

# ANTIMICROBIAL ACTIVITY OF LACTOBACTERIA AGAINST OPPORTUNITICAL MICROORGANISMS OF THE ORAL CAVITY

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## Abstract

The data on a comparative study of the antimicrobial activity of lactic acid bacteria strains isolated from the oral cavity of healthy people and lactobacilli strains isolated from various ecological niches are given in this article. Strains with high antagonistic activity against isolates of opportunistic microorganisms isolated in acute tonsillitis were selected. Their probiotic properties, such as sensitivity to antibiotics, adhesiveness and resistance to high salt content, have been studied.

**Keywords:** lactobacilli, probiotic properties, antagonism, antibiotic resistance, biological products.

## INTRODUCTION

Antibiotics are used to treat infectious diseases, including oral diseases, but their use should be limited, as the spread of antibiotic-resistant bacteria has become a major problem. As a result of the overuse and misuse of antibiotics, as well as the emergence of antibiotic resistant strains, probiotic therapy has gradually been used to prevent and alleviate infectious diseases [1, 2, 3]. In addition, *Lactobacillus* is one of the major genera of probiotics that has been extensively studied for its potential role in oral health. Available data show that consumption of milk enriched with *Lactobacillus rhamnosus* GG reduces the risk of caries in children [4]. Similarly, *Lactobacillus reuteri* reduces the risk of gingivitis [5]. Therefore, lactobacilli with probiotic properties can prevent the colonization of oral pathogens through various mechanisms. Probiotic strains produce antimicrobial components such as hydrogen peroxide and lactic acid that inhibit the growth of oral pathogens (for example, *Streptococcus mutans*).

Increased commensal colonization may interfere with colonization by otopathogens leading to disease prevention. The researchers studied the effects of selected commensal bacteria, i.e. *Bifidobacterium* and *Lactobacillus*, in the prevention of acute tonsillitis, but the results are mixed [6]. Perhaps this effective probiotic component requires a combination of protective commensals. The improvement of probiotics for the prevention of acute tonsillitis depends on better knowledge and understanding of the respiratory microbiota and pathogenic, commensal interactions [7, 8].

Evidence is accumulating showing the health benefits of oral probiotics. For example, one or more strains of lactobacilli, streptococci and/or bifidobacteria isolated from human oral samples reduce bad breath caused by volatile sulfur compounds (VSC) [9, 10] and prevent dental caries [11, 12, 13], periodontal disease [14] and other infections such as human oral candidiasis [15].

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Conversely, the presence of some oral streptococci and lactobacilli is associated with an increased risk of caries and infective endocarditis in humans. Mutant streptococci (*Streptococcus sobrinus* and *S. mutans*) have the ability to convert sucrose into a sticky, water-insoluble glucan (WIG), one of the main cariogenic factors [16]. In addition, they are well known and strong causative agents of dental caries caused by the accumulation of lactic acid in dental plaques after they have been attached to the tooth surface. Therefore, it is believed that strong acid producers can cause dental caries.

It has been established that acute tonsillitis (AT) can be caused by both viral and bacterial microorganisms. Viruses such as Epstein-Barr, adeno-, influenza, parainfluenza, Coxsackie A, herpes simplex, rhino- and coronaviruses have been reported. The most common bacterial pathogens in this context are *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Corynebacterium diphtheriae*, and *Borrelia vincenti* [17].

#### Purpose of the research

This study is aimed at screening new probiotic candidates from oral isolates obtained from healthy people and from strains of lactobacilli isolated from plant and animal sources by evaluating antibacterial activity against pathogenic bacteria involved in the development and course of acute tonsillitis.

## Materials and Methods

Thirty-two healthy volunteers (21 males, 11 females, and mean age  $39.4 \pm 10.3$  years, range 26 to 66 years) were included in the study for the isolation of oral bacteria.

Collection of samples from the oral cavity and isolation of lactobacilli from the oral cavity. Plaque and tongue coating were collected from each subject, mixed and diluted in an anaerobic vehicle. Immediately after sampling, the samples were plated on de Man, Rogosa and Sharp agar (MPC agar) plates (HiMedia, India). The inoculations were incubated at 37°C for 3 days under anaerobic conditions in an anaerobic balloon. Colonies grown on plates 2 or 3 with similar morphology were collected and cultured in MPC broth (HiMedia, India) at 37°C for 24–48 hours. The primary identification of selected cultures was carried out according to classical methods for determining the morphology, physiology and biochemical properties of lactobacilli. After the identification of the species of cultures using the MALDI TOF-MC technology, the cultures were stored at -80°C.

Antimicrobial activity. The antimicrobial activity of candidate probiotic strains isolated from the oral cavity and strains of lactobacilli stored in the laboratory collection was determined by the agar plaque method [18].

21 isolates of opportunistic and pathogenic microorganisms served as test cultures: *Enterobacter bugandensis* (1 strain),

*Escherichia coli* (3 strains), *Klebsiella pneumoniae* (1 strain), *Staphylococcus aureus* (3 strains), *Rothia mucilaginosa* (2 strains), *Shewanella putrefaciens* (1 strain), *Acinetobacter pittii* (1 strain), *Staphylococcus epidermidis* (2 strains), *Neisseria subflava* (2 strains), *Candida albicans* (2 strains) isolated from patients with acute tonsillitis. Before the experiment, the viability of the test cultures was restored by three subcultures in nutrient broth (HiMedia, India) and incubation at 37°C for 24 hours.

Adhesive ability of strains of lactobacilli isolated from the oral cavity.

The evaluation of the adhesive properties of lactobacilli was carried out by the method of V. I. Brilis [19]. Human erythrocytes of the 0(I) Rh+ blood group served as a substrate for adhesion of lactobacilli. Lactobacilli were grown for 24 hours on a dense nutrient medium MRS at a temperature of  $(37 \pm 1)^\circ\text{C}$ . The grown cultures were washed in physiological sodium chloride solution and suspensions were prepared with an optical density of 1.0. 1 ml of a suspension of 3 times washed erythrocytes with a concentration of 108 cells/l and 1 ml of a suspension of the studied cultures of lactobacilli were added to the test tubes. A phosphate buffer solution (pH 7.2–7.3) was used to prepare a suspension of microorganisms and erythrocytes. The samples were incubated at a temperature of  $(37 \pm 1)^\circ\text{C}$  for 30 minutes. To precipitate erythrocytes, the samples were centrifuged at 1000 rpm. within 2 minutes.

When determining the adhesive properties of lactobacilli, 0.02–0.03 ml of a buffer solution was applied to a glass slide, in which an equal volume of a suspension of erythrocytes and a suspension of lactobacilli was suspended. A mixture of suspensions of erythrocytes and lactobacilli was incubated in a thermostat for 30 min. at a temperature of  $(37 \pm 1)^\circ\text{C}$ , and then a dried preparation was prepared, fixed with 96% ethyl alcohol and stained according to Romanovsky-Giemsa for 30 minutes. The evaluation of the adhesive properties of the studied cultures of lactobacilli was carried out under a light microscope by determining the average adhesion index, which is equal to the average number of lactobacilli attached to one erythrocyte. The criteria for assessing the adhesive properties of microorganisms according to the method of V. I. Brilis were as follows: a high degree of adhesion - 4.01 or more bacteria, an average degree of adhesion - from 2.01 to 4.0 bacteria, a 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.

Evaluation of the production potential of selected strains.

Determination of resistance to NaCl. The ability of the studied strains to grow at elevated concentrations of sodium chloride was determined by the method described in MUK 4.2.2602-10 [20].

The study of sensitivity to antibiotics. The antibiotic susceptibility of the strains was determined by the disk diffusion method using the following antibiotics adsorbed on paper disks (HiMedia): cefotaxime (5 µg), gentamicin (10

µg), rifampicin (5 µg), kanamycin (30 µg), erythromycin (15 µg), levofloxacin (5 µg), amikacin (30 µg), vancomycin (30 µg), amoxiclav (30 µg), ofloxacin (5 µg) [21].

### Results and Discussion

The number of isolated bacteria. A total of 100 oral lactic acid bacteria isolates were obtained from 32 healthy volunteers. The isolates were deposited as an oral bacterial library for use in the next stages of research.

All isolated isolates are non-motile Gram-positive rods. Isolate No. 11, 28, 29 and 30 aniline dyes are perceived heterogeneously, so metachromatic granularity and bipolar bodies are detected. When grown on dense nutrient media on the second day of cultivation at a temperature of (37 ± 1) ° C under microaerophilic conditions, the studied isolates: Nos. 1, 3, 4, 32, 24, 33, 31 on a dense nutrient medium MPC form white colonies, shiny, round in shape with a convex bumpy surface. The edge of the colony is uneven. Colonies of some isolates Nos. 11, 25, 29, 27 and 30 are opaque, round, off-white. The surface of the colonies is smooth, matte; the edges are clear and even. The diameter of the colonies is 2–3 mm. Bacteria of isolate No. 16 form colonies 2–4 mm in diameter, opaque, beige. The shape of the colony is round; the edges are clear, but uneven. The surface of the colony is smooth, matte. The center of the colony is cone-shaped, smoothly turning into a flattened peripheral part. All isolated isolates are microaerophiles with respect to oxygen. They do not form spores or capsules and they do not have flagella.

One of the important criteria in assessing the prospects of using microorganism strains to create a new probiotic based on them is the ability of bacteria to suppress the growth and reproduction of opportunistic microorganisms involved in the development and course of acute tonsillitis. Test strains of opportunistic bacteria were isolated from the nasopharynx of patients with acute tonsillitis; An analysis of the data on the antagonistic activity of the main groups of lactobacilli shown in Figures 1, 2, 3, 4 indicates that all the studied strains of lactobacilli have a pronounced antagonistic activity against opportunistic microorganisms. The most significant indicators were obtained against strains of *Pediococcus acidilactici* (4 strains). Comparative analysis of the antimicrobial activity of strains of the genus *Pediococcus* isolated from the oral cavity of healthy people and strains previously isolated from other sources of lactobacilli (dairy products, epiphytic microflora of plants, infant feces). It has been established that strains isolated from the oral cavity more effectively than strains from non-indigenous microflora inhibit the growth of *Enterobacter bugandensis* (1 strain), *Escherichia coli* (3 strains), *Klebsiella pneumoniae* (1 strain), *Staphylococcus aureus* (3 strains), *Rothia mucilaginosa* (2 strains), *Shewanella putrifaciens* (1 strain), *Candida albicans* (2 strains). The antimicrobial activity of strains from non-indigenous microflora against *Acinetobacter pittii* (1 strain), *Staphylococcus epidermidis*

(2 strains), *Neisseria subflava* (2 strains) was higher than that of strains isolated from the oral cavity (Fig. 1).

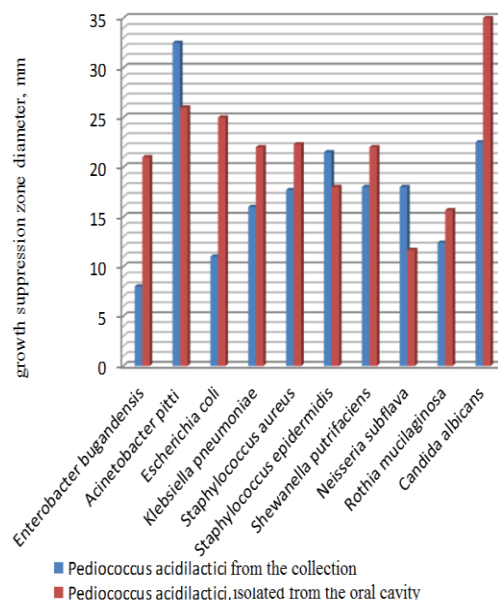


Fig. 1. Antimicrobial activity of *Pediococcus acidilactici* strains

Strains of *L. rhamnosus* isolated from non-indigenous microflora more effectively than strains from the oral cavity inhibit the growth of strains of *Acinetobacter pittii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Rothia mucilaginosa*, *Candida albicans*. The antimicrobial activity of strains isolated from the oral cavity against *Enterobacter bugandensis*, *Neisseria subflava* was higher than that of strains from non-indigenous microflora (Fig. 2).

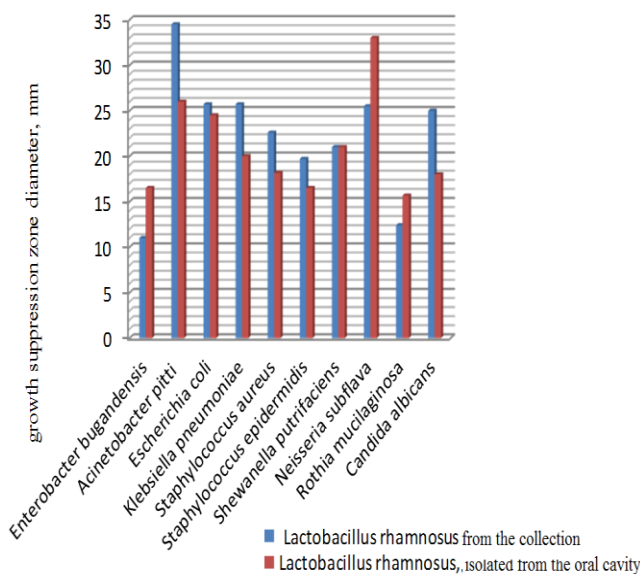


Fig.2. Antimicrobial activity of *Lactobacillus rhamnosus* strains

Figure 3 shows that strains of *Lactobacillus fermentum* have less pronounced antagonistic activity. Strains isolated from the indigenous microflora of the oral cavity inhibit the growth

of strains of *Escherichia coli*, *Staphylococcus aureus*, *Neisseria subflava*, *Rothia mucilaginosa*. The antimicrobial activity of strains from non-indigenous microflora was higher in relation to *Enterobacter bugandensis*, *Acinetobacter pittii*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Shewanella putrifaciens* and *Candida albicans* than in strains isolated from the oral cavity.

Among the studied species of lactobacilli, strains of *Lactobacillus brevis* showed the lowest antagonistic activity. Strains, *L. brevis*, isolated from the indigenous microflora of the oral cavity, inhibit the growth of strains of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Neisseria subflava*, *Rothia mucilaginosa*. The antimicrobial activity of strains from non-indigenous microflora was higher in relation to *Acinetobacter pittii*, *Shewanella putrifaciens* and *Candida albicans* than in strains isolated from the oral cavity. No culture inhibits the growth of *Enterobacter bugandensis* (Figure 4).

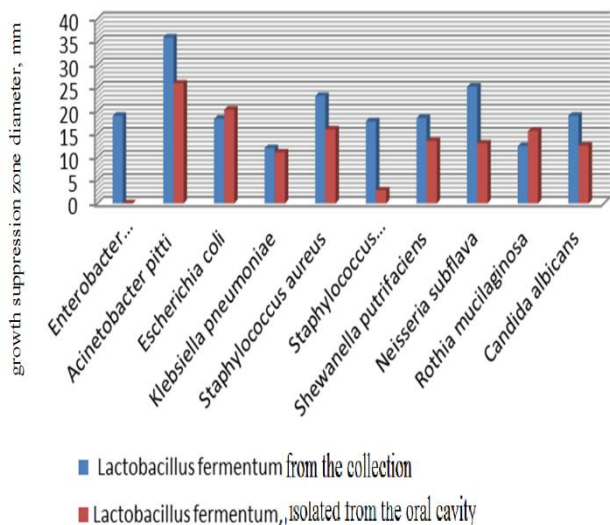


Fig. 3. Antimicrobial activity of *Lactobacillus fermentum* strains

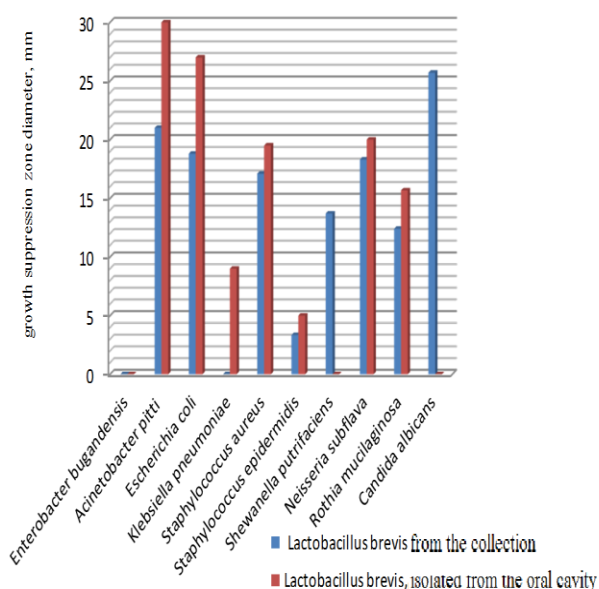


Fig.4. Antimicrobial activity of *Lactobacillus brevis* strains

Conditionally pathogenic microflora of patients with acute respiratory diseases remains an urgent problem of practical health care. It is accompanied by the development of oral dysbiosis. To combat endogenous opportunistic infection, it is recommended to use probiotics, the active principle of which are representatives of the normal microflora of the body, in particular, bifidobacteria and lactobacilli. Many representatives of the normal microflora have antagonistic properties in relation to pathogenic microbes and UPM. The highest activity was noted in lactobacilli, which produce various antibacterial substances, such as organic acids, hydrogen peroxide, muramidase, bacteriocins, microcins, etc., which determines their antagonistic activity. Lactobacilli provide the resistance of the mucous membranes of the body to colonization by allochthonous microflora.

Adhesive activity of strains of lactobacilli isolated from the oral cavity. According to the results of studies to determine the antagonistic activity of lactobacilli strains, 42 cultures were selected to determine the adhesive properties. Adhesive activity is one of the important criteria in assessing the prospects for using strains in probiotic preparations, since it directly affects the duration of lactobacilli persistence in the intestine. Data analysis of the adhesive properties of lactobacilli against human erythrocytes, presented in Table 1, indicates that the strains of *Lactobacillus paracasei* OC2 and *Lactobacillus brevis* OC1 have high adhesive activity against human erythrocytes, in total, 17.6% of strains have high adhesiveness, 41.2% have low adhesive activity against human erythrocytes and 41.2% of the studied strains have an average level of adhesion to human erythrocytes.

Table 1. Adhesion of lactic acid bacteria strains

No	Lactic acid bacteria isolated from the oral cavity	Degree of adhesion	Lactic acid bacteria isolated from other sources	Degree of adhesion
1.	<i>Pediococcus acidilactici</i> OC1	3, 4	<i>Lactobacillus plantarum</i> mal	4
2.	<i>Pediococcus acidilactici</i> OC2	4	<i>Lactobacillus plantarum</i> TK1	8



3.	<i>Pediococcus</i> sp	4	<i>Lactobacillus plantarum</i> KA3	6
4.	<i>Lactobacillus fermentum</i> OC1	7	<i>Lactobacillus kunkeei</i> 1	8
5.	<i>Enterococcus durans</i> OC1	2,3	<i>Enterococcus faecium</i> 1	7
6.	<i>Lactobacillus salivarius</i> OC1	3	<i>Enterococcus durans</i>	6
7.	<i>Lactobacillus gasseri</i> OC1	1	<i>Lactobacillus rhamnosus</i> 925	4
8.	<i>Lactobacillus paracasei</i> OC1	1	<i>Lactobacillus fermentum</i> X	3
9.	<i>Lactobacillus paracasei</i> OC2	8	<i>Lactobacillus acidophilus</i> D	8
10.	<i>Lactobacillus paracasei</i> OC3	0	<i>Lactobacillus acidophilus</i> 2	4
11.	<i>Lactobacillus fermentum</i> OC2	0	<i>Bifidobacterium animalis</i>	2
12.	<i>Lactobacillus brevis</i> OC1	8	<i>Bifidobacterium bifidum</i>	1,2
13.	<i>Lactobacillus delbrueckii</i> OC1	0	<i>Lactobacillus fermentum</i> F	3
14.	<i>Lactobacillus rhamnosus</i> OC1	4	<i>Lactobacillus reuteri</i> 2	8
15.	<i>Lactobacillus rhamnosus</i> OC2	5	<i>Lactobacillus rhamnosus</i> LGG	9
16.	<i>Lactobacillus rhamnosus</i> OC3	0	<i>Pediococcus acidilactici</i> B	6
17.	<i>Pediococcus acidilactici</i> OC3	3	<i>Weissella cibaria</i> R1	2
18.			<i>Lactobacillus brevis</i> F1	3
19.			<i>Lactobacillus plantarum</i> B1	6
20.			<i>Lactobacillus sakei</i> KS	3
21.			<i>Lactobacillus brevis</i> 2	4
22.			<i>Lactobacillus brevis</i> Fr2	8
23.			<i>Leuconostoc lactis</i> Sh	4
24.			<i>Pediococcus acidilactici</i> B2	0
25.			<i>Lactobacillus rhamnosus</i> Sh5	1,2

Some previous studies have shown that there was no correlation between the surface hydrophobicity of *Lactobacillus* and its ability to attach to the intestinal mucosa [22]. However, some studies report a correlation between hydrophobicity and adhesiveness. The higher the value of surface hydrophobicity, the higher the ability of bacterial cells to attach to the intestinal mucosa [23].

Although the detailed mechanism explaining the correlation between surface hydrophobicity of bacterial cells and adhesion to the intestinal mucosa is unclear, hydrophobicity and autoaggregation tests can be used as indicators in initial screening for potential lactic acid bacteria adhesion. The present study suggests that the probiotic adhesion test may provide important screening tools to determine the applicability of lactic acid bacteria as probiotics. The resistance of lactobacilli cultures to various concentrations of NaCl.

Based on the results of studies to determine antimicrobial activity and adhesive properties, 12 cultures of lactic acid bacteria were selected: isolated from the oral cavity: *Pediococcus acidilactici* OC1, *Pediococcus acidilactici* OC2, *Pediococcus* sp., *Lactobacillus salivarius* OC1, *Lactobacillus rhamnosus* OC1, *Lactobacillus rhamnosus* OC2; *Lactobacillus plantarum* mal, *Lactobacillus rhamnosus* 925 ak, *Lactobacillus fermentum* X, *Lactobacillus fermentum* F, *Lactobacillus acidophilus* 2, *Leuconostoc lactis* Sh. Resistance to NaCl determines the survival of probiotic strains of microorganisms during

processing and in the finished dosage form.

To study salt tolerance, the number of lactobacilli cells grown in the presence of various salt concentrations was counted. It was shown that all 12 strains are resistant to the presence of 0%, 2%, 4% and 6.5% NaCl in the medium, the cell titer is not lower than 10<sup>5</sup> CFU/ml. It should be noted that in the presence of 6% NaCl in the medium, the cell titer does not decrease in the majority of cultures isolated from the oral cavity of *Pediococcus acidilactici* OC1, *Pediococcus* sp., *Lactobacillus rhamnosus* OC1, and *Lactobacillus rhamnosus* OC2. Among the studied 6 cultures of lactobacilli isolated from other sources, the culture of *Lactobacillus fermentum* F, the number of living cells of which did not change under the influence of various concentrations of sodium chloride in the medium.

Thus, all the studied isolates were resistant to the presence of 6.5% NaCl in the medium.

Sensitivity of strains of lactobacilli to antibiotics.

LAB are found in many natural sources, but antibiotic resistance of these bacteria is a concern. Therefore, before recommending ICD strains for use, it is necessary to determine their sensitivity to antibiotics. Antibiotic-resistant strains can harm the human or animal body, as they are able to transfer resistance genes to pathogenic bacteria. In our study, we studied the sensitivity of lactobacilli strains to 12 antibiotics - cefazolin, kanamycin, erythromycin, rifampicin, ciprofloxacin, cefatoxime, levofloxacin, amikacin,

vancomycin, amoxiclav, gentamicin, ofloxacin.

The results showed that all cultures are sensitive to gentamicin. All cultures are resistant to ofloxacin: 8 strains are resistant, 1 strain is moderately resistant and 1 strain -

Lactobacillus rhamnosus OC1 is susceptible. It was revealed that 9 out of 10 cultures are resistant or moderately resistant to streptomycin L.salivarius OC1. The antibiotic sensitivity profile of the studied cultures of lactobacilli is presented in Table 2.

Table 2. Sensitivity of lactobacilli strains to antibiotics

№	Antibiotic	Diameter of the culture growth inhibition zone, mm										
		The content of the drug in the disk, mcg	<i>Pediococcus acidilactici OC1</i>	<i>Pediococcus acidilactici OC2</i>	<i>Pediococcus sp</i>	<i>Lactobacillus rhamnosus OC1</i>	<i>Lactobacillus rhamnosus OC2</i>	<i>L.salivarius OC1</i>	<i>L.fermentum X</i>	<i>L.fermentum F</i>	<i>L. acidophilus 2</i>	<i>Leuconostoc lactis Sh</i>
1	Cefozalin	30	20	30	32	18	30	30	20	28	28	28
2	Cefotaxime	15	22	26	32	28	28	32	26	30	28	30
3	Ciprofloxacin	5	0	0	0	26	0	12	18	0	0	10
4	Azithromycin	15	16	20	22	26	22	22	22	20	20	22
5	Streptomycin	10	0	0	0	12	0	24	10	0	0	12
6	Ampicillin	2	20	22	28	26	26	28	24	24	22	30
7	Erythromycin	15	18	26	30	32	24	28	28	24	22	30
8	Ofloxacin	5	0	0	8	20	0	0	14	0	0	0
9	Amoxiclav	30	20	28	32	30	28	30	32	24	28	40
10	Levofloxacin	5	10	8	14	22	12	10	16	10	14	12
11	Lincomycin	15	14	14	22	28	20	18	20	12	0	28
12	Gentamicin	10	12	14	14	14	14	12	12	12	8	18

The studied strains of lactobacilli demonstrated resistance to streptomycin and ciprofloxacin; sensitivity to this antibiotic was found only in the *Lactobacillus rhamnosus* OC1 strain. Despite the fact that gentamicin, which also belongs to aminoglycoside antibiotics, showed moderate resistance (diameters of growth inhibition zones from 10 to 18 mm). The reduced sensitivity of lactobacilli to aminoglycosides is also considered natural and is associated with the low permeability of antibiotics of this group through the membranes of lactobacilli [24]. The potential mobility of the genetic determinants of resistance to ofloxacin and aminoglycosides has not been clearly established [25]. According to M. Danielsen, A. Wind, genes of resistance to chloramphenicol and erythromycin are especially susceptible to horizontal transport [26]. Bacteria of the genus *Lactobacillus* are sensitive to these antibiotics [24; 26; 27] and, in general, to inhibitors of protein biosynthesis.

## Conclusion

Thus, it has been shown that 12 studied strains of lactobacilli have high antimicrobial activity against isolates of

opportunistic microflora in patients with acute tonsillitis. cultures isolated from the oral cavity of *Pediococcus acidilactici* OC1, *Pediococcus sp.*, *Lactobacillus rhamnosus* OC1 and *Lactobacillus rhamnosus* OC2, *Lactobacillus fermentum* F6 culture isolated from non-indigenous microflora are resistant to high salt content in the medium, medium adhesiveness to human erythrocytes and lack of acquired resistance to antibiotics. In this regard, these strains are promising for further study of their properties in order to recommend as components of effective and safe probiotic preparations for the treatment of acute diseases of the human upper respiratory tract.

## REFERENCES

1. Oo KM, Lwin AA, Kyaw YY, Tun WM, Fukada K, Goshima A, et al. Safety and long-term effect of the probiotic FK-23 in patients with hepatitis C virus infection. *BiosciMicrobiota Food Health*. 2016;35(3):123–8.
2. Michalickova D, Minic R, Dikic N, Andjelkovic M, Kostic-Vucicevic M, Stojmenovic T, et al. *Lactobacillus helveticus* Lafti L10 supplementation reduces respiratory infection duration in a cohort of elite athletes: a randomized, double-blind, placebo-controlled trial. *ApplPhysiolNutrMetab*. 2016;41(7):782–9.
3. Karlsson M, Scherbak N, Khalaf H, Olsson PE, Jass J. Substances

- released from probiotic *Lactobacillus rhamnosus* GR-1 potentiate NF-kappa B activity in *Escherichia coli*-stimulated urinary bladder cells. *FEMS Immunol Med Microbiol*. 2012;66(2):147–56.
4. Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res*. 2001;35(6): 412–20.
  5. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*. *Swed Dent J*. 2006;30(2):55–60.
  6. Nagalingam N.A., Cope E.K., Lynch S.V. Probiotic strategies for treatment of respiratory diseases. 2013. *Trends Microbiol*; 21(9):485±92. <https://doi.org/10.1016/j.tim.2013.04.008> PMID: 23707554
  7. Lehtoranta L, Pitka Èranta A, Korpela R. Probiotics in respiratory virus infections. 2014. *Eur J Clin Microbiol Infect Dis*; 33(8):1289±302. <https://doi.org/10.1007/s10096-014-2086-y> PMID: 24638909
  8. Laufer AS, Metlay JP, Gent JF, Fennie KP, Kong Y, Pettigrew MM. Microbial communities of the upper respiratory tract and otitis media in children. 2011. *MBio*; 2(1):e00245±10. <https://doi.org/10.1128/mBio.00245-10> PMID: 21285435
  9. Burton JP, Chilcott CN, Moore C, Speiser G, Tagg JR. A preliminary study of the effect of probiotic *Streptococcus salivarius* K12 on oral malodour parameters. *J Appl Microbiol*. 2006; 100: 754–764. PMID: 16553730
  10. Zahradnik RT, Magnusson I, Walker C, McDonell E, Hillman CH, Hillman JD. Preliminary assessment of safety and effectiveness in humans of ProBiora3, a probiotic mouthwash // *J Appl Microbiol*. 2009; 107: 682–690. doi: 10.1111/j.1365-2672.2009.04243.x PMID: 19486429
  11. Caglar E, Kuscü OO, SelviKuvvetli S, Kavaloglu Cildir S, Sandalli N, Twetman S. Short-term effect of ice-cream containing *Bifidobacterium lactis* Bb-12 on the number of salivary mutans streptococci and lactobacilli. *Acta Odontol Scand*. 2008; 66: 154–158. doi: 10.1080/00016350802089467 PMID: 18568474
  12. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res*. 2001; 35: 412–420. PMID: 11799281
  13. Nikawa H, Tomiyama Y, Hiramatsu M, Yushita K, Takamoto Y, Ishi H, et al. Bovine milk fermented with *Lactobacillus rhamnosus* L8020 decreases the oral carriage of mutans streptococci and the burden of periodontal pathogens. *J Invest Clin Dent*. 2011; 2: 187–196.
  14. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K, et al. Improvement of periodontal condition by probiotics with *Lactobacillus salivarius* WB21: a randomized, double-blind, placebo-controlled study. *J Clin Periodontol*. 2008; 35: 897–905. doi: 10.1111/j.1600-051X.2008.01306.x PMID: 18727656
  15. Hatakka K, Ahola AJ, Yli-Knuuttila H, Richardson M, Poussa T, Meurman JH, et al. Probiotics reduce the prevalence of oral candida in the elderly—a randomized controlled trial. *J Dent Res*. 2007; 86: 125–130. PMID: 17251510
  16. Hamada S., Slade H.D. Biology, immunology, and cariogenicity of *Streptococcus mutans* // *Microbiol Rev*. 1980; 44: 331–384. PMID: 6446023
  17. Mims C.A., Playfair J.H.L., Roitt I.M., Wakelin D., Williams R., Anderson R.. *Upper Respiratory Tract Infections*. Medical Microbiology. Mosby-Year Book Europe Ltd, Hong Kong, 1993, pp.19.1–20.10
  18. Harris LJ, Daeschel MA, Stiles ME, Klaenhammer TR. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. *J Food Prot*. 1999; 52:384-87.
  19. Брилис, В. И. Методика изучения адгезивного процесса микроорганизмов / В. И. Брилис, Т. А. Брилис, Х. П. Ленцнер, А. А. Ленцнер // *Лабораторное дело*. – 1986. – № 4. – С. 210–212
  20. МУК 4.2.1890-04. Определение чувствительности микроорганизмов к антибактериальным препаратам.
  21. МУК 4.2.2602-10 Система предрегистрационного доклинического изучения безопасности препаратов. Отбор, проверка и хранение производственных штаммов, используемых при производстве пробиотиков.
  22. Ramos, C.L.; Thorsen, L.; Schwan, R.F.; Jespersen, L. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiol*. 2013, 36, 22–29.
  23. Tokatl, M.; Gülgör, G.; Bağder Elmac, S.; Arslankoz İşleyen, N.; Özçelik, F. In Vitro Properties of Potential Probiotic Indigenous Lactic Acid Bacteria Originating from Traditional Pickles. *BioMed Res. Int*. 2015, 2015, 315819
  24. Kirtzalidou E., Pramateftaki P., Kotsou M., Kyriacou A. Screening for lactobacilli with probiotic properties in the infant gut microbiota. *Anaerobe* 2011; 17: 6: 578-585.
  25. Rojo-Bezares B., G de Los Reyes-Gavilan C., Margolles A. Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. *Int J Food Microbiol*; 2006; 111: 3: 234-240.
  26. Danielsen M., Wind A. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *Int. J. Food Microbiol*. 2003; 82;1:1-11.
  27. Katla A.K., Kruse, Johnsen G., Herikstad H. Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. *Int. J. Food Microbiol*. 2001; 67: 1-2: 147-152.