UDC:573.864.1:615.331

V.V. Mokrozub, L.M. Lazarenko, L.P. Babenko, L.M. Shinkarenko, O.M. Demchenko, M.Ya. Spivak, V.V. Bila

Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154, Acad. Zabolotny St., Kyiv, D03680, Ukraine

ANTISTAPHYLOCOCCAL ACTION OF LACTO- AND BIFIDOBACTERIA AND INTERLEUKIN-2

Antistaphylococcal action of Lactobacillus delbrueckii subsp. bulgaricus IMV B-7281, L. acidophilus IMV B-7279 and Bifidobacterium animalis VKB as well as interleukin-2 (IL-2) has been determined on the model of experimental staphylococcal infection in mice. It has been established that peroral administration of certain probiotic strains of lacto- and bifidobacteria or composition L. acidophilus IMV B-7279 – L. delbrueckii subsp. bulgaricus IMV B-7281 with IL-2 to mice favored the reducing of terms of staphylococcus persistence in the kidneys of experimental mice. The studied schemes are promising for treatment of patients with surgical infection.

*Key words***:** *Lactobacillus, Bifidobacteria, staphylococcal infection, interleukin-2*

In recent years the rapid growth of infectious-inflammatory diseases caused by pathogenic and opportunistic bacteria, viruses, fungi and others was marked all over the world. And as a result there are persistent quantitative and qualitative changes in normoflora of various body cavities (dysbiosis), the overall decrease of body defenses, immune system imbalances (hypo- or hyperactivation), possibility of secondary immunodeficiencies and autoimmune diseases which create favorable conditions for the development of infectious and inflammatory processes caused by opportunistic microorganisms – by human commensal microflora. Dysfunction of the immune system, in turn, is one of the major causes of increasing aggresiveness of opportunistic commensal microorganism with subsequent development of infectious diseases, and leads to the formation of cancer or to the occurrence of chronic inflammatory and allergic conditions (overexcited state of the immune system), etc.

Considering this, it is important not only to create new drugs for antimicrobial therapy, but also to find fundamentally new approaches for treatment and prevention of diseases caused by both pathogenic and opportunistic microorganisms. The areas, that attract more and more attention in recent years, are creation and use of products based on various strains of probiotic microorganisms that are capable, in addition to direct effect on pathogens, of raising defenses of macroorganism. These drugs are called immunobiotics [1, 2, 3]. Since the immunosuppression caused by pathogenic microorganisms affects the cytokine net, then its fast normalization requires the appropriate adjuvant therapy consisting of probiotics and immunoregulatory cytokines, including interleukin-2 (IL-2).

Materials and Methods. Experimental studies were performed on Balb/c line mice 18-20 g weight, obtained from the vivarium of the Institute of Molecular Biology and Genetics of NAS of Ukraine. All studies were performed taking into account the rules of the European Convention for the protection of vertebrate animals used for research and other scientific purposes of 18.03.1986 and the Law of Ukraine № 3447-IV *About animals protection from cruel treatment* [4].

Staphylococcosis was modeled through intraperitoneal injection of the *Staphylococcus aureus* strain 8325-4 (kindly provided to us by Professor V.S. Zuyeva, N.F. Gamaleya Institute of Epidemiology and Microbiology, Russian Federation) culture to mice daily, in a dose of 1 x 10^9 cells per animal. *S. aureus* strain 8325-4 had plasmid-based resistance to gentamicin, allowing it to be separated from other strains of staphylococcus obtained from the environment through the use of selective media containing this antibiotic. The following clinical manifestations of the infection process were observed in the infected mice: elevation of body temperature, inactivity, and loss of appetite.

Lyophilized strains of lactobacilli – *Lactobacillus acidophilus* IMV B-7279, *Lactobacillus delbrueckii* subsp. *bulgaricus* IMV B-7281 and bifidobacteria – *Bifidobacterium animalis* VKB and also IL-2 (Sigma) were used. The viability of probiotic cultures was determined by monitoring their growth on the Man-Rogosa-Sharpe (MRS) nutrient medium at 37 °C for 24-48 hours. Twenty-four hours after the infection, mice were given an *per os* a suspension of lyophilized lactobacillus and/or bifidobacteria cells in saline solution in a dose of 1 x 10^6 cells per animal, once a day for 7 days.

Strains were administered individually and in the combination *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – *L. acidophilus* IMV B-7279 (in equal proportions). IL-2 dose was calculated by the method proposed by Y.R. Rybolovlev et al. [5] and tested in experimental animals with staphylococcal infection. The drug was administered intraperitoneally in concentration of 1,000 IU / per animal twice – on the 1 and 3rd days after *S. aureus* 8325-4 injection.

A separate group of comparison included infected mice that did not receive any probiotic cultures, but instead, they were administered *per os* and intraperitoneally with the appropriate amount of saline. The control group included intact animals. All experimental studies were carried out in three repetitions.

On the 1, 3, 6 and 9th days after the start of probiotic cultures administration, kidneys were obtained from the mice; aliquots of their homogenates were plated on elective medium for staphylococci with gentamicin to determine the number of *S. aureus* 8325-4 colonies in the kidneys.

All digital data obtained were processed with the help of the Microsoft Excel-2003 through the variance analysis. Numerical data were represented as arithmetic average and standard error (M ± 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. It was previously shown [6] that the use of lactobacilli and bifidobacteria probiotic strains in mice with generalized staphylococcal infection reduced the time of *S. aureus* 8325-4 persistence in the kidneys of tested animals (Fig.1).

The results of our studies of lacto- and bifidobacteria probiotic strains and IL-2 effect on the *S. aureus* 8325-4 persistence in the kidneys of experimental animals are shown in Fig. 1. Thus, it was found that the investigated strains *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and *B. animalis* VKB (alone) and their composition *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and/or injection of IL-2 caused a reduction of staphylococcus infection in the kidneys of mice.

It should be noted that the number of gentamicin resistant *S. aureus* 8325-4 which was collected from the kidneys of animals that did not receive any treatment remained at a high level during the whole period of observation, representing: 3.18 ± 0.02; 3.23 ± 0.03; 3.10 ± 0.04 and 3.01 ± 0.04 lg CFU/ml on the 2, 4, 7 and 10th days of infection, respectively.

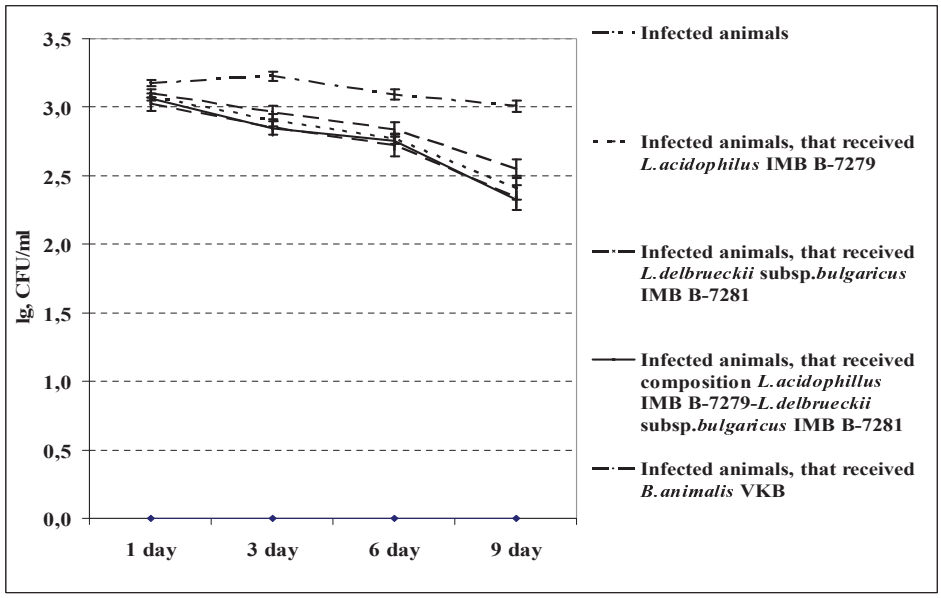

Fig. 1. The number of *S. aureus* 8325-4 colonies which were collected from renal aliquot of infected animals after oral administration of probiotic strains

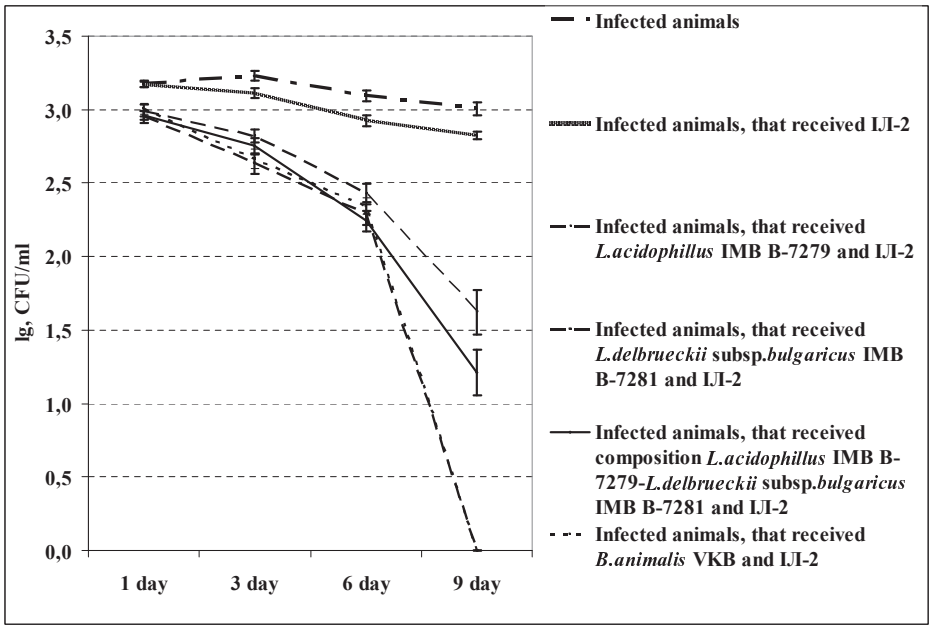

Fig. 2. The number of *S. aureus* 8325-4 colonies which were collected from renal aliquot of infected animals after oral administration of probiotic strains and IL-2

However, oral administration of all the investigated strains and composition to infected animals helped to rapidly reduce the number of *S. aureus* 8325-4 colonies in the kidneys of experimental animals from the 3rd day and the next 6 days after the start of probiotic strains administration. It should also be noted that *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 composition showed the most expressive antistaphylococcal effect.

However, double intraperitoneal injection of IL-2 contributed to a more rapid elimination of *S. aureus* 8325-4 from the kidneys of experimental animals (3.17 ± 0.02 lg CFU/ml – on the 1st day; 3.11 ± 0.03 lg CFU/ml – on the 3rd day; 2.92 ± 0.04 lg CFU/ml – on the 6th day and 2.82 ± 0.02 lg CFU/ml – on the 9th day), and the difference of those parameters in infected mice was reliable throughout the observation period starting from the 3rd day, but did not exceed the value specified for infected animals that received probiotic strains or their composition.

Complete application of probiotic strains and IL-2 further accelerated the elimination of the pathogen (Fig. 2). Thus, starting from the first day the number of *S. aureus* 8325-4 colonies, which were collected from renal aliquot of animals that received combined treatment using *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or composition *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and IL-2 was significantly lower than in animals with staphylococcal infection (2.95 ± 0.04 and 2.96 ± 0.03 against 3.18 ± 0.02 lg CFU/ml in the control group), and since the 6th day – lower than in animals with staphylococcal infection, treated only with these probiotic strains without IL-2 (2.29 ± 0.07 and 2.24 ± 0.07 against 2.72 ± 0.08 and 2.75 ± 0.07 lg CFU/ml, respectively).

It should also be noted that since the third day after the start of probiotic strains administration and throughout the next observation time the difference between the staphylococcus colonies number that were collected from renal aliquot of experimental animals with staphylococcal infection and infected animals that received probiotic strains or probiotic strains with IL-2 was accurate.

It was found that in the groups of animals which, except *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and composition *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, received injections of IL-2 as well as in infected animals which were treated only with IL-2 on the 6th and 9th days *S. aureus* 8325-4 was collected from renal aliquot in a much lesser number (2.43 ± 0.06; 1.60 ± 0.14; 2.29 ± 0.07; 0; 2.24 ± 0.07; 1.21 ± 0.16; 2.92 ± 0.04 and 2.82 ± 0.02 lg CFU/ml, respectively) compared with the groups of infected animals which received only probiotic strains as a means of their condition correcting (2.77 ± 0.04; 2.41 ±

0.09; 2.72 ± 0.08; 2.34 ± 0.09; 2.75 ± 0.07; 2.33 ± 0.06; 3.10 ± 0.04 and 3.01 ± 0.04 lg CFU/ml, respectively), and for some groups this difference was reliable only on the 3rd day after the start of probiotic strains administration (2.96 ± 0.05 lg CFU/ml for animals treated with *B. animalis* VKB against 2.66 ± 0.07 lg CFU/ml for animals treated with *B. animalis* VKB and IL-2).

Thus, we have found that probiotic strains administration to mice with staphylococcal infection leads to its elimination from the kidneys of experimental animals. Double injection of recombinant IL-2 combined with oral administration of probiotic strains accelerated the process. The most effective antistaphylococcal effect showed *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and *B. animalis* VKB, and the composition of probiotic strains *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 in combination of their oral administration with injections of IL-2.

We have previously investigated antagonistic properties of lacto- and bifidobacteria probiotic strains on the *in vitro* model. It was found that antagonistic action in relation to *S. aureus* 8325-4 between the studied strains was distributed as follows: *L. acidophilus* IMV B-7279 > *B. animalis* VKB > *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 > *B. animalis* VKL / *L. casei* IMV B-7280 [7]. The difference between antibacterial properties of probiotic strains in experiments *in vitro* and *in vivo* is associated with different mechanisms of its implementation [8], since it is known that antagonism to pathogenic and opportunistic microorganisms *in vitro* is directly related to the synthesis of biologically active substances with bactericidal and/or bacteriostatic properties – bactericins, short-chains fatty acids (lactic, acetic, formic), lysozyme, hydrogen peroxide [9, 10, 11] whereas *in vivo* antibacterial activity of lacto- and bifidobacteria probiotic strains in addition to the above factors is indirectly related to their immunomodulatory properties [12].

In the analyzed literature the data was found about the effectiveness of recombinant IL-2 use in complex therapy of various cancers (renal cell carcinoma, melanoma etc.), prevention and treatment of purulent surgical pathology (wound infection, sepsis) [13, 14, 15] and local use in the nidus of infection [16]. However, we have not found any mention about complex use of probiotics and IL-2 for treatment of purulent-inflammatory diseases caused by gram-positive opportunistic microorganisms, including staphylococci.

Conclusions

1. Probiotic strains *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKB and composition *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 had antagonistic properties against staphylococcus *in vivo*, as evidenced by accelerating the *Staphylococcus aureus* 8325-4 elimination from the kidneys of infected mice.

2. Double intraperitoneal injection of IL-2 in the complex therapy with probiotic strains *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKB and *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 composition accelerated the elimination of *Staphylococcus aureus* 8325-4 from the kidneys of experimental animals.

3. Further study of the effect of the combined use of probiotic strains and immunoregulatory cytokines in case of generalized staphylococcal infection in mice holds much promise.

В.В. Мокрозуб, Л.М. Лазаренко, Л.П. Бабенко, Л.М. Шинкаренко, О.М. Демченко, М.Я. Співак, В.В. Біла

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, Київ

АНТИСТАФІЛОКОКОВА ДІЯ ЛАКТО- ТА БІФІДОБАКТЕРІЙ ТА ІНТЕРЛЕЙКІНУ-2 ПРИ ЇХ СУМІСНОМУ ЗАСТОСУВАННІ

Р е з ю м е

Визначено антистафілококову дію *Lactobacillus delbrueckii* subsp. *bulgaricus* IMB B-7281, *L. acidophilus* IMB B-7279 та *Bifidobacterium animalis* VKB, а також інтерлейкіну-2 (ІЛ-2) на моделі експериментальної стафілококової інфекції у мишей. Встановлено, що пероральне введення інфікованим стафілококом мишам окремих пробіотичних штамів лакто- та біфідобактерій або композиції *L. acidophilus* IMB B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMB B-7281 з ІЛ-2 сприяли скороченню строків персистенції стафілококу в нирках піддослідних мишей. Досліджені схеми є перспективними для лікування хворих на хірургічну інфекцію.

К л ю ч о в і с л о в а**:** *Lactobacillus*, *Bifidobacteria*, стафілококова інфекція, інтерлейкін-2

В.В. Мокрозуб, Л.Н. Лазаренко, Л.П. Бабенко, Л.Н. Шинкаренко, О.Н. Демченко, Н.Я. Спивак, В.В. Била

Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, Киев

АНТИСТАФИЛОКОККОВОЕ ДЕЙСТВИЕ ЛАКТО- И БИФИДОБАКТЕРИЙ И ИНТЕРЛЕЙКИНА-2 ПРИ ИХ СОВМЕСТНОМ ИСПОЛЬЗОВАНИИ

Р е з ю м е

Исследована антистафилококковая активность *Lactobacillus delbrueckii* subsp. *bulgaricus* IMB B-7281, *L. acidophilus* IMB B-7279 и *Bifidobacterium animalis* VKB, а также интерлейкина-2 (ИЛ-2) на модели экспериментальной стафилококковой инфекции у мышей. Установлено, что введение инфицированным мышам отдельных пробиотических штаммов лакто- и бифидобактерий или их композиции *L. acidophilus* IMB B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMB B-7281 с ИЛ-2 способствовало сокращению сроков персистенции стафилококка в почках экспериментальных мышей. Исследованные схемы являются перспективными для лечения больных с хирургической инфекцией.

К л ю ч е в ы е с л о в а**:** *Lactobacillus*, *Bifidobacteria*, стафилококковая инфекция, интерлейкин-2.

А д р е с а в т о р а: *Мокрозуб В.В.,* Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины; Киев, ГСП, Д03680, ул. Акад. Заболотного 154, Украина.

1. *Shirobokov V.P., Jankowskij D.S., Dyment G.S.* Human microbial ecology (Russian). – Kyiv: LLC “Ruta-Tours”, 2010. – 340 p.
2. *Khoroshilova N.V*. Immunomodulatory and therapeutic effect of probiotics // Immunology (Russian). – 2003. – N 6. – P. 352–356.
3. *Fuller R*. Probiotics in human medicine // Gut. – 1991. – **32.** – P. 439–442.
4. *Reznikov O*. The problem of ethics in carrying out of experimental medical and biological research on animals (Ukrainian) // Bulletin of the National Academy of Sciences of Ukrainian. – 2001. – N 1. – P. 5–7.
5. *Rybolovlev Y.R., Rybolovlev R.S.* Dosing of substances for mammals using constants of biological activity (Russian) // Reports of the Academy of Sciences of the USSR. – 1979. – **247**, N 6. – P. 1513–1516.
6. *Mokrozub V.V., Lazarenko L.M., Babenko L.P., Shynkarenko L.M., Spivak M.Ja*. Antibacterial and immunomodulating properties of lactobacilli and bifidobacteria strains on the experimental model of staphylococcal infection (Ukrainian) // Biotechnology. – 2012. – **5**, N 2. – P. 98–105.
7. *Starovojtova S.A., Lazarenko L.N., Avdeeva L.V*. Strains of bacteria of the *Lactobacillus* and *Bifidobacterium* genera search, looking to create probiotics (Russian) // Science messenger of Uzhgorod University (Biol. Series). – 2009. – **26**. – P. 216–219.
8. *Gerritsen J., Smidt H., Rijkers G.T., de Vos W.M*. Intestinal microbiota in human health and disease: the impact of probiotics // Genes Nutr. – 2011. – N 6. – P. 209–240.
9. *Gatesoupe F.J*. Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments // J. Mol. Microbiol. Biotechnol. – 2008. – **14**. – P. 107–114.
10. *Ringo E., Gatesoupe F.J*. Lactic acid bacteria in fish: a review // Aquaculture. – 1998. – **160**. – P. 177–203.
11. *O’Shea E.F., Cotter P.D., Ross R.P., Hill C*. Strategies to improve the bacteriocin protection provided by lactic acid bacteria // Current opinion in biotechnology. – 2013. – **24,** Iss. 2. – P. 130–134.
12. *Dunne C., O’Mahony L., Murphy L., Thornton G., Morrissey D., O’Halloran S., Feeney M., Flynn S., Fitzgerald G., Daly C., Kiely B., O’Sullivan G.C., Shanahan F., Collins J.K. In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings // Amer. J. Clinical Nutrition. – 2001. – **73**, N 2. – P. 386–392.
13. *Kozlov V.K*. Immunopathogenesis of Surgical Sepsis: Manual for Physicians (Russian). – St. Petersburg: Publisher “Clear Light”, 2002. – P. 48.
14. *Zakrevskaya A.V., Berezin L.A., Kulyashova L.B*. Roncoleukin® using in the treatment of pyogenic infections (Russian) // Medical Immunology. – 1999. – **1**, N 3-4. – P. 122–123.
15. *Spivak M.Ja., Belotskyi S.M., Karlov V.A*. Sepsis: Immunology and Immunotherapy (Russian). – Kiev: Phytosociocenter, 2007. – 304 p.
16. *Egorova V.N., Popovich A.M.* Roncoleukin. Comprehensive Treatment of Infectious Diseases (Russian). – St. Petersburg: «Alternative printing», 2004. – 48 p.

Отримано 10.09.2012