

## EFFECTS OF ORAL AND VAGINAL ADMINISTRATION OF PROBIOTIC BACTERIA ON THE VAGINAL MICROBIOTA AND CYTOKINES PRODUCTION IN THE CASE OF EXPERIMENTAL STAPHYLOCOCCOSIS IN MICE

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**Objective.** The objective of this study was to elucidate the effects of the probiotic strain *Lactobacillus casei* IMV B-7280 and *L. casei* IMV B-7280 - *Bifidobacterium animalis* VKL - *B. animalis* VKB (*Lc* - *VKL* - *VKB*) or *B. animalis* VKL - *B. animalis* VKB (*VKL* - *VKB*) strain compositions that administrated both orally and vaginally or only orally, on the vaginal microbiota and cytokines production in experimental vaginal staphylococcosis in BALB/c mice. **Methods.** Probiotic bacteria administrated into staphylococcus-infected mice vaginally and orally simultaneously or only orally. Material from the vagina was plated on selective media; the number of colony forming units was counted. The concentration of cytokines in the blood serum of mice was determined by enzyme immunoassay method. **Results.** It was found, that the number of aerobic and facultative anaerobic microorganisms in the vagina of staphylococcus-infected mice was decreased after administration of these probiotic bacteria or its compositions both into vagina and orally or only orally. The increase in the number of lactic acid bacteria (LAB) and bifidobacteria in the vagina of these mice was also observed. However, the number of coliform bacteria and microscopic fungi was smaller and the number of LAB was greater after administration of *L. casei* IMV B-7280 or *Lc* - *VKL* - *VKB* and *VKL* - *VKB* compositions into staphylococcus-infected mice vaginally and orally simultaneously, compared to only oral administration. A change of the vaginal microbiota of staphylococcus-infected mice that received these probiotic bacteria was accompanied with higher level of interferon- $\gamma$  and interleukine-12 production, which favors the development of Th1 immune response and decrease of the production of pro-inflammatory cytokine tumor necrosis factor- $\alpha$ . The immunomodulatory effectiveness of *L. casei* IMV B-7280 and *Lc* - *VKL* - *VKB* composition will also be higher if they are administered into staphylococcus-infected mice both orally and vaginally than only orally.

**Conclusions.** Probiotic strain *L. casei* IMV B-7280 and *Lc* - *VKL* - *VKB* or *VKL*-*VKB* strain compositions can be a good candidate for the creation of immunobiotics for treatment of urogenital infections.

**Keywords:** lactic acid bacteria, bifidobacteria, microbiota, vagina, staphylococci, cytokines, mice.

Traditionally, probiotics, based of commensal microbiota, in particular lactic acid bacteria (LAB) and bifidobacteria, are widely used for the treatment and prevention of intestinal or vaginal microbiota imbalance in many infectious and non-infectious human diseases [1, 2, 3, 4]. Most studies have shown the favor

of probiotics in the prevention or treatment as alternatives or co-treatments of urogenital infections in adults and children. Although antibacterial therapy using antibiotics is usually effective, a high frequency of relapses of urogenital infections and increasing microbial resistance are observed due to the multiple use of antimicrobial drugs, that justify the search for new approaches for treatment of patients. So, it has been shown that probiotics, including some selected strains of *Lactobacillus* may be an effective strategy to prevent bacterial vaginosis, urinary tract infections, vulvovaginal candidiasis, papillomavirus infection, etc. [1, 5, 6, 7]. It was also justified the joint use of probiotics with antibiotics in the treatment of bacterial vaginosis for more effective microbiota restoring in the urogenital tract [8].

However, a number of experimental and clinical studies demonstrated that some probiotic strains of LAB and bifidobacteria are able to effect the immune response in infectious, allergic, neoplastic and other diseases, therefore, they can be used to create high-efficient immunobiotics. So, these probiotic strains activated the innate (increased the activity of dendritic cells, neutrophils, macrophages, natural killer cells etc.) and adoptive immunity, and altered the production of the Th1 (interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-2 (IL-2), IL-12, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) etc.) or Th2 (IL-4, IL-10 etc.) type cytokines, which determine, in what direction the immune response will develop – humoral or cellular [9, 10]. Th1 cells and Th1 type cytokines play crucial roles in host health against viral and bacterial infections, alleviate allergic diseases and suppress tumors [11]. It should be noted that immunomodulatory properties of LAB and bifidobacteria are species- and strain-specific. Different species or variants within the same species can interact with the immune system on local or systemic levels in different ways [10]. Probably the mode of administration of these probiotic strains is also important. Thus, before LAB and bifidobacteria strains using as immunobiotics, it is necessary to carefully examine the mechanisms of their immunomodulatory action in various experimental conditions.

We previously found that the probiotic strains *Lactobacillus casei* IMV B-7280, *Bifidobacterium animalis* VKL and *B. animalis* VKB had anti-staphylococcal and immunomodulatory efficacy in the case of experimental staphylococcosis in BALB/c mice. After administration of these probiotic bacteria or compositions with its presence into vagina of staphylococcus-infected mice, the normalization of the vaginal microbiota, as well as the activation of the cellular immune response were observed [12].

The objective of this study was to elucidate the effects of oral or oral and vaginal administration of probiotic strain *L. casei* IMV B-7280 and *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB (Lc - VKL - VKB) or *B. animalis* VKL - *B. animalis* VKB (VKL - VKB) compositions on the vaginal microbiota and cytokines production in the case of experimental staphylococcosis in mice.

**Materials and methods.** Experimental studies were performed on six-week-old female BALB/c mice, synchronized in their estral cycle. All studies were performed taking into account the rules of the “European Convention for the protection of vertebrate animals used for experimental and other scientific purposes” (Strasbourg, 1986) and in accordance with “General ethical

principles of experiments on animals". Mice were kept in standard vivarium conditions at a temperature of  $22 \pm 1$  °C, they were provided with the full mixed feed and had free access to automatic water bowls.

The probiotic strains used were: *L. casei* IMV B-7280 (deposited in the Depository of microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine), *B. animalis* VKL and *B. animalis* VKB that isolated from content of gut of healthy people. The lyophilized probiotic bacteria were used in our study. The concentration of the LAB and bifidobacteria were tested by plating on the Man-Rogosa-Sharpe agar (MRSA) medium and Bifidum-agar (BA) in aerobic and anaerobic conditions respectively at 37 °C for 24-48 h and prepared suspension in phosphate-buffered saline (PBS) of given concentration.

*Staphylococcus aureus* strain 8325-4 (kindly provided to us by Professor V.S. Zuyeva, N.F. Gamaleya Institute of Epidemiology and Microbiology, Russian Federation), which contains the plasmid of resistance to gentamicin was chosen for modeling of the intravaginal staphylococcosis in mice. Suspension of the *S. aureus* strain 8325-4 daily culture in PBS was administered once into the vagina of BALB/c mice in the dose of  $5 \times 10^7$  cells per animal. On the 1<sup>st</sup> day after infection a suspension of *L. casei* IMV B-7280 (individually) or Lc - VKL - VKB, or VKL - VKB compositions (in equal proportions of each bacteria) in PBS was administrated into vagina or/and orally in a dose of  $1 \times 10^6$  cells per animal, once a day for 7 days.

8 experimental groups were formed from BALB/c mice (12 mice in each group): 1) intact mice; 2) infected mice; 3) infected mice that obtained *L. casei* IMV B-7280 orally; 4) infected mice that obtained *L. casei* IMV B-7280 orally and vaginally; 5) infected mice that obtained VKL - VKB orally; 6) infected mice that obtained VKL - VKB orally and vaginally; 7) infected mice that obtained Lc - VKL - VKB orally; 8) infected mice that obtained Lc - VKL - VKB orally and vaginally.

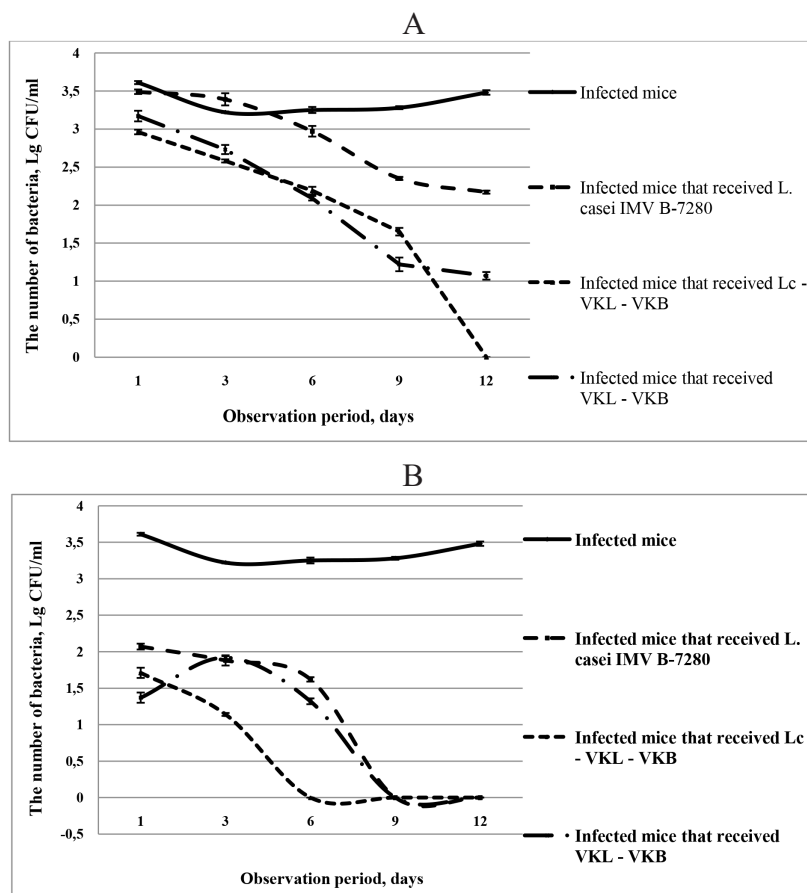
The material from vagina was collected using standardized sterile cotton tampons on the 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 30<sup>th</sup> days after first administration of the probiotic bacteria. Swabs from each tampon were performed with 1 mL of 0.5 M NaCl and plated 0.1 ml onto agar nutrient media: Meat-Peptide agar (MPA, medium for aerobic and facultative anaerobic bacteria), 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), Sabouraud agar without antibiotics (selective medium for fungi), MRSA (selective medium for lactobacilli) and BA (selective medium for bifidobacteria). 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. Swabs from the vagina were also plated on elective medium for staphylococci (BAIRD-PARKER-Agar, Merck, Germany) containing gentamicin at a concentration of 15 µg/ml to identify the growth of *S. aureus* strain 8325-4, which has a plasmid-based resistance to gentamicin, so unlike other strains of staphylococci it is able to grow on the medium with this antibiotic.

On the 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> days after probiotic bacteria administration the peripheral blood was taken from the tail vein of each mouse of all groups

who had previously received anesthesia. We determined the concentration of cytokines in the blood serum of mice by enzyme immunoassay method (ELISA). Method was performed according to the manufacturer's recommendations (Thermo Fisher Scientific Inc. (Bender MedSystems GmbH), Austria). ELISA test kits for determination of mice IL-12 (p40 + p70), mice IFN- $\gamma$ , mice TNF- $\alpha$  were used. The concentration of cytokines in serum was expressed as pg./ml.

All digital data received were processed with the help of the Epi Info software (version 6.0) through analysis of variance. Numerical data were represented as arithmetic average and standard error ( $M \pm m$ ) using Student's t-test. The differences between the groups were considered statistically significant at  $P < 0.05$ .

**Results.** The acceleration of elimination of *S. aureus* strain 8325-4 from the vagina of staphylococcus-infected mice was observed after *L. casei* IMV B-7280 or Lc - VKL - VKB and VKL - VKB compositions oral and intravaginal or only oral administration compared with staphylococcus-infected mice that did not obtain probiotic bacteria (control group). We found that the anti-staphylococcal efficiency of *L. casei* IMV B-7280 or Lc - VKL - VKB and VKL - VKB compositions depended on the mode of their administration into staphylococcus-infected mice (Fig. 1).



**Fig. 1.** The cell counts of *S. aureus* strain 8325-4 recovered from the vagina of infected BALB/c mice receiving *L. casei* IMV B-7280 or probiotic compositions orally (A) or orally and vaginally (B)

Complete elimination of *S. aureus* strain 8325-4 from the vagina was observed only on the 12<sup>th</sup> day after oral administration of Lc - VKL - VKB composition. But, *S. aureus* 8325-4 was eliminated completely from the vagina of staphylococcus-infected mice after oral and vaginal administration of Lc - VKL - VKB composition on the 6<sup>th</sup> day and *L. casei* IMV B-7280 or VKL - VKB composition on the 9<sup>th</sup> day. Our data show that the anti-staphylococcal efficiency of these probiotic bacteria was higher after simultaneous oral and vaginal administration into staphylococcus-infected mice than after only oral administration.

The number of aerobic and facultative anaerobic microorganisms, staphylococci, streptococci, coliform bacteria and microscopic fungi in the vagina of staphylococcus-infected mice was decreased after oral or simultaneous oral and vaginal administration of *L. casei* IMV B-7280 strain, as well as Lc - VKL - VKB or VKL - VKB compositions-compared with control group (Table 1). However, the number of coliform bacteria and microscopic fungi was more rapidly decreased after simultaneous vaginal and oral administration of probiotic bacteria than after only oral administration ( $P < 0.05$ ).

The increase in the number of LAB and bifidobacteria in the vagina of staphylococcus-infected mice that obtained these probiotic bacteria was also observed (Fig. 2). But, after vaginal and oral administration of *L. casei* IMV B-7280 or Lc - VKL - VKB and VKL - VKB compositions, the number of LAB was greater throughout the observation period (30 days) than after only oral administration ( $P < 0.05$ ). The level of LAB on the 30<sup>th</sup> day was the highest in the group of infected mice that obtained *L. casei* IMV B-7280 into vagina and orally. *L. casei* IMV B-7280 or Lc - VKL - VKB and VKL - VKB compositions increased the level of bifidobacteria in case of both vaginal and oral introduction throughout the observation period (30 days), but on the 30<sup>th</sup> day the level of bifidobacteria was higher in the groups of infected mice that obtained Lc - VKL - VKB and VKL - VKB compositions into vagina and orally and VKL - VKB composition only orally.

Also we observed changes in the production of the Th1 type cytokines (IL-12 and IFN- $\gamma$ ) in different periods of observation (Table 2).

It should be noted that the level of IL-12 in the serum of staphylococcus-infected mice that did not obtain the probiotic bacteria or probiotic compositions was decreased on the 1<sup>st</sup> day.

A slight decrease in the level of IFN- $\gamma$  in serum of these staphylococcus-infected mice was also observed throughout the observation period but the difference compared with the intact mice was not statistically significant. The infection with *S. aureus* strain 8325-4 also caused a significant increase in the level of TNF- $\alpha$  on the 3<sup>rd</sup> day, which probably confirms the development of inflammation.

As shown in Table 2, the oral administration of *L. casei* IMV B-7280 into staphylococcus-infected mice did not affect the level of IL-12 in the serum throughout the observation period. But, the level of IFN- $\gamma$  in the serum of mice of this group was increased compared with control group throughout the observation period. At the same time, the level of IL-12 (throughout the observation period) and the level of IFN- $\gamma$  (on the 3<sup>rd</sup> and 6<sup>th</sup> days) were significantly higher in serum of staphylococcus-infected mice treated with

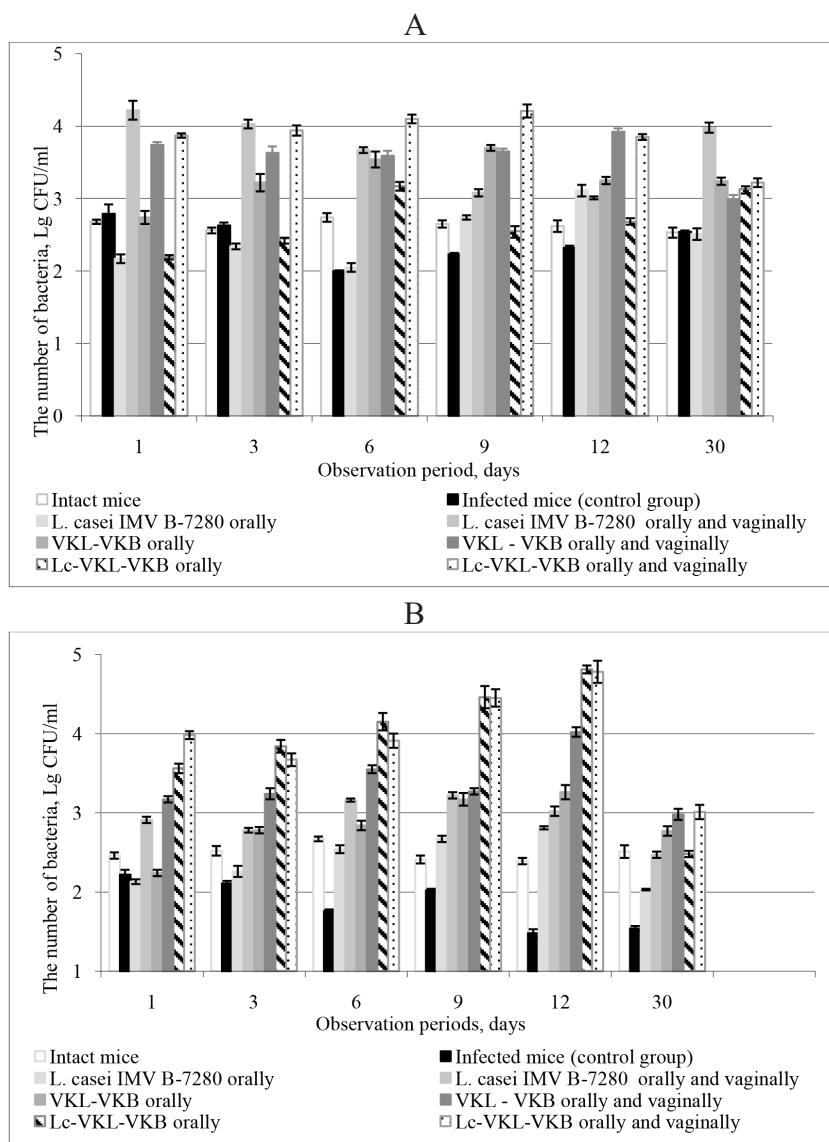
**Table 1**  
**Spectrum of microorganisms in the vagina of staphylococcus-infected mice that received probiotic bacteria orally or vaginally,  $M \pm m$**

Group of mice/ day of study	The number of microorganisms, Lg CFU/ml					
	MPA	BAIRD-PARKER-Agar	KF-Streptococcus agar	ENDO	Sabouraud agar	
Intact mice	1	2.24 ± 0.12	2.51 ± 0.08	2.08 ± 0.04	1.08 ± 0.05	2.07 ± 0.04
	3	2.17 ± 0.06	2.45 ± 0.07	2.02 ± 0.05	1.03 ± 0.02	2.11 ± 0.04
	6	2.33 ± 0.05	2.39 ± 0.03	2.14 ± 0.03	1.06 ± 0.01	2.09 ± 0.02
	9	2.27 ± 0.07	2.61 ± 0.05	2.17 ± 0.07	1.09 ± 0.03	2.12 ± 0.05
	12	2.19 ± 0.02	2.58 ± 0.02	2.09 ± 0.01	1.05 ± 0.05	2.03 ± 0.02
	1	3.78 ± 0.13*	4.54 ± 0.08*	3.53 ± 0.05*	1.60 ± 0.04*	2.63 ± 0.05*
Infected mice (control group)	3	3.82 ± 0.09*	4.30 ± 0.05*	3.45 ± 0.03*	1.48 ± 0.04*	1.48 ± 0.02*
	6	3.83 ± 0.11*	4.25 ± 0.07*	3.34 ± 0.07*	1.48 ± 0.06*	2.18 ± 0.08
	9	3.51 ± 0.08*	4.22 ± 0.11*	3.26 ± 0.11*	1.30 ± 0.00*	2.34 ± 0.05*
	12	3.36 ± 0.08*	4.19 ± 0.09*	3.22 ± 0.08*	1.33 ± 0.08*	1.77 ± 0.04
	1	2.17 ± 0.02•	3.82 ± 0.05*•	3.62 ± 0.01*	1.24 ± 0.04•	1.69 ± 0.05*•
	3	2.16 ± 0.03•	3.92 ± 0.03*•	3.04 ± 0.05*•	1.37 ± 0.02*•	< 1
<i>L. casei</i> IMV B-7280 orally	6	2.23 ± 0.01•	2.84 ± 0.02*•	3.10 ± 0.03*•	1.23 ± 0.03•	1.25 ± 0.03*•
	9	2.54 ± 0.04*•	2.67 ± 0.06•	3.14 ± 0.09*	1.31 ± 0.04*•	< 1
	12	2.68 ± 0.05*•	2.61 ± 0.03•	3.22 ± 0.06*•	1.32 ± 0.05	1.08 ± 0.02*•
	1	2.73 ± 0.02•	2.72 ± 0.01•	2.58 ± 0.04*•	1.28 ± 0.08	1.26 ± 0.03*•
	3	2.25 ± 0.07•	1.85 ± 0.08*•	2.45 ± 0.03•	< 1	1.12 ± 0.04*•
	6	2.36 ± 0.05•	1.89 ± 0.05*•	2.28 ± 0.08•	< 1	1.03 ± 0.02*•
<i>L. casei</i> IMV B-7280 orally and vaginally	9	2.33 ± 0.03	1.73 ± 0.03*•	2.72 ± 0.05*•	< 1	< 1
	12	2.45 ± 0.02•	2.10 ± 0.02*•	2.74 ± 0.05*•	< 1	< 1

Group of mice/ day of study		The number of microorganisms, Lg CFU/ml				
		MPA	BAIRD-PARKER-Agar	KF-Streptococcus agar	ENDO	Sabouraud agar
VKL-VKB orally	1	2.78 ± 0.08*	3.82 ± 0.04*	3.79 ± 0.04*	1.65 ± 0.05*	2.14 ± 0.08•
	3	3.12 ± 0.06*	3.51 ± 0.02*	4.01 ± 0.05*	1.48 ± 0.03*	1.25 ± 0.03*
	6	3.52 ± 0.08*	3.29 ± 0.07*	3.86 ± 0.06*	2.57 ± 0.02**	1.06 ± 0.02*
	9	3.15 ± 0.09*	3.31 ± 0.03*	3.70 ± 0.08*	< 1	< 1
	12	3.19 ± 0.11*	3.14 ± 0.02*	3.22 ± 0.05*	< 1	1.13 ± 0.04*
VKL - VKB orally and vaginally	1	3.08 ± 0.03*	1.96 ± 0.07*	3.11 ± 0.04*	1.25 ± 0.01*	< 1
	3	3.13 ± 0.11*	2.04 ± 0.06*	2.76 ± 0.03*	1.23 ± 0.04*	< 1
	6	3.06 ± 0.07*	2.19 ± 0.03*	3.42 ± 0.03*	< 1	1.23 ± 0*
	9	2.93 ± 0.10*	1.32 ± 0.04*	3.99 ± 0.09*	< 1	< 1
	12	2.91 ± 0.05*	1.67 ± 0.06*	4.15 ± 0.02*	< 1	1.54 ± 0.06*
Lc-VKL-VKB orally	1	2.78 ± 0.05*	3.92 ± 0.10*	3.96 ± 0.05*	1.64 ± 0.04*	2.24 ± 0.07*
	3	2.96 ± 0.06*	2.90 ± 0.06*	3.52 ± 0.04*	1.82 ± 0.03**	< 1
	6	3.03 ± 0.06*	2.55 ± 0.03•	3.16 ± 0.03*	< 1	2.09 ± 0.02
	9	3.13 ± 0.02*	2.41 ± 0.02•	3.42 ± 0.06*	1.13 ± 0.02•	1.31 ± 0.06*
	12	2.73 ± 0.03*	2.11 ± 0.04*	2.93 ± 0.02*	< 1	< 1
Lc-VKL-VKB orally and vaginally	1	2.92 ± 0.02*	2.06 ± 0.12*	3.77 ± 0.12*	1.15 ± 0.04**	1.11 ± 0.02**
	3	3.21 ± 0.06*	2.74 ± 0.06•	3.54 ± 0.05*	< 1	< 1
	6	3.06 ± 0.07*	2.12 ± 0.04*	3.22 ± 0.05*	< 1	< 1
	9	2.65 ± 0.03*	1.12 ± 0.02*•	2.84 ± 0.07*•	< 1	< 1
	12	2.60 ± 0.09*•	1.47 ± 0.05*•	2.65 ± 0.02*•	< 1	< 1

Significant differences with the intact mice are represented by \* ( $P < 0.05$ ), while differences with the indicators of staphylococcus-infected mice who did not receive probiotic bacteria or probiotic compositions (control group) are represented by • ( $P < 0.05$ ). The agar nutrient media: Meat-Peptide agar (MPA, medium for aerobic and facultative anaerobic bacteria), BAIRD-PARKER-Agar (Merek, Germany; selective medium for staphylococci), KF-Streptococcus agar (Merek, Germany; selective medium for streptococci), ENDO (NSCAMB, Obolensk, Russia; selective medium for coliform bacteria), Sabouraud agar without antibiotics (selective medium for fungi).

*L. casei* IMV B-7280 orally and vaginally than after this strain were administered only orally ( $P < 0.05$ ). We observed an increase in the levels of IL-12 (on the 1<sup>st</sup> day) and IFN- $\gamma$  (on the 1<sup>st</sup> and 6<sup>th</sup> days) after oral administration of Lc - VKL - VKB composition into staphylococcus-infected mice compared with control group. The increase in the levels of IL-12 (throughout the observation period) and IFN- $\gamma$  (on the 6<sup>th</sup> and 9<sup>th</sup> days) was more substantial in serum of staphylococcus-infected mice that obtained this probiotic composition orally and vaginally than only orally ( $P < 0.05$ ). On the contrary, in the case of using VKL - VKB composition for the treatment of staphylococcus-infected mice, the level of IL-12 (on the 1<sup>st</sup> and 3<sup>rd</sup> days) was higher if this composition was administered only orally than orally and vaginally. This probiotic composition increased the level of IFN- $\gamma$  on the 1<sup>st</sup> and 6<sup>th</sup> days and only on the 3<sup>rd</sup> day if it



**Fig. 2.** The cell counts of LAB (A) and bifidobacteria (B) recovered from the vagina of infected BALB/c mice receiving *L. casei* IMV B-7280 or probiotic compositions orally or orally and vaginally



was administered orally or orally and vaginally respectively.

Additionally, the production of TNF- $\alpha$  was examined to confirm that these probiotic strains had anti-inflammatory effect in the case of experimental staphylococcosis. After their oral and vaginal or only oral administration into staphylococcus-infected mice, the level of the TNF- $\alpha$  significantly decreased or was not detected at all in the serum of mice from these experimental groups.

Our findings suggest that the elimination of *S. aureus* strain 8325-4 from the vagina and normalization of the vaginal microbiota of staphylococcus-infected mice that received *L. casei* IMV B-7280 and Lc - VKL - VKB or VKL - VKB compositions orally and vaginally or only orally occurs at higher level of IFN- $\gamma$  and IL-12 production, which favors the development of the Th1 type immune response, and decrease of the production of pro-inflammatory cytokine TNF- $\alpha$ . It should be noted that the level of IFN- $\gamma$  significantly increased on the 3<sup>rd</sup> day if *L. casei* IMV B-7280 or studied probiotic compositions were administered into staphylococcus-infected mice orally and vaginally compared with their oral administration.

**Table 2**

**The cytokines level in the serum of infected mice that obtained probiotic bacteria,  $M \pm m$**

Groups of mice	Observation period after injection of probiotic strains	Concentration of cytokine, pg./ml		
		IL-12	TNF- $\alpha$	IFN- $\gamma$
Intact mice	-	613.8 $\pm$ 56.9	5.1 $\pm$ 0.2	3.7 $\pm$ 0.5
Infected mice (control group)	1 day	350.9 $\pm$ 11.9*	4.0 $\pm$ 0.4	2.1 $\pm$ 0.6
	3 day	678.8 $\pm$ 32.1	12.3 $\pm$ 0.7*	2.3 $\pm$ 0.6
	6 day	587.3 $\pm$ 42.9	6.1 $\pm$ 0.1	2.7 $\pm$ 0.9
Infected mice that obtained <i>L. casei</i> IMV B-7280 orally	1 day	385.6 $\pm$ 13.7*	6.2 $\pm$ 0.6	22.1 $\pm$ 0.2*•
	3 day	760.0 $\pm$ 44.3	5.4 $\pm$ 0.4	8.2 $\pm$ 0.3*•
	6 day	625.5 $\pm$ 52.4	0	6.1 $\pm$ 0.5•
<i>L. casei</i> IMV B-7280 orally and vaginally	1 day	618.3 $\pm$ 47.9•	0	12.1 $\pm$ 0.7*•
	3 day	855.8 $\pm$ 38.0*•	0	97.5 $\pm$ 1.2*•
	6 day	1365.3 $\pm$ 76.8*•	0	29.9 $\pm$ 1.4*•
VKL - VKB orally	1 day	1045.0 $\pm$ 65.3*•	1.1 $\pm$ 0.1*	11.3 $\pm$ 0.2*•
	3 day	1227.0 $\pm$ 54.1*•	2.6 $\pm$ 0.4	2.4 $\pm$ 0.2*
	6 day	499.5 $\pm$ 37.2	6.1 $\pm$ 0.2	9.5 $\pm$ 0.1*•
VKL - VKB orally and vaginally	1 day	855.6 $\pm$ 25.4*•	0	5.2 $\pm$ 1.2
	3 day	469.2 $\pm$ 19.3•	0	87.1 $\pm$ 2.6*•
	6 day	403.2 $\pm$ 42.5*	0	2.3 $\pm$ 2.2*
Lc - VKL - VKB orally	1 day	454.3 $\pm$ 13.7•	0	36.0 $\pm$ 0.4*•
	3 day	615.3 $\pm$ 23.9	0	2.9 $\pm$ 0.1
	6 day	512.3 $\pm$ 33.8	0	12.0 $\pm$ 0.5*•
Lc - VKL - VKB orally and vaginally	1 day	1850.3 $\pm$ 76.1*•	0	7.4 $\pm$ 0.6*•
	3 day	1328.0 $\pm$ 60.5*•	0	85.5 $\pm$ 0.5*•
	6 day	865.6 $\pm$ 59.3*•	0	13.4 $\pm$ 0.4*•

Significant differences with the intact mice are represented by \* (P < 0.05), while differences with the indicators of the staphylococcus-infected mice who did not receive probiotic bacteria or probiotic composition (control group) are represented by • (P < 0.05).

**Discussion.** Today, many probiotic preparations are recommended for intravaginal use in the treatment of patients with urogenital infections [1, 6, 13]. Previously, clinical studies have shown that probiotic bacteria can be delivered to the vagina after oral intake [18]. It should be noted that the oral application of probiotics is more practical for the treatment of patients with urogenital infections, so it is a more effective strategy. That is why in our present studies we have investigated whether the *L. casei* IMV B-7280 and Lc - VKL - VKB or VKL - VKB compositions normalize the vaginal microbiota and affect the production of cytokines in case of their oral use in the model of vaginal staphylococcosis in mice. It was suggested that these probiotic bacteria after their oral administration, probably, are able to colonize not only the intestines but also the vaginal tract of staphylococcus-infected mice, and create less supportive environment for *S. aureus* strain 8325-4 and other pathogens survival by producing antimicrobial substances and stimulate the Th1 type immune response. Therefore, *S. aureus* strain 8325-4 elimination from the vagina and vaginal microbiota normalization as well as induction the production of the Th1 type cytokines at the system level were observed in staphylococcus-infected mice that were orally treated with the *L. casei* IMV B-7280 and Lc - VKL - VKB or VKL - VKB compositions.

Our data coincide with the data of other authors. So, recently has been reported that strains of LAB, isolated from human or animal vagina or gut, have functional properties that can support their use as a potential probiotic for oral and vaginal applications [14, 15, 16]. These probiotic strains after oral application are able to colonize the vagina and effectively modulate vaginal microbiota and immunological profile. This was first proved for such strains as *L. rhamnosus* GR-1 and *L. fermentum* RC-14, that after oral administration were delivered to the vagina and effectively treated women with bacterial vaginosis [17, 18, 19].

After oral application of the combination of three probiotic strains, *L. fermentum* 57A, *L. plantarum* 57B and *L. gasseri* 57C, with high adhesive ability to both vaginal and intestinal epithelial cells, also observed the colonization by them of a woman's vagina within a few weeks, which was associated with a significant improvement of parameters such as the total number of vaginal LAB, pH etc. [14]. The frequency of recurrence of bacterial vaginosis and aerobic vaginitis in women decreased after the use of oral probiotic preparation (prOVag®) containing three strains of *Lactobacillus* together with a standard treatment with metronidazole, and targeted antibiotic treatment (after failure of metronidazole therapy). The reduced and maintained low vaginal pH and increased vaginal *Lactobacillus* number following this treatment were observed [20]. It should be noted that obviously there are differences in ability to colonize the vagina and intestines even for strains of the same species. Thus, it was found that *L. rhamnosus* GR-1 effectively colonized intestine and vagina, in contrast to the strain *L. rhamnosus* GG, which poorly

colonizes vagina [21], but well colonizes intestine [22]. Oral use of probiotics may be useful for modulation of the vaginal microbiota and immunological profiles during pregnancy because violation of the vaginal microbiota is one of the most important mechanisms for preterm birth and perinatal complications [23].

But, in our studies we assumed that the antistaphylococcal effectiveness of *L. casei* IMV B-7280 or Lc - VKL - VKB and VKL - VKB compositions will be higher if they are administered simultaneous orally and vaginally. In the experimental model of vaginal staphylococcosis we have found that oral and vaginal administration of *L. casei* IMV B-7280 and Lc - VKL - VKB or VKL - VKB compositions into staphylococcus-infected mice was more effective in normalization of vaginal microbiota. *L. casei* IMV B-7280 and Lc - VKL - VKB composition more efficiently stimulated aslo Th1 type cytokine production after simultaneous oral and vaginal administration than only oral administration. But oral administration of VKL - VKB composition into staphylococcus-infected mice more effectively increased the IL-12 production than oral and vaginal separately.

*L. casei* IMV B-7280 has a high and *B. animalis* VKL and *B. animalis* VKB – an average adhesiveness to epitheliocytes [24]. Therefore, this difference in adhesiveness to epitheliocytes may be due to the increase in the ability of *L. casei* IMV B-7280 and its composition with bifidobacteria to colonize the epithelium of the vagina and gut after oral and vaginal administration to staphylococcus-infected mice. So, after oral and vaginal administration of Lc - VKL - VKB composition we found more effective elimination of *S. aureus* strain 8325-4 from the vagina compared with VKL - VKB composition. Our analyses confirm that the study of the effectiveness of different methods of probiotic strains and their various compositions administration may be useful for their selection when creating immunobiotics.

Thus, *L. casei* IMV B-7280 and Lc - VKL - VKB or VKL - VKB compositions after oral as well as oral and intravaginal administration restored the microbiome of the vagina on the model of vaginal staphylococcosis in mice. This probiotic strains and their compositions are also should be regarded as immunomodulators, as it was evidenced the activation of Th1 type cytokines production after their use, so they can serve as support for an active immune system. However, simultaneous oral and vaginal administration of *L. casei* IMV B-7280 and Lc - VKL - VKB composition was more effective than oral. So, our results showed that the *L. casei* IMV B-7280 and Lc - VKL - VKB or VKL - VKB compositions can be a good candidate for the creations of immunobiotics for treatment of urogenital infections. However, additional studies regarding immunomodulatory properties of these probiotic bacteria are needed to confirm this. Such immunobiotics in the long term can also be used in the treatment of other infectious diseases.

**ДІЯ ПРОБІОТИЧНИХ БАКТЕРІЙ ПРИ ПЕРОРАЛЬНОМУ І  
ВАГІНАЛЬНОМУ ВВЕДЕННІ НА ВАГІНАЛЬНУ МІКРОБІОТУ  
ТА ПРОДУКЦІЮ ЦИТОКІНІВ ЗА ЕКСПЕРИМЕНТАЛЬНОЇ  
СТАФІЛОКОКОВОЇ ІНФЕКЦІЇ У МИШЕЙ**

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**Резюме**

**Мета.** Метою цього дослідження було визначення впливу пробіотичного штаму *L. casei* IMB B-7280 та композицій *L. casei* IMB B-7280 – *Bifidobacterium animalis* VKL – *B. animalis* VKB (Lc – VKL – VKB) або *B. animalis* VKL – *B. animalis* VKB (VKL – VKB), які вводили вагінально і перорально або лише перорально, на вагінальну мікробіоту та продукцію цитокінів за експериментальної вагінальної стафілококової інфекції у мишей лінії BALB/c. **Методи.** Пробіотичні бактерії або пробіотичні композиції вводили інфікованим стафілококом мишам одночасно перорально та вагінально або лише перорально. Матеріал із піхви висівали на селективні середовища; підраховували кількість колонієутворюючих одиниць, враховуючи, що одна така колонія відповідає одній бактерії. Концентрацію цитокінів у сироватці крові мишей визначали методом імуноферментного аналізу. **Результати.** Встановлено, що кількість аеробних і факультативно-анаеробних мікроорганізмів у піхві інфікованих стафілококом мишей зменшувалась у різні періоди спостереження після введення цього пробіотичного штаму та пробіотичних композицій одночасно у піхву і перорально або лише перорально. Також спостерігали збільшення кількості лактобацил і біфідобактерій у піхві цих мишей. Однак кількість коліморфних бактерій та мікроскопічних грибів була меншою, а кількість лактобацил більшою після одночасного вагінального і перорального введення *L. casei* IMB B-7280, а також композицій Lc – VKL – VKB або VKL – VKB інфікованим стафілококом мишам порівняно з пероральним введенням. Зміна мікробіоти піхви інфікованих стафілококом мишей, які отримували цей пробіотичний штам або пробіотичні композиції, відбувалася за підвищення рівня продукції інтерферону- $\gamma$  та інтерлейкіну-12, що сприяє розвитку Th1 типу імунної відповіді, а також зниження продукції прозапального цитокіну фактору некрозу пухлин- $\alpha$ . Імуномодульовальна ефективність *L. casei* IMB B-7280 і композицій Lc – VKL – VKB також була вищою, якщо їх вводили інфікованим стафілококом мишам вагінально і перорально, ніж лише перорально. **Висновки.** *L. casei* IMB B-7280 і пробіотичні композиції Lc – VKL – VKB або VKL – VKB можуть бути хорошими кандидатами для створення імунобіотиків для лікування урогенітальних інфекцій.

**Ключові слова:** лактобацили, біфідобактерії, мікробіота, піхва, стафілокок, цитокіни, миші.

# ДЕЙСТВИЕ ПРОБИОТИЧЕСКИХ БАКТЕРИЙ ПРИ ПЕРОРАЛЬНОМ И ВАГИНАЛЬНОМ ВВЕДЕНИИ НА ВАГИНАЛЬНУЮ МИКРОБИОТУ И ПРОДУКЦИЮ ЦИТОКИНОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ У МЫШЕЙ

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## Резюме

**Цель.** Целью данного исследования было определение влияния пробиотического штамма *L. casei* ИМВ В-7280 и композиций *L. casei* ИМВ В-7280 - *Bifidobacterium animalis* VKL – *B. animalis* VKB (Lc – VKL – VKB) или *B. animalis* VKL – *B. animalis* VKB (VKL – VKB), которые вводили вагинально и перорально или только перорально, на вагинальную микробиоту и продукцию цитокинов при экспериментальной вагинальной стафилококковой инфекции у мышей линии BALB/c. **Методы.** Пробиотические бактерии или пробиотические композиции вводили инфицированным стафилококком мышам одновременно перорально и вагинально или перорально. Материал из влагалища высевали на селективные среды; подсчитывали количество колониеобразующих единиц, учитывая, что одна такая колония соответствует одной бактерии. Концентрацию цитокинов в сыворотке крови мышей определяли методом иммуноферментного анализа. **Результаты.** Установлено, что количество аэробных и факультативно-анаэробных микроорганизмов во влагалище инфицированных стафилококком мышей снижалось в различные периоды наблюдения после введения этого пробиотического штамма или пробиотических композиций одновременно во влагалище и перорально или только перорально. Также наблюдалось увеличение количества лактобацилл и бифидобактерий во влагалище этих мышей. Однако количество колиформных бактерий и микроскопических грибов было меньшим, а количество лактобацилл было большим после одновременного вагинального и перорального введения *L. casei* ИМВ В-7280, а также композиций Lc – VKL – VKB или VKL – VKB инфицированным стафилококком мышам по сравнению с пероральным введением. Изменение микробиоты влагалища инфицированных стафилококком мышей, получавших этот пробиотический штамм или пробиотические композиции, происходило при повышении уровня продукции интерферона- $\gamma$  и интерлейкина-12, что способствует развитию Th1 типа иммунного ответа, а также при снижении продукции провоспалительного цитокина фактора некроза опухолей- $\alpha$ . Иммуномодулирующая эффективность *L. casei* ИМВ В-7280 и композиции Lc – VKL – VKB также была выше, если их вводили инфицированным стафилококком мышам одновременно вагинально и перорально, чем только перорально. **Выводы.** Пробиотический штам *L. casei* ИМВ В-7280 и композиции Lc – VKL – VKB или VKL – VKB могут быть хорошими кандидатами для создания иммунобиотиков для лечения урогенитальных инфекций.

**Ключевые слова:** лактобациллы, бифидобактерии, микробиота, влагалище, стафилококк, цитокины, мыши.

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