# ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ ИММУНОТРОПНЫХ СВОЙСТВ МЕТАБОЛИТОВ ШТАММА *BACILLUS SUBTILIS B-9909*, ПЕРСПЕКТИВНОГО В КОНСТРИРОВАНИИ НОВОГО ГЕПАТОПРОТЕКТОРА

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**Резюме.** Настоящее краткое сообщение посвящено вопросам экспериментального изучения иммуннотропной активности нового соединения – метабиотика, на основе метаболитов (биологически активных веществ, БАВ), продуцируемых сапрофитным и безопасным стандартизированным штаммом ВКПМ *Bacillus subtilis B-9909*. Цель исследования – экспериментальная оценка иммунотропного действия метаболитов, продуцируемых пробиотическими микроорганизмами рода Bacillus культуры пробиотических микроорганизмов ВКПМ *Bacillus subtilis* B-9909 на лабораторных животных при моделировании у них токсического поражения. Метаболиты выделяли из культуральной жидкости бактериальной культуры *Bacillus subtilis*, штамм ВКПМ B-9909, при его глубинном культивировании в среде, состоящей из соляно-кислотного гидролизата соевой муки или панкреатического гидролизата казеина. Культура в это время находилась в конце экспоненциальной фазы роста (16-18 часов культивирования). Исследование показателей гуморального статуса у экспериментальных групп животных при оценке терапевтической эффективности экспериментального образца метабиотика, по отношению к группе лабораторных животных, получавших препарат сравнения урсосан, проводили путем определения таких количественных показателей сыворотки крови, как титры иммуноглобулинов M, G, A, E, титр α-интерферона и концентрации циркулирующих иммунных комплексов. Поражение печени изучали путем моделирования острого токсического гепатита у белых крыс. Экспериментальный токсический гепатит моделировали на лабораторных животных – белых крысах. Внутрижелудочно вводили 40%-ный раствор $CCl_4$ в вазелиновом масле в течение 2 недель из расчета 0,2 г.кг$^{-1}$. Полученные результаты экспериментальных исследований свидетельствуют, что возникновение иммунного воспалительного синдрома в значительной степени было в контрольной группе подопытных животных с воспроизведенным токсическим гепатитом. В группе, в которой лабораторным животным были назначены метаболиты (БАВ), патологический процесс был существенно менее выражен, чем в группе, получавшей препарат сравнения. Важно отметить тот факт, что по окончании срока наблюдения (30-е сутки), в группе лабораторных животных, получавших метаболиты отмечали нормализацию всех исследуемых показателей, в отличие от группы с урсосаном, в которой показатели воспалительного иммунного синдрома не были до конца восстановлены. Таким образом, проведенные исследования по изучению гуморального статуса лабораторных животных, получавших метаболиты,

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**Образец цитирования:**

*Н.А. Забокрицкий «Экспериментальное изучение иммунотропных свойств метаболитов штамма Bacillus subtilis B-9909, перспективного в конструировании нового гепатопротектора» // Медицинская иммунология, 2023. Т. 25, № 3. С. 601-604.*
*doi: 10.15789/1563-0625-ESO-2772*



**For citation:**

*N.A. Zabokritskiy "Experimental study of immunotropic properties of metabolites of Bacillus subtilis B-9909 strain, promising in the formulation of a new hepatoprotector", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 3, pp. 601-604.*
*doi: 10.15789/1563-0625-ESO-2772*



**DOI:** 10.15789/1563-0625-ESO-2772

продуцируемых пробиотическими микроорганизмами рода *Bacillus subtilis* В-9909 на лабораторных животных при моделировании у них токсического поражения, дают основания выполнить заключение о наличии у испытуемого образца метабиотика существенного иммуномодулирующего эффекта, в сравнении с урсосаном. Данное обстоятельство позволяет рекомендовать данное соединение, как перспективное для создания нового лекарственного кандидата гепатопротектора с иммунотропным эффектом.

*Ключевые слова: метабиотик, Bacillus subtilis, метаболиты, пробиотик, иммунотропная активность, гуморальный иммунитет, гепатотоксичность, гепатопротектор*

# EXPERIMENTAL STUDY OF IMMUNOTROPIC PROPERTIES OF METABOLITES OF *BACILLUS SUBTILIS B-9909* STRAIN, PROMISING IN THE FORMULATION OF A NEW HEPATOPROTECTOR

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**Abstract.** This brief report is devoted to the experimental study of the immunotropic activity of a new compound, a metabiotic, based on metabolites (biologically active substances, BAS) produced by the saprophytic and safe standardized strain of *Bacillus subtilis B-9909*. The aim of the study was to experimentally evaluate the immunotropic effect of metabolites produced by probiotic microorganisms of the genus Bacillus of the culture of probiotic microorganisms of the *Bacillus subtilis B-9909* on laboratory animals when modeling their toxic lesion. Metabolites were isolated from the culture fluid of the bacterial culture *Bacillus subtilis*, strain RNCIM (Russian National Collection of Industrial Microorganisms) *B-9909*, during its deep cultivation in a medium consisting of hydrochloric acid hydrolysate of soy flour or pancreatic hydrolysate of casein. The study of humoral status indicators in experimental groups of animals in assessing the therapeutic efficacy of an experimental metabiotic sample, in relation to the group receiving the ursosan comparison drug, was carried out by determining such quantitative serum parameters as the titers of immunoglobulins M, G, A, E, the IFNα titer and the concentration of circulating immune complexes. Liver damage was studied by modeling acute toxic hepatitis in white rats. Thus, the studies conducted to study the humoral status of laboratory animals receiving metabolites produced by probiotic microorganisms of the genus Bacillus of the culture of probiotic microorganisms of the *Bacillus subtilis B-9909* on laboratory animals when modeling toxic damage in them, give grounds to conclude that the test sample of BAS has a significant immunomodulatory effect, in comparison with ursosan. This circumstance allows us to recommend this compound as promising for the creation of a new hepatoprotector drug candidate with an immunotropic effect.

*Keywords: metabiotic, Bacillus subtilis, metabolites, probiotic, immunotropic activity, humoral immunity, hepatotoxicity, hepatoprotector*

## Introduction

Modern domestic and foreign scientists agree that it is very advisable to create new medical immunological preparations. It is especially important that such drug candidates implement specific pharmacological mechanisms in a variety of biological models. All this will allow in the future to extrapolate the obtained results to practical public health.

Today it is necessary to take into account that the human body is exposed to adverse factors, such as: ecology, climatic and geographical features, social, professional. In combination with a bad epidemiological situation, they cause various pathological disorders of both individual tissues and organs, and entire systems of the human body. All this affects the morbidity and mortality of the population of the country.

In connection with the foregoing, the creation and use of preparations of microbiological origin is especially promising. This pharmacological group is probiotics in various pharmacological dosage forms.

This confirms the fact that many probiotics, such as bactisubtil, biosporin, sporobacterin, etc. already implemented in practical healthcare. The proportion of the use of probiotics based on non-pathogenic bacteria of the genus Bacillus and their metabolites is increasing. These metabolites are considered biologically active substances (BAS). And drugs based on metabolites are called metabiotics [1, 3, 5, 7].

Thus, metabiotics derived from biologically active substances of bacteria of the genus Bacillus are of considerable scientific interest [3, 8].

**The aim of the study** was to experimentally evaluate the immunotropic effect of metabolites produced by

probiotic microorganisms of the genus Bacillus of a culture of probiotic microorganisms RNCIM *Bacillus subtilis B-9909* on laboratory animals when modeling their toxic damage.

## Materials and methods

Metabolites of microorganisms of the RNCIM strain *Bacillus subtilis B-9909* were used in the work.

The complex of biologically active substances was obtained under laboratory conditions according to the recommendations currently available in the scientific literature [2, 5, 6, 8].

Metabolites were isolated from the cultural liquid of the bacterial culture of *Bacillus subtilis*, strain RNCIM *B-9909*, during its deep cultivation in a medium consisting of hydrochloric acid hydrolysate of soy flour or pancreatic hydrolysate of casein. The culture at that time was at the end of the exponential growth phase (16-18 hours of cultivation) [2, 5].

Cultivation was carried out in 250.0 mL flasks on a temperature-controlled plant for growing microorganisms UVMT-12-250. To obtain the culture liquid in large volumes, a BIOR-0.1 fermenter was used.

Subsequently, the culture liquid was subjected to the following technological operations:

– centrifugation (8000 rpm-1 for 15 minutes) or, with large volumes of culture liquid, separation (to separate the cell mass) using an ASG-3MB separator;

– ultrasonic disintegration (to destroy the remaining bacterial cells of *Bacillus subtilis*) for which an ultrasonic disperser UZD2-0.1/22 was used [2, 4, 8];

– sterilizing ultrafiltration using membrane filters "Millipor" with a pore diameter of 0.22 μm and "Sartorius" with a pore diameter of 0.3 μm;

– freeze-drying (to a residual moisture level of 3-5%) in a laboratory freeze-drying unit LSS-2. The yield of freeze-dried BAS complex, freed from cell biomass (from 1 liter of centrate liquid), was 10-15 g.

The qualitative and quantitative content of metabolites was determined by high performance liquid chromatography. Separation was performed at room temperature using a SupelcosilTM LC-18 column (250 × 4.6 mm, particle size 5 μm).

The study of indicators of the humoral status in experimental groups of animals in assessing the therapeutic efficacy of the experimental sample, in relation to the comparison drug ursosan, was carried out by determining such quantitative indicators of blood serum as the titers of immunoglobulins M, G, A, E, the titer of α-interferon and the concentration of circulating immune complexes.

Liver damage was studied by modeling acute toxic hepatitis in albino rats.

Experimental toxic hepatitis was modeled on laboratory animals, white rats. A 40% solution of $CCl_4$ in vaseline oil was injected intragastrically for 2 weeks at the rate of 0.2 $g.kg^{-1}$.

The humoral status in the experimental groups of animals in assessing the therapeutic efficacy of the test preparations was determined by studying such quantitative indicators of blood serum as:

– titer of immunoglobulins M, $mg \cdot cm^{-3}$;
– titer of immunoglobulins G, $mg \cdot cm^{-3}$;
– titer of immunoglobulins A, $mg \cdot cm^{-3}$;
– titer of immunoglobulins E, $mg \cdot cm^{-3}$;
– titer of α-interferon (IFNα), $pg \cdot cm^{-3}$;
– concentration of circulating immune complexes (CIC), opt. units

Quantitative determination of the concentration of CEC was carried out using the precipitation method with a 3.5% solution of polyethylene glycol. Quantitative determination of immunoglobulins (IgM, IgG, IgA, IgE) and α-interferon in blood serum was performed using enzyme immunoassay. The sampling of material for research (blood serum) was carried out on the 1st and 7th days after the start of treatment with experimental samples of the tested drugs.

The results were statistically analyzed using Microsoft Office Excel 2010 and Statistica 12.0 software packages. In this case, the method of dispersion analysis (ANOVA) was used. The normality of the distribution of the obtained data was assessed using the Kolmogorov–Smirnov method. Statistical assessment of the significance of intergroup differences was performed using Fisher's parametric test, depending on the normality of the data distribution. Statistical hypotheses were evaluated at the critical significance level $p < 0.05$.

## Results and discussion

The experimental data obtained indicate that in the group of experimental animals with a reproduced model of acute toxic hepatitis, compared with the control group of animals, on the third day of the experiment, there is a significant increase in the values of humoral immunity indicators that are directly involved in the immediate hypersensitivity reaction. Thus, the IgM titer increased by 3.5 times, the IgE titer – by 2 times, and, accordingly, the CIC concentration – by 3.2 times. This immunosuppressive effect is caused by toxicity of carbon tetrachloride on immunity, its effect on sIgA and IgG, as well as a decrease in the titer of IFNα – 2.5 times in the experimental group with a reproduced model of acute toxic hepatitis, compared with the control.

Further observations show us that on the third day in the groups of animals that, against the background of the development of acute toxic hepatitis, intragastrically administered metabolites (BAS) and the reference drug ursosan saw the development of an immunoinflammatory syndrome. It is important to note that only small changes in humoral immunity parameters were observed in the group of animals treated with metabolites in relation to animals that were prescribed ursosan.

The study of the immunoinflammatory syndrome on the eighth day of the experiment showed a decrease in its severity in all groups of experimental animals.

It was found that the concentration of IgM and IgE remained quite high (1.8 times increase). At the same time, an increase in the concentration of IgA and IgG was also noted. In addition, in all groups of experimental animals with toxic hepatitis, the CIC titer remained without correlation changes. It is important to note that we were able to establish an increase in the concentration of α-interferon in the group of experimental animals treated with BAS (41.6±2.8 pg·cm$^{-3}$), a 2-fold increase compared to the control group (20.4±1.7 pg·cm$^{-3}$). At the same time, in the group of experimental animals treated with the reference drug ursosan, an increase in the concentration of IFNα correlated with an increase in its concentration in the group without treatment, respectively, 18.8±1.5 pg·cm$^{-3}$ and 16.6±1.5 pg·cm$^{-3}$. The increase in CIC titer persisted in all groups of animals with acute toxic hepatitis.

Further observations on the fourteenth day of the experiment showed a return to the normal studied parameters of humoral immunity. In all experimental groups, the concentration of IgM remained 2-2.5 times higher than in the control group of animals. Other studied indicators were also increased by 1.6 – 1.8 times. A higher concentration of IFNα was registered in the group with BAS – 34.2±1.8 pg·cm$^{-3}$, compared with the ursosan group – 19.1±1.5 pg·cm$^{-3}$ and in the group without treatment – 18,7±1.5 pg·cm$^{-3}$.

The thirtieth day of observation was the final one. It was found that in the group with BAS there was a restoration of all indicators of humoral immunity to the initial values. In the ursosan group, the IgM titer and CIC concentration remained elevated. All other indicators of humoral immunity in this group corresponded to the upper limit of normal.

The obtained results of experimental studies prove that the occurrence of the immune inflammatory syndrome was largely in the control group of experimental animals with reproduced toxic hepatitis. In the group in which metabolites (BAS) were prescribed to laboratory animals, the pathological process was significantly less pronounced than in the group receiving the reference drug. It is important to note that at the end of the observation period (day 30), in the group with BAS, normalization of all studied parameters was noted, in contrast to the group with ursosan, in which the indicators of the inflammatory immune syndrome were not fully restored.

## Conclusion

Thus, the conducted studies on the study of the humoral status of laboratory animals treated with metabolites produced by probiotic microorganisms of the genus Bacillus of the culture of probiotic microorganisms RNCIM *Bacillus subtilis B-9909* on laboratory animals when modeling their toxic damage, give grounds to conclude that the test sample has significant BAS immunomodulatory effect, in comparison with ursosan.

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*Поступила 14.04.2023*
*Отправлена на доработку 25.04.2023*
*Принята к печати 26.04.2023*

*Received 14.04.2023*
*Revision received 25.04.2023*
*Accepted 26.04.2023*