BIOLICAL INDICATORS OF ORAL LACTOBACILLI IN WOMEN OF FERTILE AGE: NORMAL AND CAVITIES.

Khaldarbekova G.Z.1, Muhamedov I.M.2, Tarinova M.V.3, Shigakova L.A.4

1 Khaldarbekova Guljahon, a graduate student, Tashkent State Dental Institute, Uzbekistan
2 Muhamedov Ilaman - Doctor of Medical Sciences, Professor, Scientific Director of the Department of Microbiology and Pharmacology of Tashkent State Dental Institute, Uzbekistan
3 Tarinova Margarita - Ph.D. in Biology, Associate Professor of the Department, Uzbekistan
4 Shigakova Luciya - Assistant of the Department of Histology and Medical Biology of Tashkent State Dental Institute, Uzbekistan

Abstract. Scientists have been researching for a long time, where they have found evidence of a connection between “Lactobacillus - Caries”. According to the results of some countries, the qualitative and quantitative composition of lactoflora of the oral cavity is already known in the norm and case of diseases caused by disbiotic conditions of the mouth. This article presents the results of the quantitative and qualitative composition of oral lactoflora of women of fertile age, the state of biological properties of each particular representative and their impact on cavities formation, which can explain the microbiological etiology of cavities. Each of the following indicators was statistically processed and compared with the results of foreign countries.

Keywords: microflora, lactobacilli, cavity, biological properties, resistance, fertile age, dysbiosis.

Relevance. It is known that the human body is colonized by a huge number of microorganisms, all together, they constitute a human microbiota. Therefore, one of the most important tasks of modern clinical microbiology is the study of microbiotas of the main body biotopes: oral cavity, intestines and vagina, which is an indicator of the reproductive health of women [2].

In recent years, with the development of science in various fields, more and more attention is paid to women of fertile, that is, of fertile age, and this is explained by the fact that the future of each nation is provided by women. While maintaining women’s health, it is important to pay special attention to the state of the oral cavity, in particular the microflora of the mouth. Because healthy teeth and mucous membranes show how healthy a person is. From the microbiological point of view, the oral cavity is considered to be the most important, original, huge in many microbes, biotopes of the human body. The oral cavity, as the beginning of the...
digestive system, is a complex and diverse microflora. According to different authors, the number of species of bacteria in the oral cavity is more than 700, including anaerobes [11,12]. The number of microorganisms changes during the day, with saliva production playing a leading role. Factors causing changes in the content of individual representatives of the flora are antibiotics, change of the dents, physiological effects, elimination of all dental caries and removal of damaged teeth, various somatic diseases [15].

One of the components of this microflora is considered to be the \textit{Lactobacillus} genus. Their quantitative content in the mouth of healthy people is lg 4.60±0.2 KOE/ml. According to our data, the content of resident lactobacillus in the saliva of healthy women aged 20-40 years (n=40) is lg 4.60±0.2 KOE/ml.

Microorganisms belonging to the genus \textit{Lactobacillus} are currently one of the most important and promising objects in both medical microbiology and the national economy. Lactobacillus are microorganisms widely spread in the environment. They have high biological and functional activity, which determines their practical use as probiotics and in food production [4,8,18,19].

The specific and quantitative composition of lactoflora of the oral cavity differ and may vary depending on the state of the body. The problem of identification and classification of lactobacilli is particularly relevant due to their wide use as probiotic drugs and in the production of fermented products of traditional and functional nutrition. It has been established that the species composition of human lactoflora can vary significantly depending on national origin, the territory of residence, food ration and many other factors [13].

The study of the basic biological properties of lactobacilli, in particular the ability to ferment carbohydrates, is essential for species identification of these bacteria.

Cilia located on the cell surface and consisting of proteins and polysaccharides [25]. According to data from different authors, all types of lactobacilli have adhesive activity, but they all exhibit different manifestations [14]. Despite this, many researchers believe that lactobacilli with high adhesive activity can lead to cavities because when they are adhesive on the surface of tooth enamel, they release organic acids (products of life), which in turn serve as a factor in the destruction of enamel.

According to different authors, it is known that lysozyme can inhibit lactic acid production with lactobacilli [27]. Antilisocime activity makes lactobacilli synthesize acids (products of life activity) under constant exposure to lysozyme in the oral cavity [5]. It is also known that acid-producing bacteria are classified as pathogenetic factors determining the development of caries [26].

Antagonistic properties in relation to pathogenic and conditionally pathogenic microflora possess many representatives of normoflora. However, the highest activity is observed in lactobacilli. They secrete various antibacterial products, organic acids, H$_2$O$_2$, muroimidase, bacteriocins, microcins, etc., which provide resistance to colonization of oral mucosa by transitor microflora, which is directly related to their antagonistic activity [16]. Besides, scientists have found that the ability to form biofilms in the oral cavity is typical for all antagonistically active strains of lactobacilli [17].

As a rule, the antagonistic properties of lactobacilli are due to the production of lactic and acetic acids, hydrogen peroxide, and the formation of substances similar to antibiotics. Many
scientists believe that the formation of these organic acids from carbohydrates leads to a decrease in the pH of the oral environment, thereby inhibiting the development of other microorganisms [20].

In aerobic conditions, lactobacillus bacteria grow much worse than anaerobic ones, as they secrete hydrogen peroxide (H$_2$O$_2$) and do not form catalases [6]. The ability to produce H$_2$O$_2$ plays an important role in the antimicrobial and virulicide mechanisms of lactobacilli [9]. Lactobacilli capable of producing H$_2$O$_2$ are characterized by bactericidal action concerning representatives of pathogenic and allochton microflora, thus showing antibacterial ability [21,23]. The ability of lactobacilli to produce hydrogen peroxide (H$_2$O$_2$) is considered as one of the most important factors in the mechanism of antagonistic activity [22]. Having information about the microbiological etiology of caries, it is important to determine the state of lactobacilli ability to produce H$_2$O$_2$, as it was found that the number of representatives of pathogenic microflora increases significantly in this disease. This indicates a decrease in antibacterial ability, which is directly related to their antagonistic activity.

The ability of lactic acid bacteria to form lysozyme-like enzymes and thus have a bactericidal and bacteriostatic effect on pathogenic microflora is widely used in the food industry, medicine, veterinary medicine and agriculture. According to V.V.Pospelova et al. (1992), the most important positive side of lactobacilli as a member of autochthonous microflora providing colonization resistance is their ability to produce lysozymoid enzymes. This ability was first described by N.Kaufman and K.Bauer back in 1956. As we know, lysozyme-like enzymes are considered to be antibacterial substances, enzymes of the hydrolase class, which destroy cell walls of bacteria by hydrolysis of peptidoglycan (murein). Thus, the production of lysozymeproducing lactobacillus-like enzymes hinders the existence and colonization of representatives of allochton microflora.

Under the influence of cellular and humoral factors of body protection, such as immunoglobulins A and G, lysozyme, lactoferrin, polymorphonucleara, saliva peroxidase, myeloperoxidase, sialic acids, etc., the question of survival of lactobacilli in the mouth of a healthy person remains open. But in conditions of increased immune protection of the macroorganism in any pathological processes, it is important to find out qualitative data on lactobacilli that contribute to their survival and functioning, which could explain the microbiological etiology of the emergence of this pathology in the oral cavity.

Due to the lack of accurate data, as well as inaccurate information on oral lactobacilli in women of fertile age in the Republic of Uzbekistan, there is a need for a more in-depth study on the identification of oral lactobacilli, as well as their biological properties in the normal and caries (due to wide distribution among the population of RUz) in women of fertile age.

According to the results of many studies, it is known that the number of lactobacteria in the oral cavity in the case of dental caries is significantly increased. In some countries, a “lactobacillus test” (determination of the number of lactobacteria) has been proposed to assess the “activity” of the dental caries process [15]. Unfortunately, the true significance of lactobacillus in dental caries development has not been determined. It would be correct to consider that lactobacillus bacteria play a small role in the process of plaque formation and at the initial stages of bacteria attachment to tooth enamel. Still, with the increasing severity of caries, the value of lactobacillus in its progression also increases [11].
The above-mentioned characteristics and results obtained in the study of biological properties of each type of lactobacteria in women of fertile age with and without caries would help to form a unified standard of microbiological assessment and analysis for our region. Since strains that are part of probiotics for the correction of dysbiosis in the oral cavity, as well as products of functional nutrition, should not have biological properties that harm the oral cavity and the body as a whole.

**Research objective.** To study and analyze oral lactoflora in women of fertile age, paying special attention to the state of quantitative and qualitative criteria, as well as the role of basic biological properties in normal and caries.

**Material and methods.** A transverse cohort study was carried out, which included a comprehensive dental examination, study of microbiological features of the oral cavity, quantitative and species composition of lactobacteria in healthy and caries patients in women aged 20-40 years. A total of 90 women were involved in the study. To be able to compare the results, the subjects were divided into two groups: healthy - with caries. The material was collected 1-2 hours after meals. In the laboratory, the test material was grinded in an isotonic solution of sodium chloride and sown to dense nutrient media. Identification of lactobacteria was carried out by studying morphology, tinctorial, cultural and biochemical properties on Gissa media (a motley row of carbohydrates), as well as by the definition of the Bergey’s Manual of Systematic Bacteriology [7,28].

The adhesive properties of lactobacilli were studied by V.I.Brilis et al [1]. Native erythrocytes of human 0 (I) - the first group, Rh “+” will be used as a cellular substance. The adhesive activity was estimated by the average adhesion index (SPA), which is the average number of microbes attached to one erythrocyte when counting at least 25 erythrocytes, taking into account no more than 5 erythrocytes in one field of vision.

The qualitative presence and quantitative expression of the antilysozyme activity sign were studied by O.V. Bukharin et al. [3].

The antagonistic activity was determined using the method of vertical strokes.

The ability of lactobacillus to produce hydrogen peroxide was determined using the Graf method with et al. [24], the principle of which is based on potassium peroxide oxidation in the presence of ammonium molybdate with the formation of iodine starch complex. The state of antipericidal protection of the body was judged by the activity of enzymes.

Determination of lactobacillus ability to produce lysozyme-like enzymes was performed by A.A.Lenzner et al.[10]. The degree of lysozyme activity was judged by the presence and size of the lysis zone radius of the test strain around the growing culture under study: 1-3 mm - low activity, 4-7 mm - average activity and > 7 mm - high activity. In static treatment, the activity degree was expressed in conventional units 0 (no LA), 1 (low LA), 2 (medium LA), and 3 (high LA).

**The results and their discussion.** As a result of our previous work, we can consider the composition of microflora and lactobacteria of the oral cavity in healthy women of fertile age to be quite stable both quantitatively and qualitatively. And these results fully meet the established early criteria, and the micro-ecological balance of the oral cavity is maintained in a normal way. It was found out that 5 species dominate the oral lactoflora structure of women of fertile age: *L.acidophilus; L.casei; L.salivarius; L.fermentum; L.rhamnosus.*
It was found out that the species composition of oral lactoflora in healthy women of fertile age is dominated by *L. acidophilus*, *L. casei* and, *L. rhamnosus* in equal shares, while in caries patients women are dominated by *L. salivarius* and *L. casei*.

Lysocime activity, antilysocime activity, antagonistic ability, adhesion index, the ability to produce H$_2$O$_2$ - these properties are considered the most important indicators of lactobacilli, which explain their survival in the oral cavity, as well as are considered influential factors in the microbial semination of the mouth in general.

A study of adhesive ability has shown that all lactobacilli have this property. The strain *L. acidophilus* 4.15 ±0.21 bakt/ery was the most adhesive in women with normal oral cavity, and *L. casei* 2.26 ±0.44 bakt/er was the most low-adhesive, and all other species showed the average adhesiveness of *L. salivarius* 3.87 ±0.21 Bact/ ery, *L. fermentum* 3.0 ±0.16 Bact/ ery, *L. rhamnosus* 2.91 ±0.2 Bact/ ery. In women with tooth decay, *L. casei* 4.67 ±0.03 Bact/ery had a high adhesiveness, while all other species showed an average adhesiveness of *L. acidophilus* 2.52 ±0.12 Bact/ ery, *L. salivarius* 3.67 ±0.41 Bact/ ery, *L. fermentum* 3.80 ±0.16 Bact/ ery, and *L. rhamnosus* 3.0 ±0.15 Bact/ ery.

When determining the antilysocime activity (ALA), oral lactoflora revealed that 98% of healthy lactobacilli and 80% of caries patients had this feature. At the same time, ALA cultures from the former were significantly higher than those from the latter, and were 7.76 ±0.22 and 4.73 ±0.31 µg/ml, respectively. The maximum ALA indicator in the healthy group was *L. salivarius* 8.3 ±0.04 µg/ml and the low ALA indicator was *L. rhamnosus* 7.0 ±0.3 µg/ml, while all others showed intermediate positions of *L. acidophilus* 7.6 ±0.5 µg/ml, *L. casei* 7.9 ±0.06 µg/ml, *L. rhamnosus* 8.0 ±0.2 µg/ml. Strains isolated from caries patients showed lower ALA levels: *L. acidophilus* 4.8 ±0.3 µg/ml, *L. casei* 4.3 ±0.28 µg/ml, *L. salivarius* 4.59 ±0.26 µg/ml, *L. rhamnosus* 5.02 ±0.12 µg/ml.

Five main lactobacillus species were isolated from the mouth cavity - *L. acidophilus*, *L. casei*, *L. salivarius*, *L. fermentum*, *L. rhamnosus*. Antagonism to E.coli, Staphilococcus aureus, Candida albicans, Staphilococcus haemolyticus was studied. According to the results, all the species showed different antagonistic properties, such as *L. acidophilus* - 50% to E.coli, 75% to St.aureus, 100% to St. hayemolyticus, 0% to Candida albicans; *L. casei* - 100% to E.coli, St.aureus, St. hayemolyticus and 25% to Candida albicans; *L. salivarius* - 0% to E.coli, St. hayemolyticus and St. hayemolyticus, *L. fermentum* - 25% to E.coli, 0% to St. aureus, 75% to St. hayemolyticus, 50% to Candida albicans; *L. rhamnosus* - 75% to E.coli, 25% to St. aureus, 100% to St. hayemolyticus, 0% to Candida albicans; whereas the figures for women with caries were relatively lower: *L. acidophilus* - 0% to E.coli, St.aureus, 25% to St. hayemolyticus, 50% to Candida albicans; *L. casei* - 50% to E.coli and St. hayemolyticus, 0% to St. aureus, 25% to Candida albicans; *L. salivarius* - 0% to E.coli, St. aureus, St. hayemolyticus, St. hayemolyticus, St. hayemolyticus, and St. hayemolyticus. haemolyticus and 25% to Candida albicans; *L. fermentum* - 0% to E.coli and St.aureus, 25% to St. haemolyticus and Candida albicans; *L. rhamnosus* - 25% to E.coli, St. haemolyticus, Candida albicans and 0% to St. aureus.

As a result of the conducted research it was found out that 91% of lactobacillus isolated from the oral cavity of healthy women and 71% of caries women had the ability to produce hydrogen peroxide. At the same time, the index of each species in two groups did not differ much, for example, the indexes of lactobacilli in the healthy group were *L. acidophilus* -
3.53±0.03 mmol/l; L.casei - 1.99±0.04 mmol/l; L.salivarius - 3.19±0.03 mmol/l; L. fermentum - 3.44±0.02 mmol/l; L.rhamnosus - 2.02±0.1 mmol/l, while those studied with caries had the following L. acidophilus - 3.69±0.05 mmol/l; L.casei - 1.71±0.04 mmol/l; L.salivarius - 3.53±0.03 mmol/l; L.fermentum - 3.18±0.04 mmol/l; L.rhamnosus - 2.24±0.08 mmol/l.

In the research, 94% of the strain from healthy and 63% of the strain from caries were found to have the ability to produce a lysozyme-like enzyme. The oral lactobacillus L.acidophilus, L.casei, L.salivarius, L.fermentum, L.rhamnosus mainly had average activity. For example, the lactobacillus indices of healthy subjects were L.acidophilus - 6,3 mm; L.casei - 6 mm; L.salivarius - 8,3 mm; L.fermentum - 8,0 mm; L. rhamnosus - 7,3 mm whereas the indices of the isolated from the investigated with caries were slightly decreased: L.acidophilus - 5,0 mm; L.casei - 4,6 mm; L.salivarius - 6,3 mm; L.fermentum - 6,0 mm; L.rhamnosus - 6,0 mm.

The average value of lactobacilli to produce a lysozyme-like enzyme in normal and caries was 2.6±0.15 and 2.0±0.22, respectively.

Table №1

**Biological properties of healthy oral lactobacteria investigated**

<table>
<thead>
<tr>
<th>№</th>
<th>Biological features</th>
<th>L.acidophilus</th>
<th>L.casei</th>
<th>L.salivarius</th>
<th>L.fermentum</th>
<th>L.rhamnosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LA - Lysozyme activity, (mm)</td>
<td>6,3</td>
<td>6,0</td>
<td>8,3</td>
<td>8,0</td>
<td>7,3</td>
</tr>
<tr>
<td>2</td>
<td>AI - Adhesion Index</td>
<td>4,15±0,21</td>
<td>2,26±0,44</td>
<td>3,87±0,21</td>
<td>3,0±0,16</td>
<td>2,91±0,2</td>
</tr>
<tr>
<td>3</td>
<td>AA - antilysozyme activity, (µg/ml)</td>
<td>7,6±0,5</td>
<td>7,9±0,06</td>
<td>8,3±0,04</td>
<td>7,0±0,3</td>
<td>8,0±0,2</td>
</tr>
<tr>
<td>4</td>
<td>H₂O₂ products (mol/l)</td>
<td>3,53±0,03</td>
<td>1,99±0,04</td>
<td>3,19±0,03</td>
<td>3,44±0,02</td>
<td>2,02±0,10</td>
</tr>
<tr>
<td>5</td>
<td>Antagonism rate</td>
<td>E.coli</td>
<td>50%</td>
<td>100%</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>St.aureus</td>
<td>75%</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>St.hemolyt</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida albicans</td>
<td>0%</td>
<td>25%</td>
<td>0%</td>
<td>50%</td>
</tr>
</tbody>
</table>
Table №2

**Biological properties of lactobacteria of the oral cavity studied with caries**

<table>
<thead>
<tr>
<th>№</th>
<th>Biological features</th>
<th>L.acidophilus</th>
<th>L.casei</th>
<th>L.salivarius</th>
<th>L.fermentum</th>
<th>L.rhamnosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LA - Lysozyme activity, (mm)</td>
<td>5.0</td>
<td>4.6</td>
<td>6.3</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>AI - Adhesion Index</td>
<td>2.52±0,12</td>
<td>4.67±0.03</td>
<td>3.67±0.41</td>
<td>3.8±0.16</td>
<td>3.0±0.15</td>
</tr>
<tr>
<td>3</td>
<td>AA - antilysozyme activity, (µg/ml)</td>
<td>4.8±0,3</td>
<td>4.3±0.28</td>
<td>4.59±0.63</td>
<td>4.95±0.26</td>
<td>5.02±0.12</td>
</tr>
<tr>
<td>4</td>
<td>H₂O₂ products (mol/l)</td>
<td>3.69±0.05</td>
<td>1.71±0.04</td>
<td>3.53±0.03</td>
<td>3.18±0.04</td>
<td>2.24±0.08</td>
</tr>
<tr>
<td>5</td>
<td>Antagonism rate</td>
<td>E.coli</td>
<td>0%</td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>St.aureus</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>St.hemolyt</td>
<td>25%</td>
<td>50%</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida albicans</td>
<td>50%</td>
<td>25%</td>
<td>25%</td>
<td>25%</td>
</tr>
</tbody>
</table>

**Conclusion.** Based on our results, we found a significant variation in the biological properties of one species in two study groups. For example, we observed a decrease in the lysozyme and antilysozyme activity (AA, ALA) of all strains in women with caries. And this directly proves the microbiological etiology of the disease. Also, the adhesion index (AI) decreases sharply in *L.acidophilus* and increases in *L.casei* in the group of caries patients. In general, the highest rate of oral lactobacillus adhesive activity in women with caries once again shows their participation in caries formation.

All types of lactobacilli had similar indices for hydrogen peroxide production. Based on these data, we can conclude that the decrease in antagonistic properties of caries is not due to a decrease in the ability to produce hydrogen peroxide in general.

Lactobacilli isolated from the mouth of healthy women showed antagonism to conditionally pathogenic and pathogenic microflora to a greater extent than lactobacilli isolated from the investigated patients with caries. Although not all isolates from healthy women showed maximum antagonistic activity, we can consider that this property plays an important role in maintaining the normal state of the mouth. But it should be remembered that the manifestation of antagonistic activity depends on some factors, among which we should first of all mention the variety of interactions between the antagonist and his victim in specific environmental conditions.
References:


