BACTERIA NEW PROBIOTIC STRAINS IMPACT ON INTERFERON PRODUCTION

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The immunomodulatory properties of L. casei IMB B-7280, L. acidophilus IMB B-7279, B. animalis VKL and B. animalis VKB probiotic strains were studied and their effects on indicators of immunity were determined. Probiotic strains of L. casei IMB B-7280, L. acidophilus IMB B-7279, B. animalis VKL and B. animalis VKB under their intragastric injection in intact mice at a dose of 1×10^6 cells per animal once a day for 7 days had immunomodulatory properties. was strengthened All of these probiotic strains potentized interferonogenesis under conditions of physiological norm in different periods of observation. The investigated strains are promising for of body immunoreactivity correction affected due to infectious and inflammatory diseases.

Probiotic strains, lactobacilli, bifidobacteria, interferon immunomodulating properties.

Problem definition. Immune system disfunction emerging due to the changing environment, wide use of new chemotherapeutic agents of different nature, violation of normal microflora etc. is one of the major causes of increased aggressiveness opportunistic commensal microorganisms with the subsequent development of infectious and inflammatory diseases in humans and animals [11].

Obtaining modern imunobiotic based medications based on the representatives of normal microflora, especially of lacto- and bifidobacteria strains is an important issue of modern biotechnology [1].

Recent research and publications analysis. Literature data indicate that the imunomodulating0 properties of some lakto- and bifidobacterias cultures differ from each other significantly, it is their individual characteristics. In the course of creating drugs based on lacto- and bifidobacterias with elevated immunomodulatory activity (immunobiotics), it is correct to ensure that all conditions realized in biological potential of these bacterias are embedded [1]. In recent years, special attention has been paid to studying the mechanisms of modulating influence of lactobacilli on the immune response. Being associated with the intestinal mucosa, lactobacilli have universal immunomodulatory properties, including both immune stimulation and immune suppression [2-3,7-8]. Stimulating effects of lactic acid bacteria occur in the mechanisms of activation of the reticule-endothelial system tract and producing a number of cytokines which provide a balance between humoral and cell-mediated immunity [2-4, 9]. The most important mechanism of interaction between lactobacilli and representatives of generally obligate microflora of the host, aimed to support homeostasis, is to stimulate a number of cytokines production [9,13]. Cytokines are divided into several groups: interleukins (IL) – factors of interactions between leukocytes; interferon (IFN) – cytokines with antiviral activity; tumor necrosis factor (TNF) – cytokines with cytotoxic activity; colony stimulating factor (CSF) – hematopoietic cytokines and hemokyny (HC) – chemotactic cytokines [2,5-6,12-13].

The aim of the study was to determine the immunomodulatory properties of probiotic strains *L. casei* IMB B-7280, *L. acidophilus* IMB B-7279, *B. animalis* VKL. and *B. animalis* VKB by examining their impact on the production of endogenous interferon.

Material and methods research. *L. casei* IMB B-7280, *L. acidophilus* IMB B-7279, *B. animalis* VKL and *B. animalis* VKB, strains were used in the research. They were obtained from the collection of the Institute of Microbiology and

Virology named by D.K. Zabolotnyy NAS of Ukraine. The study was performed using freeze-dried bacteria. Before each experiment the viability of probiotic cultures by monitoring their growth on medium Man-Rogosa-Sharpe (MRS) at 37° C for 24-48 h was tested.

Experimental studies of the biological activity of probiotic bacteria agents was conducted in vivo. The study was conducted on the female mice BaLb/c animals weighing 18-20 g. Animals were divided on the basis of analogies into 5 groups of 15 animals each. Probiotic strains of bacteria (individually) were administered to mice per os for 7 days once a day. The dose of probiotic preparations per animal (*per os*) was 1×10^6 cells. Interferonogenesis dynamics was studied according to the changes in the contents of interferon in serum 6 h, 1, 3, 6 and 12 days.

Studying interferon status indicators. Interferon-α production was investigated with immune cells (peripheral blood leukocytes, spleen cells) in vitro in response to adequate induction, the spontaneous production of interferon and the contents of endogenous interferon in biological fluids were determined.

To induce interferon-α into the wells of sterile flat-bottomed 24-well plate made 400 uL of appropriate culture medium, 500 ml of cell suspension and 100 ml of solution rydostyne (100 micrograms/ml) were imputed. s100 ml of culture medium were added instead og rydostyne into the control well. The cells were incubated at 37° C for 24 h in a water-saturated atmosphere with a constant level of CO2 (5%). After incubation, the cells were transferred into sterile centrifuge tubes and centrifuged (10 min, 1500 r/min.). Supernatant was taken from each well to another sterile tube.

Interferon production was determined by micromethod [4] in the intact and experimental mice at 8, 24 and 72 hours after drug administration. For this purpose, 5 mice from each group were chosen by cerkvicale dislocation method and blood serum [4], spleen [8] were obtained from which splenocytes [7] were obtained.

All the datas received were worked out with Erie Info (version 6.0) computer program by variation statistics method. The numerical data were presented in the form of the average arithmetic mean value and standard deviation (M±m). The difference between groups was considered statistically significant at $P<0,05$.

Results. The studies proved that Lactobacillus or bifidumbacillus preparations injections to mice caused endogenous interferone production stimulation (Fig.1). However, the power and dynamics of interferone production were significantly different depending on the strain of Lactobacillus and bifidumbacillus. Among the lactobacilli the biggest interferon producing activity was noted in *L. acidofillus* IMB B-7279, and among the bifidumbakteria - in *B. animalis* VKL.

Significant accumulation of interferon in serum under the influence of *L. acidofillus* IMB B-7279 and *B. animalis* VKL was observed as soon as in 6 hours, interferon titers increased from 5,30±0,40 $\log_2$ U/ml in the control to 7,05±0,01 and 7,00±0,04 $\log_2$ U/ml, respectively ($P<0,05$). High levels of serum interferon was maintained for 1-st day (8,06±0,01 and 6,9±0,01 $\log_2$ U/ml), 3-d day (9,10±0,05 and 9,30±0,20 $\log_2$ U/ml) and 6^{th} day (9,00±0,01 and 8,04±0,02 $\log_2$ U/ml). In the mice treated with *L. acidofillus* IMB B-7279 concentration of this cytokine in serum appeared to increase by the 12-th day as well (6,75±0,02 $\log_2$ U/ml). However, interferon titers on the 12^{th} day decreased to the level of controls (4,4±0,30 $\log_2$ U/ml; $P>0,05$) after injecting the mice with *B. animalis* VKL.

Under the influence of *L. casei* IMB B-7280 serum interferon concentrations did not change compared with those of control after 6 h (5,0±0,09 $\log_2$ U/ml) and 1-st day (5,30±0,07 $\log_2$ U/ml), but it was increased by 3-d (6,30±0,01 $\log_2$ U/ml) and 6-th day (5,9±0,04 $\log_2$ U/ml). After 12 days, the contents of interferon in the serum of mice treated with *L. casei* IMB B-7280, was reduced to the level of performance in the control (4,25±0,30 $\log_2$ U/ml). After the injecting the mice with *B. animalis* VKB the serum interferon titers did not change inr 6 h and in 1 day (respectively 6,00±0,01 and 4,30±0,10 $\log_2$ U/ml), but they increased at 3^{rd} day to 7,30±0,30 $\log_2$ U/ml ($P<0,05$). At 6^{th} and 12^{th} days of interferon titers in the serum of these mice decreased to control levels (respectively 4,8±0,04 and 4,50±0,30 $\log^2$

U/ml). The results indicate that preparations of *L. acidofillus* IMB B-7279 and *B animalis* VKL were effective inducers as "early" and "late" interferon, *L. casei* IMB B-7280 after its injecting to mice induced the formation of "late" interferon.

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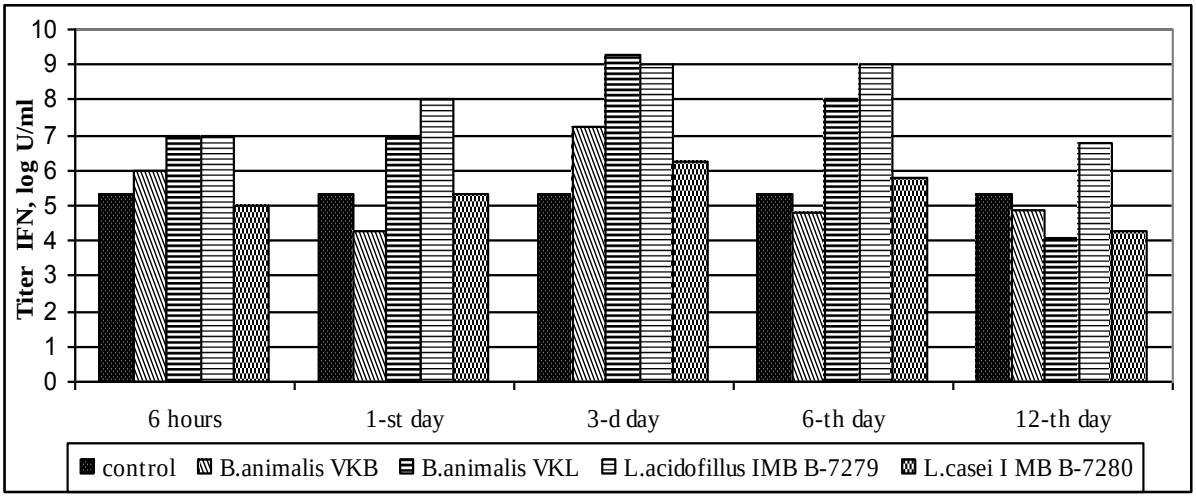

Figure 1. Changes in the content of serum interferon after administration of intact mice with probiotic bacteria strains

Spontaneous interferon production by cells of the spleen. It has been found out that injecting probiotics caused changes in interferon production activity of spleen cells (Fig. 2). Increase in the ability of splenocytes to the spontaneous production of interferon *in vitro* was observed with the injecting *B. animalis* VKL. Splenocytes, obtained on day 1, in vitro spontaneously produced interferon in the credits 7,10±0,06 $\log_2$ U/ml vs 5,00±0,20 $\log_2$ U/ml ($P<0,01$) in control. At 3rd and 6th days the tendency to increase spontaneous production of interferon was found, the titers were respectively 6,4±0,01 $\log_2$ U/ml, but the difference with the control was not significant (respectively 6,00±0,01 and 5,00±0,01 $\log_2$ U/ml). Interferon titers in supernatants of unstimulated splenocytes were obtained 12 days after injecting the mice with B. animalis VKL, which kept at the level of controls (4,8±0,01 $\log_2$ U/ml).

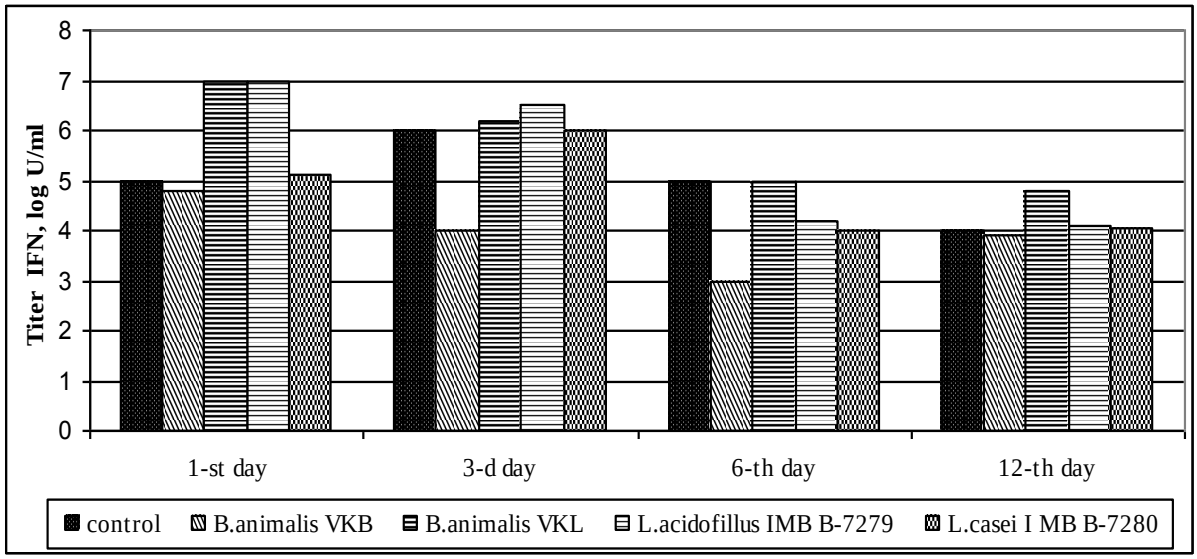

Figure 2. Interferon spontaneous production in vitro by spleen cells of intact mice treated with probiotic bacteria strains.

However, under the influence of *B. animalis* VKB a decrease in the ability to spontaneous interferon production was observed in splenocytes obtained at 3rd and 6th days after the injecting, titers were equal to 4,00±0,01 and 3,00±0,01 log_2 U/ml respectively versus 6,00±0,01 ($p<0,01$) and 5,00±0,01 ($P<0,01$) log_2 U/ml in the control. Interferon titers in supernatants of unstimulated splenocytes obtained on day 1 and 12 did not differ significantly from the control ones (4,8±0,01 and 3,9±0,02 log_2, U/ml respectively; in the control it made 5,00±0,01 and 4,00±0,01 log_2 U/ml.

Spontaneous production of interferon by spleen cells in vitro increased when under the mice injecting with *L. acidophillus* IMB B-7279. By the 1st day interferon titers increased to 7,00±0,04 log_2 U/ml vs 5,00±0,01 log_2 U/ml ($P<0,01$) in control. However, by 3rd, 6th and 12th days, they probably are not distinguished from that of controls (respectively 6,5±0,01; 4,2±0,02 and 4,1±0,02 log_2 U/ml).

Under the influence of *L. casei* IMB B-7280 1 night spontaneous production of interferon by splenocytes *in vitro* slightly increased (5,30±0,06 log_2 U/ml) by the 1st day, but the difference compared with those of control was insignificant.

By 3rd, 6th and 12th days after the injecting the mice with *L. casei* IMB B-7280 the splenocytes produced interferon spontaneously in the ctitres at the ampont of

6,00±0,01; 4,10±0,02 and 4,00±0,03 $\log_2$ U/ml respectively, which did not differ from those of controls.

Thus, the effective interferon production among the medications studied, were *B. animalis* VKL and *L. acidophillus* IMB B-7279. After injecting mice, with *B. animalis* VKL and *L. acidophillus* IMB B-7279 on the 1st day there was significant increase in the ability of splenocytes to spontaneous production of interferon *in vitro*. *B. animalis* VKB, *L. casei* IMB B-7280 after mice injecting did not affect the spontaneous production of interferon splenocytes *in vitro*.

Production of interferon-α by cells of the spleen. The mice injecting was accompanied by a change in the ability of spleen cells to the production of interferon-*α in vitro* (Fig. 3).

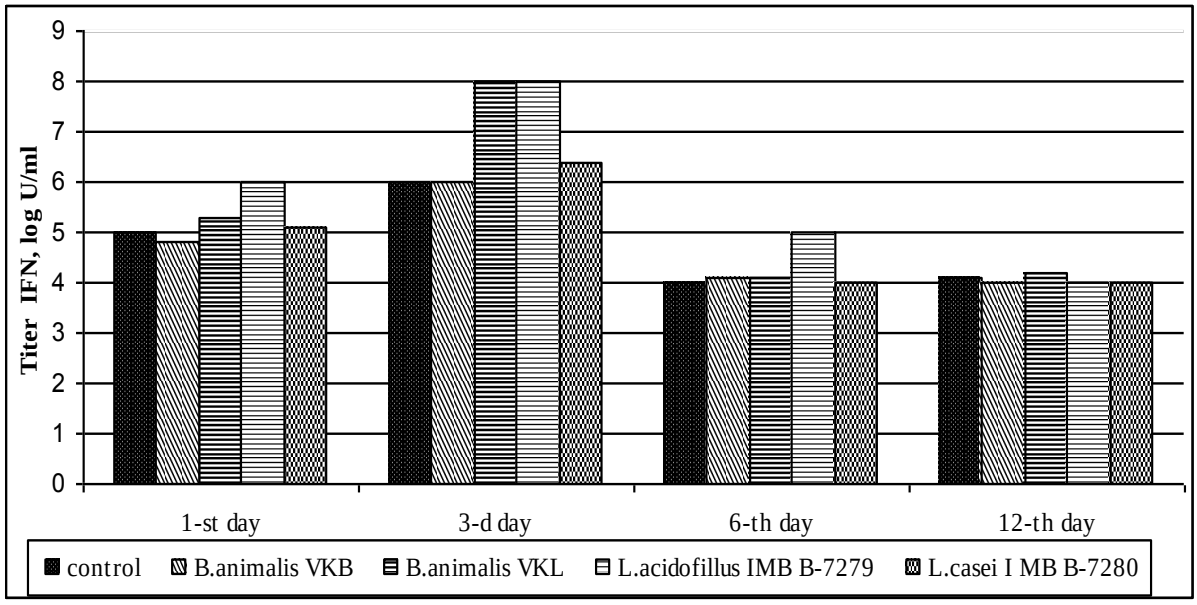

Figure 3. In vitro IFN production by spleen cells of intact mice that received probiotic strains of bacteria

It has been established that interferon-α production by activated splenocytes in vitro was increased under the influence of *B. animalis* VKL. The trend to increasing titers of interferon-α in supernatants of stimulated splenocytes on 1-st day after its introduction was shown (5,4±0,12 $\log_2$ U/ml in the control of 5,00±0,01 $\log^2$ U/ml; P>0,05). By the 3rd day titers of interferon-α increased significantly - up to 8,05±0,01 $\log^2$ U/ml vs 6,00±0,08 $\log_2$ U/ml (P<0,001) in control. By 6th and 12th days after injecting the mice with *B. animalis* VKL the

splenocytes produced interferon-α level control - in the titres under 4,1±0,01 and 4,3±0,18 $\log_2$ U/ml compared to 4,00±0,01 and 4,1±0,02 $\log_2$ U/ml in the control.

B. animalis VKB did not affect the production of interferon-α in vitro in response to adequate induction of splenocytes obtained at 1, 3, 6 and 12 days after its introduction, titers equaled respectively 4,8±0,01; 6,05±0,02; 4,1±0,02 and 4,10±0,01 $\log_2$ U/ml.

L. acidophillus IMB B-7279 increased the ability of splenocytes to produce interferon *in vitro*. After the injecting *L. acidophillus* IMB B-7279 on the 1st day it was showed a trend to increase titers of interferon-α in supernatants of activated splenocytes (6,05±0,36 $\log_2$, U/ml in the control of 5,00±0,01 $\log_2$ U/ml), whereas on 3rd day they increased significantly - up to 8,00±0,23 $\log_2$ U/ml vs 6,00±0,01 $\log_2$ U/ml ($P<0,001$) in the control. By 6th and 12th days the titers of interferon-α decreased to the targets level - according to 5,06±0,09 and 4,10±0,06 $\log_2$ U/ml.

Under the influence of *L. casei* IMB B-7280 production of interferon-α by spleen cells *in vitro* on 1st day did not change significantly (5,1±0,08 $\log_2$ U/ml) but increased slightly on 3 rd day (6,4±0,21 $\log_2$ U/ml; $P>0,05$).

Titers of interferon-α in supernatants of the stimulated splenocytes did not differ from that of controls at 6 and 12 days (respectively 4,1±0,09 and 4,00±0,01 $\log_2$ U/ml).

Conclusions. Probiotic strains of *L. casei* IMB B-7280, *L. acidophilus* IMB B-7279, *B. animalis* VKL and *B. animalis* VKB under intragastric injecting intact mice at a dose of 1×10^6 cells per the animal once a day for 7 days have immunomodulating properties. Yes, all of these probiotic strains under conditions of physiological norm in different periods of observation encreased the potentiality of interferon production in mice *B. animalis* VKL, *L. acidophillus* IMB B-7279, *L. casei* IMB B-7280 led to an increase in the ability of splenocytes to produce interferon-α *in vitro* in response to adequate induction for 1st and 3rd day days. The investigated Lacto- and Bifidobacterium strains can be further recommended as probiotyc medications with immunomodulating properties.

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