

ANTIBACTERIAL ACTIVITY OF *Lactobacillus casei* IMV B-7280 IN CASES OF EXPERIMENTAL UROGENITAL STAPHYLOCOCCAL INFECTION

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The aim of work was to determine antibacterial activity of *Lactobacillus casei* IMV B-7280 probiotic strain on the experimental urogenital tract infection of mice. The influence of intravaginal and/or *per os* administration of this strain once per day during 7 days on the microflora of vagina, kidneys and intestinal contents of *Staphylococcus aureus* 8325-4 infected mice was studied.

It was established, that in cases of experimental staphylococcal infection of urogenital tract *L. casei* IMV B-7280 had effective antagonistic activity against *S. aureus* 8325-4 and opportunistic bacteria. After *L. casei* IMV B-7280 introduction into infected mice reduction or complete elimination of *S. aureus* 8325-4 in vagina, kidneys and intestinal contents in different periods of observation was established. Under the influence of *L. casei* IMB B-7280 the number of coliform bacteria, streptococci and staphylococci in the vagina was normalized, and fungal flora — decreased even in comparison with intact mice. Normalization of kidneys microflora was also observed. In the intestinal contents of infected mice treated with *L. casei* IMV B-7280 the number of streptococci did not change, staphylococci number decreased, but the number of fungal and coliform flora remained relatively low during the observation period.

L. casei IMV B-7280 probiotic strain is promising to create immunobiotics with antibacterial action, which can be used for the prevention and treatment of urogenital infections caused by opportunistic microorganisms.

Key words: antibacterial activity, immunobiotics, *Lactobacillus casei*, *Staphylococcus aureus*.

Combined therapy of patients with infectious and inflammatory diseases of urogenital tract, the frequency of which is high and have no clear downward tendency, including both systemic use of antibiotics and stimulation of nonspecific resistance of the organism is using immunomodulatory drugs of different nature. The largest share of urogenital pathogens, infectious agents of infectious and inflammatory diseases belongs to opportunistic microorganisms, increased aggressiveness of which often occurs as a result of vaginal microbiota violations against decrease in the number of lactic acid bacteria, including lactobacilli (LAB) and/or immunosuppression [1–4]. Increase of antimicrobial resistance of uropatogen's that causes infections of the genitourinary system in women also observed [5].

In this regard, there is considerable interest for use of immunobiotics based on lactic acid bacteria, especially LAB and bifidobacteria in cases of urogenital tract infections to restore and preserve balance of urogenital microflora and as a highly efficient natural immunomodulatory drugs [6–9]. Note that these immunobiotics can be used locally (by administrating into vagina), and the development of non-invasive treatments, as it known, is the important task of medicine.

Immunomodulatory activity of some strains of LAB and bifidobacteria is already defined. It was established that these activity is associated with interaction of the structural components of pattern-recognizing receptors expressed on the surface of immune cells. Such receptor-ligand interactions can provide effective development of humoral and cellular

immune response initiated due course of an infection by balancing the production of cytokines of different groups (Th1- and Th2-type cytokines) [10].

The results of experimental studies and clinical observations demonstrate the effectiveness and safety of probiotics in cases of urogenital infections of *Lactobacillus paracasei* CRL 1289, *L. rhamnosus* GR-1, *L. fermentum* RC-14, *L. crispatus* CTV-05 [11–16] and *L. casei* ssp. *rhamnosus* GR-1 [12], *L. casei* var *rhamnosus* [17], *L. casei* GR-1 [18] strains *in vitro* and in animal models showed high antibacterial efficacy regarding infectious diseases of the urogenital tract. Efficacy and safety of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 for patients have proved in several clinical studies in experimental intravaginal application and in their consumption with milk [19]. *L. casei* Shirota and *L. crispatus* CTV-05 strains also appeared effective in the prevention of urogenital infections of humans [20].

Ukrainian pharmaceutical market is represented by a group of probiotics for intravaginal use on the basis of lactic acid bacteria from domestic and foreign manufacturers, which have contraindication and side effects. Antibacterial effectiveness of probiotic drugs was determined, but they effect on the state of the immune system not investigated.

We have previously isolated and characterized probiotic strain *L. casei* IMV B-7280, which had an antagonistic effect *in vitro* relatively to broad spectrum of pathogenic and opportunistic organisms, causative agents of urogenital infections, and immunomodulatory properties associated with the activation of phagocytes, production of interferon (IFN) by physiological norm and normalization of the phenotypic composition of lymphocytes in staphylococcus infected animals [9, 21, 22]. Therefore, this probiotic strain is promising to create immunobiotics with antibacterial activity, which can be used for the prevention and treatment patients with urogenital tract infections caused by opportunistic microorganisms.

So, the aim of this work was to determine antibacterial activity of *L. casei* IMV B-7280 probiotic strain on the experimental urogenital tract infection of mice caused by staphylococcus, by examining the qualitative and quantitative composition of the microflora of vagina, kidneys and intestinal contents.

Materials and Methods

Experimental studies were performed on six-week-old female BALB/c mice that were kept in the vivarium in standard conditions and temperature 22 ± 1 °C during the experiment; they were provided with full-fledged animal feed and had free access to water. All studies were performed taking into account the rules of the “European Convention for the protection of vertebrate animals using for experimental and scientific aims” (Strasbourg, 1986) and “General ethics of animal experimentation”.

L. casei IMV B-7280 strain, previously selected from associated culture during laboratory studies of fermented biological material, was used. Strain was deposited in Ukrainian collection of microorganisms (Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine). Before each experiment the viability of the probiotic cultures was tested by monitoring their growth on the Man-Rogosa-Sharpe (MRS) agar medium at 37 °C for 24–48 h. The study was performed using bacteria lyophilized in Cuddon Freeze Dryer FD1500 (New Zealand).

The definition of cultural, morphological, physiological and biochemical properties of probiotic strains of bacteria were carried out according to generally accepted research methods. Their ability to ferment carbon sources in MRS solution without meat extract and carbohydrates addition was determined, as well as resistance to the action of bile, gastric juice, pancreatic enzymes and phenol [23]. Adhesive properties of the strain to oral mucosa epithelial cell were investigated by the method of [24]. The following indicators were determined: the average adhesion (AA) — the average number of microbial cells that are attached to one surface of the epithelial cells; the participation rate of epithelial cells (PR) — the percentage of epithelial cells, which contained on its surface adhesive microorganisms; index of adhesiveness of the microorganism (AI) — the average number of microbial cells attached on the surface of one epitheliocyte (include only involved in cell adhesion). AI was calculated by the formula: $IAM = (AA \cdot 100) / PR$.

Staphylococcosis was modeled through intravaginal administration of the *S. aureus* 8325-4 daily culture (kindly provided to us by Professor V. S. Zuyeva, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Federation) to mice, in doses of $5 \cdot 10^7$ cells per animal. The following clinical

manifestations of the infection process were observed in the infected mice: significant increase in whitish mucous secretions of the vagina, elevation of body temperature, inactivity, and loss of appetite. *L. casei* IMV B-7280 was administered to these mice into vagina and/or per os in a dose of $1 \cdot 10^6$ cells/animal once per day during 7 days.

5 groups of mice were formed: 1) *S. aureus* infected mice, that were administered with 0.15 M NaCl into vagina (36 mice); 2) infected mice treated with *L. casei* IMV B-7280 into vagina (36 mice); 3) infected mice treated with *L. casei* IMV B-7280 into vagina and per os (36 mice); 4) infected mice treated with *L. casei* IMV B-7280 per os (36 mice); 5) intact mice injected with 0.15 M NaCl into vagina (36 mice).

On the 1, 3, 6, 9 and 12th days after the administration of probiotic strain material was collected from the vagina, kidneys and intestine of decapitated fully anaesthetized mice. The material from vagina was collected using standardized sterile cotton tampons. Swabs from each tampon were performed with 1 ml of 0.15 M saline.

Aliquots of vaginal swabs, suspension of kidneys pounded in sterile sand and suspension of intestinal contents were plated onto nutrient mediums: BAIRD-PARKER-Agar (Merck, Germany; selective medium for staphylococci), KF-Streptococcus agar (Merck, Germany; selective medium for streptococci), ENDO (NSCAMB, Obolensk, Russia; selective medium for coliform bacteria) and Sabouraud agar (selective medium for fungi). After cultivation at 37 °C for 24 h, the number of colony forming units (CFU) was counted, given that one such colony corresponds to one bacterium.

Aliquots of vaginal swabs, suspension of kidneys pounded in sterile sand and suspension of intestinal contents were also plated onto BAIRD-PARKER-Agar nutrient mediums with gentamicin in concentration 15 µg/ml (selective medium for gentamicin-resistant staphylococci), for identification of *S. aureus* 8325-4 strain growth, that had plasmid-based resistance to gentamicin and was able to grow in the medium with these antibiotic.

All digital data received were processed with the help of the Epi Info (version 6.0) software through analysis of variance. Numerical data were represented as arithmetic average and standard error ($M \pm m$). The null hypothesis for the control and experimental comparative groups was checked using Wilcoxon-Mann-Whitney (U) and

Kolmogorov-Smirnov nonparametric criteria. The differences between the groups were considered statistically meaningful at $P < 0.05$.

Results and Discussion

L. casei IMV B-7280 is a stationary strain, does not form spores, is bacillar form, positively stained by the Gram stain and is catalase-negative. Strain is growing in the MRS (Man-Rogosa-Sharpe) medium in a wide pH range from 1.0 to 9.0 (optimum pH — from 5.5 to 6.5) in 38.0 ± 1.0 °C condition for 18–24 hours. We found that stationary phase of growth of this strain starts in 10 h after inoculation on MRS medium. Strain accumulates lactic acid (185 ± 6 °T); ferments a wide range of carbohydrates and alcohols such as glucose, fructose, sodium gluconate, lactose, maltose, galactose, cellobiose, glucose, rhamnose, ribose, sucrose, trehalose, mannose, mannitol, salicin, but not ferments starch, arabinose, raffinose, melibiose, xylose. *L. casei* IMV B-7280 has a high adhesiveness to oral mucosa epithelial cell. So, AI amounted to 7.18 ± 1.75 standard units (SU), AA and PR — 6.74 ± 1.94 and 91.30 ± 5.24 SU respectively. The strain is resistant to bile (in a concentration from 0.1 to 40%), gastric juice (in a concentration from 0.1 to 4.0%), pancreatic enzymes (in a concentration from 0.1 to 4.0%) and phenol. Previously we have shown that *L. casei* IMV B-7280 had antagonistic activity against opportunistic and pathogenic microorganisms *in vitro* [25]. To determine antibacterial activity of this strain *in vivo*, we used experimental model of intravaginal staphylococcal infection in mice induced by *S. aureus* 8325-4. The effect of *L. casei* IMV B-7280 on persistence of *S. aureus* 8325-4 and on the microflora of the vagina, kidneys and intestinal contents of these mice was investigated.

It was established that staphylococcus infection of mice led to increase in the number of opportunistic microorganisms that were seeded out from vagina to elective mediums for staphylococcus, streptococcus and coliform bacteria, and *S. aureus* 8325-4, that was plated on BAIRD-PARKER-Agar medium (Merck, Germany) with 15 mg/ml gentamicin in a stable number throughout the observation period (1–12th days) (Table 1).

The number of *S. aureus* 8325-4 in vagina of staphylococcus infected mice treated with *L. casei* IMV B-7280 by different schemes was significantly lower. Thus, after strain

Table 1. Changes in the microorganism's spectrum of mice vagina under the influence of *L. casei* IMV B-7280

Group of mice	Day of study	The number of microorganisms (lg CFU/ml)				
		BAIRD-PARKER-Agar	BAIRD-PARKER-Agar with gentamicin	KF-STREPRO-COCCUS-Agar	ENDO Agar	SABOURAUD Agar
Intact mice	–	2.51±0.08	0	2.08±0.04	1.08±0.05	2.07±0.04
<i>S. aureus</i> 8325-4 infected mice	1	4.54±0.08*	3.61±0.02	3.53±0.05*	1.60±0.04*	2.63±0.05*
	3	4.30±0.05*	3.22±0.01	3.45±0.03*	1.48±0.04*	1.48±0.02
	6	4.25±0.07*	3.25±0.04	3.34±0.07*	1.48±0.06*	2.18±0.08
	9	4.22±0.11*	3.28±0.02	3.26±0.11*	1.30±0.01	2.34±0.05
	12	4.19±0.09*	3.48±0.03	3.22±0.08*	1.48±0.04*	1.90±0.02
Infected mice that received <i>L. casei</i> IMB B-7280 into vagina	1	2.11±0.02·	2.51±0.02·	2.72±0.07·	1.00±0.00·	1.62±0.03·*
	3	1.60±0.03·	2.34±0.02·	2.26±0.20·	0	0
	6	1.90±0.04·	2.24±0.01·	3.32±0.09·	1.00±0.00·	1.29±0.07·*
	9	2.08±0.01·	1.20±0.01·	3.22±0.14·	0	1.49±0.07·*
	12	2.43±0.05·	0	2.91±0.15·	1.05±0.08·	1.06±0.05·*
Infected mice that received <i>L. casei</i> IMB B-7280 into vagina and <i>per os</i>	1	2.65±0.04·	2.07±0.04·	2.62±0.02·	1.32±0.11	1.31±0.02·*
	3	1.90±0.06·	1.88±0.07·	2.37±0.06·	0	1.09±0.02·*
	6	1.96±0.02·	1.62±0.03·	2.17±0.07·	0	1.12±0.03·*
	9	1.62±0.04·	0	2.64±0.09·	0	1.05±0.03·*
	12	1.99±0.03·	0	2.67±0.08·	0	0
Infected mice that received <i>L. casei</i> IMB B-7280 <i>per os</i>	1	3.75±0.03*	3.49±0.03	3.52±0.02	1.12±0.03	1.71±0.02
	3	3.94±0.04*	3.39±0.08	3.12±0.09	1.30±0.02	1.61±0.02
	6	2.79±0.07·	2.97±0.07	3.01±0.08	1.07±0.02	1.35±0.04
	9	2.55±0.06·	2.35±0.02·	3.00±0.06	1.27±0.03	1.21±0.03
	12	2.68±0.01·	2.17±0.02·	3.16±0.07	1.65±0.01	1.12±0.03

Note: here and later significant difference with intact mice is represented by * ($P < 0.05$) while differences with the indicators of the infected mice who did not receive probiotic strains are represented by · ($P < 0.05$).

introduction to infected mice only into vagina, *S. aureus* 8325-4 number reduced on the 1–9th days, and did not seed out from vagina on the 12th day compared to infected mice that did not receive this strain.

Antagonistic activity of *L. casei* IMV B-7280 in relation to *S. aureus* 8325-4 was increased when *L. casei* IMV B-7280 was administered to infected mice both into vagina and *per os* (*S. aureus* 8325-4 was seeded out from infected mice vagina in a much smaller number on the 1–6th days and did not seed out on the 9 and 12th days). The least effective *L. casei* IMV B-7280 was when the strain was introduced to infected mice only *per os* — *S. aureus* 8325-4 was seeded out from the vagina during the observation period, and it's number reduced only on the 9 and 12th days.

In the vagina of infected mice treated with *L. casei* IMV B-7280 into vagina or into vagina and *per os*, the number of microorganisms,

that were plated on the elective mediums for staphylococci, streptococci and coliform bacteria reduced to the level of intact mice (control), but fungal flora was seeded out even in lower numbers than in the control. As it can be seen from the presented in Table 1 data, after *L. casei* IMV B-7280 *per os* administration to infected mice, only staphylococci were seeded out from their vagina in lower number, but the number of other investigated opportunistic microorganisms in the vagina was the same as in infected mice that did not receive this strain.

We first found that intravaginal infection of mice with *S. aureus* 8325-4 likely contributed to the development of ascending *S. aureus* infection of genitourinary system and led to changes in gut because this strain of staphylococcus was seeded out also from their kidneys and intestinal contents; microflora of kidney and intestinal contents also simultaneously varied (Table 2 and 3).

Table 2. Changes in the microorganism's spectrum of mice kidneys under the influence of *L. casei* IMV B-7280

Group of mice	Day of study	The number of microorganisms (lg CFU/ml)				
		BAIRD-PARKER-Agar	BAIRD-PARKER-Agar with gentamicin	KF-STREPRO-COCCUS-Agar	ENDO Agar	SABOURAUD Agar
Intact mice	–	1.00±0.01	–	0	0	0
<i>S. aureus</i> 8325-4 infected mice	1	1.47±0.03	0	0	1.15±0.01	0
	3	1.86±0.04	1.35±0.02	0	1.00±0.03	0
	6	2.00±0.07	1.48±0.01	1.15±0.01	1.00±0.04	1.28±0.02
	9	1.55±0.02	1.15±0.01	1.65±0.03	1.34±0.02	1.30±0.02
	12	1.69±0.02	1.35±0.03	2.10±0.05	0	1.15±0.01
Infected mice that received <i>L. casei</i> IMB B-7280 into vagina	1	1.20±0.01	0	0	0	0
	3	0	0	0	0	0
	6	0	1.10±0.01	1.25±0.02	0	0
	9	0	0	0	0	0
	12	0	0	0	0	0
Infected mice that received <i>L. casei</i> IMB B-7280 into vagina and per os	1	1.00±0.01	0	0	0	0
	3	0	0	0	0	0
	6	0	0	0	0	0
	9	0	0	0	0	0
	12	0	0	0	0	0
Infected mice that received <i>L. casei</i> IMB B-7280 per os	1	1.20±0.01	0	0	0	0
	3	1.00±0.03	1.12±0.01	0	0	0
	6	1.10±0.02	1.35±0.01	1.56±0.03	0	0
	9	0	0	1.82±0.04	0	0
	12	0	0	1.52±0.02	0	0

Microorganisms that were sown to elective medium for staphylococci eliminated from kidneys from the 3rd day after *L. casei* IMV B-7280 administration into vagina and *per os* or into vagina and from the 9th day after strain administration only *per os*. Streptococci from the kidneys of infected mice did not seeded out during the observation period after administration of *L. casei* IMV B-7280 into vagina and *per os* and were seeded out in small quantities only on the 6th day, but did not seed in other periods of observation after strain administration only into vagina. However, after *per os* administration of *L. casei* IMV B-7280 the amount of streptococci in kidneys decreased only on the 12th day. Coliform bacteria and fungal flora were never seeded out from kidneys of infected mice administrated with *L. casei* IMV B-7280 by different schemes.

As it is shown in Table 3, *S. aureus* 8325-4 was seeded out from the intestinal contents of infected mice throughout the observation period. In the intestinal contents of infected mice the number of staphylococci increased, but did not change the number of

microorganisms that were plated into elective medium for streptococci and fungal flora on the 3–12th days decreased the number of coliform microorganisms.

It is shown that in the intestinal contents of infected mice the quantity of *S. aureus* 8325-4 significantly decreased after administration of *L. casei* IMV B-7280 into vagina or into vagina and *per os* compared with infected mice that did not receive this strain. *S. aureus* 8325-4 eliminated from intestinal contents of infected mice treated with *L. casei* IMV B-7280 into vagina and *per os* from 6th days, and only into vagina — eliminated from intestinal contents only on the 12th day.

L. casei IMV B-7280 influenced on the number of other opportunistic microorganisms in the intestinal contents: the level of staphylococci decreased to the level of control after intravaginal or intravaginal and *per os* administration of the strain. A slight decrease in the number of staphylococci was found on the 1, 6, 9 and 12th days in the intestinal contents of infected mice treated with *L. casei* IMV B-7280 *per os*, but these indicators remained higher than in the control group on the 1–6th days.

Table 3. Changes in the microorganism's spectrum of mice intestine under the influence of *L. casei* IMV B-7280

Group of mice	Day of study	The number of microorganisms (lg CFU/ml)				
		BAIRD-PARKER-Agar	BAIRD-PARKER-Agar with gentamicin	KF-STREPTOCOCCUS-Agar	ENDO Agar	SABOURAUD Agar
Intact mice	–	2.87±0.04	–	3.25±0.01	4.15±0.09	2.77±0.05
<i>S. aureus</i> 8325-4 infected mice	1	3.98±0.06*	1.45±0.01	3.12±0.03	4.11±0.07	2.98±0.04
	3	3.65±0.08*	2.78±0.03	3.05±0.02	3.64±0.07*	3.11±0.06
	6	3.78±0.11*	2.95±0.02	2.96±0.01	3.38±0.08*	3.14±0.09*
	9	3.42±0.06*	2.53±0.03	3.09±0.05	3.43±0.02*	2.76±0.08
	12	3.56±0.08*	2.40±0.04	3.13±0.03	3.58±0.03*	3.04±0.06
Infected mice that received <i>L. casei</i> IMB B-7280 into vagina	1	3.15±0.07·*	1.35±0.01	3.15±0.05	4.05±0.05	2.55±0.07·
	3	3.46±0.09*	1.44±0.01·	3.02±0.01	3.28±0.06*	2.87±0.04
	6	3.21±0.08·*	1.10±0.01·	2.98±0.02	3.45±0.08*	2.43±0.03·
	9	2.89±0.03·	0	3.17±0.05	3.79±0.07	2.27±0.02·*
	12	2.86±0.05·	0	3.24±0.07	4.07±0.12	2.11±0.05·*
Infected mice that received <i>L. casei</i> IMB B-7280 into vagina and <i>per os</i>	1	2.95±0.02·	1.15±0.01·	3.33±0.09	3.98±0.08	1.43±0.02·*
	3	2.66±0.05·	1.00±0.02·	3.25±0.06	3.67±0.09*	0
	6	2.99±0.02·	0	3.41±0.08	3.55±0.04*	0
	9	3.05±0.03·	0	3.17±0.07	3.69±0.07*	0
	12	2.84±0.06·	0	3.10±0.05	3.72±0.08*	0
Infected mice that received <i>L. casei</i> IMB B-7280 <i>per os</i>	1	3.25±0.08·*	1.76±0.02	3.02±0.03	3.58±0.07*	1.76±0.02·*
	3	3.54±0.09*	1.54±0.01·	2.86±0.05	3.61±0.04*	1.28±0.05·*
	6	3.26±0.07·*	1.42±0.03·	2.65±0.03	3.24±0.03*	1.13±0.04·*
	9	3.11±0.04·	1.15±0.01·	2.98±0.03	3.66±0.03*	1.10±0.07·*
	12	3.06±0.03·	0	3.12±0.05	3.79±0.07*	0

The number of streptococci appeared unchanged in the intestinal contents of infected mice of all groups treated with *L. casei* IMV B-7280, while the number of coliform bacteria in different periods of observation kept low compared to the level of control (except 1st day after this strain administrating into vagina). *L. casei* IMV B-7280 had antagonistic effect against fungal flora in the intestinal contents, its number decreased after strain administration into vagina on the 9 and 12th days compared with infected mice that did not receive probiotic strain. In the intestinal contents of infected mice treated with *L. casei* IMV B-7280 into vagina and *per os* the fungal flora were eliminated from the 3rd day. *L. casei* IMV B-7280 *per os* administration to infected mice led to a significant decrease in the number of fungal flora in the intestinal contents from the 1st day of observation period compared to infected mice that did not receive probiotic strain.

L. casei IMV B-7280, as described earlier [22], was resistant to bile (in concentration from

0.1 to 40%), gastric juice (at a concentration of 0.1–4%), pancreatic enzymes (at a concentration of 0,1–4%); some antibiotics; had high adhesiveness to the red blood cells in peripheral blood of healthy donors and epithelial cells. It was shown that after this strain introduction to staphylococcus infected mice the number of CD3+, CD4+ T cells normalized in spleen and immunoregulatory index CD4/CD8 were increased [9]. Under the influence of *L. casei* IMV B-7280 activation of effector phagocytic system cell function and the change of Th1- and Th2-type cytokine production growth also observed towards Th1-type (IFN- γ and interleukin(IL)-12) cytokine production and reduce of Th2-type (IL-4); in these mice was increased number of natural killer cells in the spleen (unpublished data). That is, in cases of experimental staphylococcal infections of urogenital tract *L. casei* IMV B-7280, on the one hand, inhibited the growth of opportunistic microorganisms, causative agents of infectious diseases of the urogenital tract, on the other — had immunomodulatory

properties associated with changes in indicators of innate and acquired immunity.

According to the literature, *L. paracasei* CRL 1289 strain also had antagonistic effect against *S. aureus* on the mice model of intravaginal staphylococcus infection, which confirmed by the decrease in the number of pathogens in the vagina, normalization of inflammation and cytomorphological structure of vaginal mucosa epithelium [26]. The group of prototype strains for *L. casei* IMV B-7280 including such as strains as: *L. casei* var *rhamnosus* [17], *L. casei* GR-1 [18], *L. casei rhamnosus* (Lcr35) [27] and *L. casei Shiota* [28]. *L. casei* GR-1 [18] inhibited the growth of uropathogen bacteria, so this strain can be used in clinic for correction of the urogenital tract microflora. *L. casei rhamnosus* (Lcr35) normalized microbiocenosis of vagina in cases of vaginosis [18]. *L. casei Shiota* strain was effective in urogenital infections preventing. From the above only *L. casei Shiota* probiotic strain has researched immunomodulatory effect associated with the induction of interferon production in low titers and impact on cellular immunity indicators [28].

However, unlike the prototype strains — *L. casei* var *rhamnosus*, *L. casei* GR-1, *L. casei rhamnosus* (Lcr35) [17, 18, 27] — *L. casei* IMV B-7280 strain has simultaneously described antibacterial and immunomodulatory properties. Unlike *L. casei Shiota* strain [28], *L. casei* IMV B-7280 strain has a wider range of immunomodulatory action, as far as it affects not only the production of interferon, but also a Th1- (IL-12, IFN- γ) and Th2- (IL-4) type cytokines production, and alter both innate and acquired immunity on local and systemic levels in cases of urogenital infections.

Thus, injection of mice with *S. aureus* 8325-4 induced infectious process in the vagina and likely contributed the development of ascending *S. aureus* infection of genitourinary system, as evidenced by: *S. aureus* 8325-4 identifying in the vagina (on the 1–12th days) and kidneys (on the 3–12th days); change in

vaginal microflora (increase in the number of staphylococci, streptococci, fungal and coliform bacteria) and appearance of streptococci in kidneys (on the 6–12th days), coliform bacteria (on the 3–9th days), fungal flora (on the 6–12th days); increasing the number of staphylococci.

After *S. aureus* 8325-4 infecting, mice intestinal microflora changed: *S. aureus* 8325-4 appeared in the intestinal contents during the observation period; increased the number of staphylococci and fungal flora and reduced the number of coliform bacteria was observed.

Probiotic strain *L. casei* IMV B-7280, which was administered to infected mice vagina or into vagina and *per os* at a dose of $1 \cdot 10^6$ cells/animal daily for 7 days, had antistaphylococcal effect *in vivo*: the number of *S. aureus* 8325-4 in the vagina decreased, full elimination of the pathogen took place in different periods of observation. However normalized number of staphylococci, streptococci and coliform bacteria and fungal flora number in the vagina decreased as compared with intact mice.

In cases of experimental *S. aureus* urogenital infection *L. casei* IMV B-7280 which was administered to infected mice vagina or vagina and/or *per os* showed antistaphylococcal effect, that was testified in reduction or complete elimination of *S. aureus* 8325-4 in kidney and intestinal contents in different periods of observation; normalization of kidneys microflora was also observed. In the intestinal contents of infected mice treated with *L. casei*-IMV B-7280 the number of streptococci was not changed, staphylococci number was decreased, but the number of coliform bacterial and fungal flora were remained low, relative to the control, during the observation period.

So probiotic strain *L. casei* IMV B-7280 is promising to create immunobiotics with antibacterial activity, which can be used for the prevention and treatment of urogenital tract infections caused by opportunistic microorganisms.

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**АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ
Lactobacillus casei IMB B-7280
ЗА ЕКСПЕРИМЕНТАЛЬНОЇ
УРОГЕНІТАЛЬНОЇ СТАФІЛОКОКОВОЇ
ІНФЕКЦІЇ**

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Метою роботи було визначення антибактеріальної активності *Lactobacillus casei* IMB B-7280 за експериментальної урогенітальної стафілококової інфекції у мишей. Досліджували вплив цього пробіотичного штаму лактобактерій, який вводили інфікованим *Staphylococcus aureus* 8325-4 мишам у піхву та/або *per os* щодоби протягом 7 діб, на мікрофлору піхви, нирок і кишкового вмісту.

Встановлено, що за експериментальної урогенітальної стафілококової інфекції *L. casei* IMB B-7280 справляв ефективну антибактеріальну дію стосовно як *St. aureus* 8325-4, так й інших умовно патогенних бактерій. Після введення *L. casei* IMB B-7280 інфікованим мишам спостерігали зменшення або повну елімінацію *St. aureus* 8325-4 із піхви, нирок та кишкового вмісту в різні терміни спостереження.

Пробіотичний штам *L. casei* IMB B-7280 є перспективним для створення імунобіотиків з антибактеріальною активністю, які можна використовувати для профілактики та лікування урогенітальних інфекцій, спричинених умовно патогенними мікроорганізмами.

Ключові слова: антибактеріальна активність, імунобіотики, *Lactobacillus casei*, *Staphylococcus aureus*.

**АНТИБАКТЕРИАЛЬНАЯ АКТИВНОСТЬ
Lactobacillus casei IMB B-7280 ПРИ
ЭКСПЕРИМЕНТАЛЬНОЙ
УРОГЕНИТАЛЬНОЙ
СТАФИЛОКОККОВОЙ ИНФЕКЦИИ**

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Целью работы было определение антибактериальной активности *Lactobacillus casei* IMB B-7280 при экспериментальной урогенитальной стафилококковой инфекции у мышей. Исследовали влияние этого пробиотического штамма лактобактерий, который вводили инфицированным *Staphylococcus aureus* 8325-4 мышам во влагалище и/или *per os* ежедневно в течение 7 сут, на микрофлору влагалища, почек и кишечного содержимого.

Установлено, что при экспериментальной урогенитальной стафилококковой инфекции *L. casei* IMB B-7280 оказывал эффективное антибактериальное действие относительно как *St. aureus* 8325-4, так и других условно патогенных бактерий. После введения *L. casei* IMB B-7280 инфицированным мышам наблюдали уменьшение или полную элиминацию *St. aureus* 8325-4 из влагалища, почек и кишечного содержимого в разные сроки наблюдения.

Пробиотический штамм *L. casei* IMB B-7280 является перспективным для создания иммунобиотиков с антибактериальной активностью, которые могут использоваться для профилактики и лечения урогенитальных инфекций, вызванных условно патогенными микроорганизмами.

Ключевые слова: антибактериальная активность, иммунобиотики, *Lactobacillus casei*, *Staphylococcus aureus*.