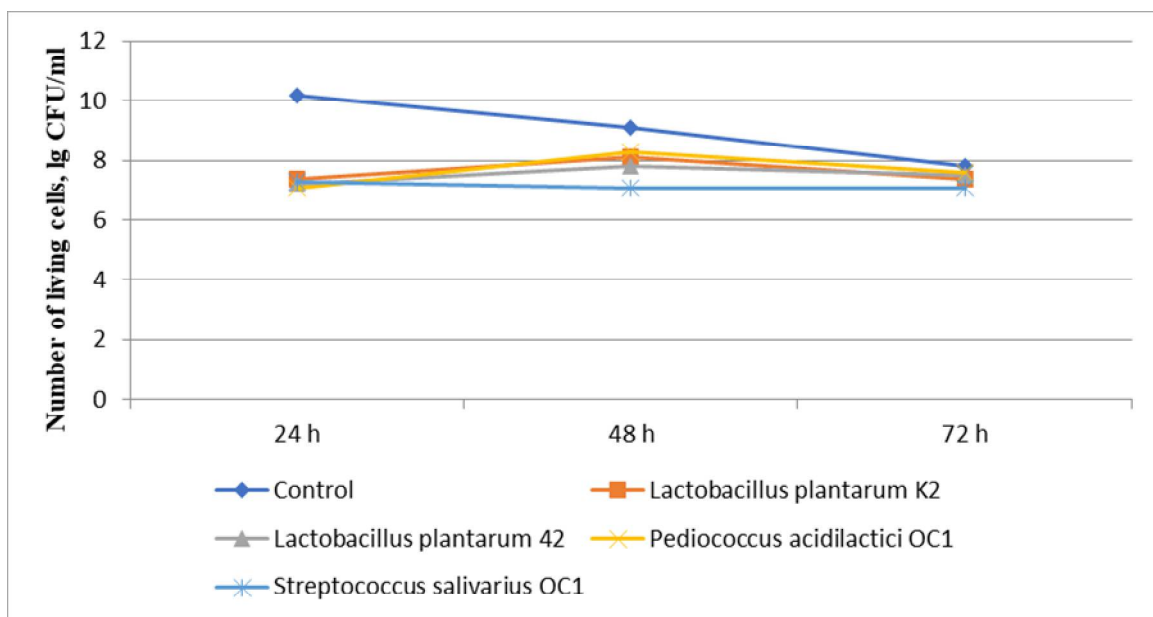
Table 2. Biofilm formation by opportunistic microorganisms isolated from the oral cavity

Types of LAB	Number of strains tested	Number of strains forming biofilm	Biofilm-forming index capacity
<i>Staphylococcus aureus</i>	4	2	3.15xODC (M)
<i>Streptococcus pyogenes</i>	4	2	1.9xODC (W)
<i>Enterobacter cloacae</i>	3	3	3.8xODC (M)
<i>Neisseria subflava</i>	3	1	2.2xODC (M)
<i>Escherichia coli</i>	2	2	2.9xODC (M)
<i>Staphylococcus epidermidis</i>	2	2	2.6xODC (M)
<i>Neisseria mucosa</i>	2	2	6xODC (S)
<i>Gemella haemolysans</i>	2	2	1.3xODC (W)
<i>Rothia mucilaginosa</i>	1	1	2xODC (M)
<i>Enterobacter bugandensis</i>	1	1	2xODC (M)
<i>Acinetobacter lwoffii</i>	1	1	1.7xODC (W)
<i>Acinetobacter pittii</i>	1	1	5.5xODC (S)
<i>Streptococcus mitis</i>	1	1	1.2xODC (W)
<i>Candida albicans</i>	1	1	4.7xODC (S)
<i>Candida guilliermondii</i>	1	1	2.5xODC (M)
<i>Klebsiella pneumoniae</i>	1	1	6.1xODC (S)
Total	37	24	

Note: (W) – weak; (M) - moderate; (S) - strong.



a)



b)

Fig. 2. Antagonistic activity of biofilms of lactobacteria strains against *Staphylococcus aureus* 1: a) suppression of planktonic cells b) suppression of cells in the biofilm.

Antagonistic activity of biofilms formed by strains of lactic acid bacteria. The results obtained in the study of antagonistic activity of biofilms formed by strains: *Pediococcus acidilactici* B2, *Lactiplantibacillus plantarum* K2, *Lactiplantibacillus plantarum* 42, *Streptococcus salivarius* OC1 showed that the biofilm formed by the cultures effectively inhibits the growth of both planktonic cells and cells in the biofilm of *Staphylococcus aureus* and *Klebsiella pneumoniae* (Fig. 2 and 3 (a, b)). In both cases, the titer of live planktonic cells and cells in biofilm decreased to 2 logarithms.

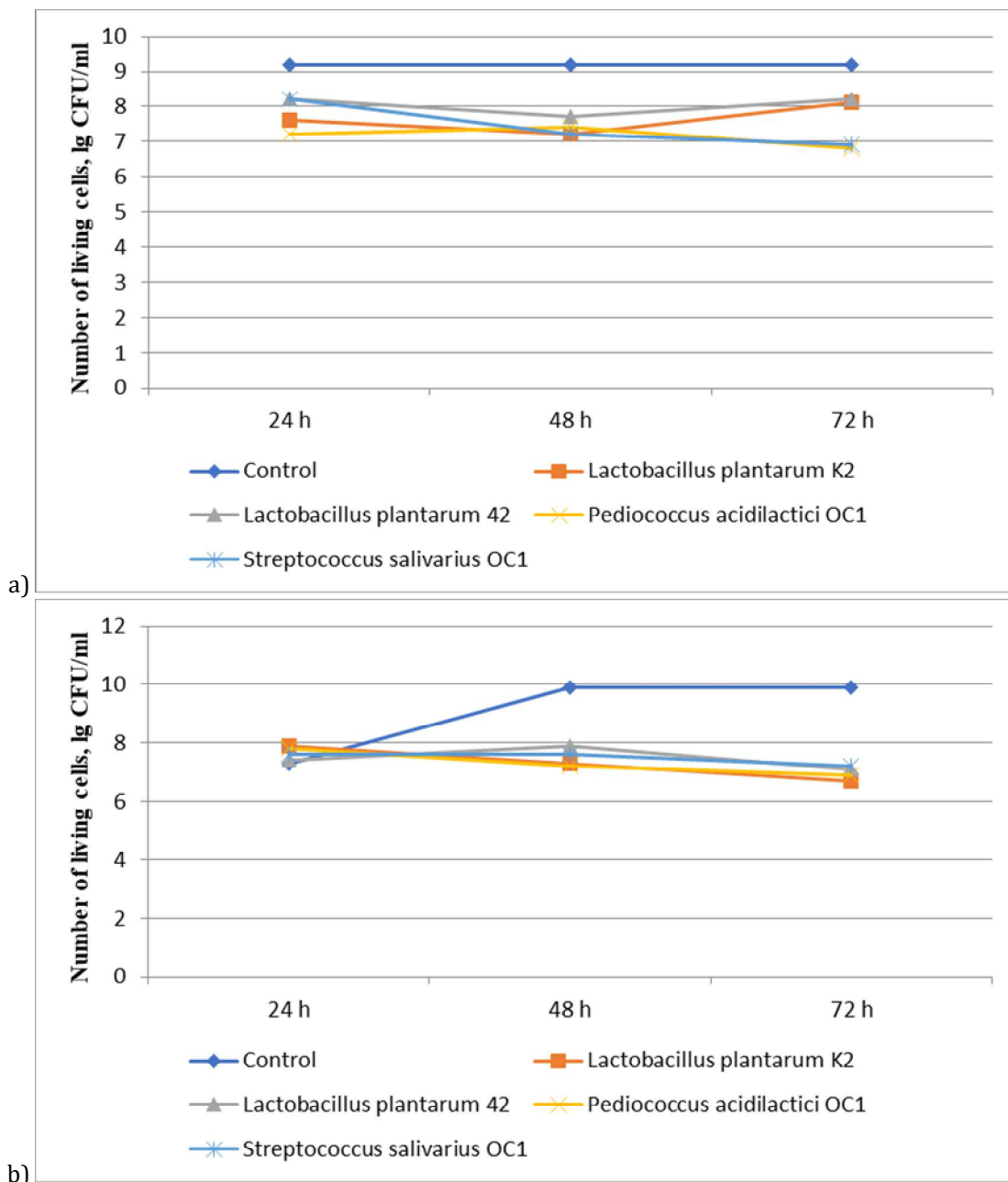


Fig. 3. Antagonistic activity of biofilms of lactobacteria strains against *K. pneumoniae*: a) suppression of planktonic cells b) suppression of cells in the biofilm.

DISCUSSION

In this investigation, we explored the autoaggregation, coaggregation, adhesive, and biofilm-forming properties of lactobacteria strains. Additionally, we examined the antagonistic activity of their biofilms against planktonic and biofilm cells of conditionally pathogenic microorganisms isolated from patients with acute tonsillitis. Previous studies have explored the biofilm-forming capabilities of *L. plantarum* and *L. rhamnosus*, focusing on their ability to inhibit biofilm formation in *Vibrio* spp. [22]. The formation of biofilms in *K. pneumoniae* is influenced by factors such as the polysaccharide capsule, fimbriae, pili, iron metabolism, and the presence of different bacterial species [23]. In a study by Satpute et al. (2016), lactic acid bacteria exhibited high antimicrobial effects against *L. monocytogenes*, possibly due to the presence of bacteriocin compounds and biosurfactants [24]. Bacteriocins, which are bactericidal proteins and similar substances, contribute to the antimicrobial properties. The formation of biofilms in *Staphylococcus aureus* involves surface proteins and the polysaccharide intercellular adhesion (PIA). The study included the identification of corresponding genes, such as those in the *icaADBC* operon (*icaA*, *icaB*, *icaC*, *icaD*). Protein factors influencing *Staphylococcus aureus* biofilm formation include the clumping factor encoded by *clfA* and *clfB* genes, collagen-binding protein (*cna* gene), and fibronectin-binding

protein (fnbA; fnbB genes) [25]. While many studies have explored the effects of probiotics on *Staphylococcus aureus*, few have delved into their impact on the various stages of biofilm formation and cell surface hydrophobicity (CSH). Consequently, our study focused on investigating the antimicrobial and antibiofilm effects of two standard strains (*L. casei* ATCC 39392 and *L. rhamnosus* ATCC 7469) on *S. aureus* ATCC 33591 [26]. Other studies have examined the effects of metabolites produced by lactobacilli. For instance, Yang et al. studied the impact of the biosurfactant produced by *L. plantarum* on the initial attachment and biofilm formation in *S. aureus*. They also assessed the effects of several genes (icaA, sarA, srtA, and cidA) involved in biofilm formation using real-time PCR. Notably, sarA induces attachment and initial biofilm formation by suppressing extracellular proteolytic and nucleolytic enzymes. Reduced sarA expression prevents the initial attachment of *S. aureus* cells. Some studies suggest that the cell-free supernatant of *L. casei* and *L. rhamnosus* increases the expression of hld and cidA genes. The increased expression of the hld gene indicates improved activity of the agr-quorum-sensing system. Researchers demonstrated that the agr system suppresses genes encoding adhesion factors and biofilm formation. Additionally, agr enhances the expression of detergent-like peptides and nucleotides, contributing to increased biofilm separation [27].

CONCLUSION

The findings from this study underscore the autoaggregation, coaggregation, adhesive capabilities, and biofilm-forming proficiency of the examined lactobacilli strains. Moreover, these strains exhibited antagonistic effects against opportunistic microorganism strains isolated from patients with acute tonsillitis. Consequently, there is a clear rationale for further research aimed at developing effective drugs for the prevention and treatment of infections stemming from biofilms of opportunistic microorganisms. These results emphasize the potential significance of leveraging lactobacilli in the development of therapeutic interventions against biofilm-associated infections.

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